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ORIGINAL PAPER

Effects of the Application of Dense Phase Carbon Dioxide Treatments on Technological Parameters, Physicochemical and Textural Properties and Microbiological Quality of Lamb Sausages

Natalia Szerman · Wei Li Rao · Xin Li · Yang Yang · Sergio R. Vaudagna · De Quan Zhang

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Abstract The effects of different dense phase carbon dioxide (DPCD) treatments were evaluated on technological parameters, physicochemical and textural properties and microbiological quality of lamb sausages. Sausages were prepared using lamb meat (*Longissimus dorsii*, 68.6 % w/w), lamb fat (17.2 % w/w), water (12.9 % w/w) and NaCl (1.37 % w/w). Raw sausages were subjected to different CO_2 pressures (10, 20 and 30 MPa) at 60 °C–30 min and treatment times (2, 14 and 25 min) at 55 °C–10 MPa. Weight loss, pH and total expressible fluid increased significantly ($p < 0.05$) as pressure increased from 0 to 30 MPa. These parameters also increased significantly ($p < 0.05$) as treatment time increased (at 10 MPa). The increase in CO_2 pressure and treatment time significantly ($p < 0.05$) modified internal and surface color parameters. Lightness and b^* values increased, whereas redness (a^*) decreased. Also, the increase in CO_2 pressure and treatment time significantly ($p < 0.05$) increased Warner–Bratzler shear force and textural parameters values. DPCD treatments may modify meat proteins, which may lead to weak interactions among proteins and formation of gel-like structures. Regarding microbial inactivation, the highest reductions in microbiological counts (2 Log CFU g^{-1}) were obtained applying a CO_2 pressure of 20 MPa at 60 °C for 30 min.

Keywords DPCD · Sausage quality · Lamb meat

Introduction

Non-thermal technologies such as high hydrostatic pressure, pulsed electric fields, dense phase carbon dioxide (DPCD), ultrasound and pulsed light have gained acceptance as food processing methods. DPCD is a cold pasteurization method that inactivates microorganisms and affects enzymes through molecular effects of CO_2 under pressures below 50 MPa, without exposing foods to adverse effects of heat and allowing foods to retain their fresh-like physical, nutritional and sensory qualities [6]. The carbon dioxide used in this process, which is a powerful solvent for a wide range of compounds in food processing, is non-toxic, non-flammable, relatively inert, low-cost, recyclable and readily available in high purity, and leaves no residue when removed after the process [6, 11].

A typical batch system has a CO_2 gas cylinder, a pressure regulator, a pressure vessel, a water bath or heater, and a CO_2 release valve. The sample is placed into the pressure vessel, and the temperature is set to the desired value. Then, CO_2 is introduced into the vessel until the sample is saturated at the desired pressure and temperature. The sample is left in the vessel for a period of time, and then the CO_2 outlet valve is opened to release the gas [6].

Natalia Szerman and Wei Li Rao have contributed equally to this work and should be considered co-first authors.

N. Szerman · S. R. Vaudagna
Instituto Tecnología de Alimentos, Centro de Investigación de Agroindustria, Instituto Nacional de Tecnología Agropecuaria (INTA), CC 77, B1708WAB Morón, Argentina

N. Szerman · S. R. Vaudagna
Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917, C1053AAY Buenos Aires, Argentina

W. L. Rao · X. Li · Y. Yang · D. Q. Zhang (✉)
Institute of Agro-Products Processing Science and Technology, Chinese Academy of Agricultural Sciences/Key Laboratory of Agro-Products Processing, Ministry of Agriculture, Beijing 100193, China
e-mail: dqzhang0118@126.com

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Some factors such as process pressure, temperature and time have significant influence on microbial inactivation in DPCD pasteurization [7]. The solubilization rate of CO_2 and its total solubility in water as well as in phospholipids are controlled by the pressure: higher pressures enhance CO_2 solubilization and induce both acidification and membrane expansion [9]. Regarding temperature, the inactivation rate increases as temperature increases: higher temperatures enhance CO_2 diffusivity and can also increase the fluidity of cell membranes to make CO_2 penetration easier [9]. However, the treatment should not be operated at temperatures far above CO_2 critical temperature because the solubility of the solvent within this region decreases quite rapidly as temperature increases and could deteriorate food quality in many applications [9, 12].

DPCD application to solid foods has been less studied than that to liquid foods, due to the complexity of the matrix, which can make CO_2 bactericidal action more difficult, and to the lack of information about the inactivation mechanism [11]. In the case of meat, CO_2 is easily absorbed and can decrease the pH. When meat is exposed to CO_2, carbonic acid is formed and then further dissociates to bicarbonate and hydrogen ions [13]. The pressure and temperature applied during treatment can affect molecular interactions and protein conformation, leading to protein denaturation and aggregation in the meat [17]. For that reason, meat quality may be affected, especially in relation to protein denaturation. Some researchers have studied the effect of DPCD on pasteurization and quality of meat products, in cured products such as cooked and dry ham [9, 10], ground beef [19], pork loins [4, 5, 22], marinated pork [3] and minced lamb meat [18].

The aim of this study was to evaluate the effects of DPCD treatments (different pressures and treatment times) on technological parameters (weight loss, total expressible fluid), physicochemical and textural properties and microbiological quality of lamb sausages.

Materials and Methods

Product Manufacture

Lamb loin (*Longissimus dorsii*) obtained from carcasses of Tan sheep crossed with Small-tail Han sheep bred in Ningxia (China) was used to manufacture the sausages. After 24 h post-slaughter, the meat was vacuum-packed and frozen at −18 °C. Sausages were prepared with the following composition: lean meat 68.6 % (w/w), lamb fat 17.2 % (w/w), ice water 12.9 % (w/w) and sodium chloride 1.37 % (w/w). Before manufacturing the sausages, the meat was thawed at 4 °C in a fridge. After trimming off the exterior fat and connective tissue, the meat was cut into cubes. Then, the meat cubes were chopped and mixed with NaCl in a 5-L cutter with a knife rotation rate of 1,450 rpm and bowl rotation rate of 14 rpm (model SZ-5, Guanzhou Xuszhong Food machinery Co. Ltd., China). Next, ice water and lamb fat were added and mixed in the same conditions described above. The meat emulsion was stuffed on a 3-L filling machine (Hakka Food Processing, China) in collagen casings (200 mm long, 25 mm in diameter). After manufacturing, the sausages were kept at 5 °C for 18 h until the DPCD treatments.

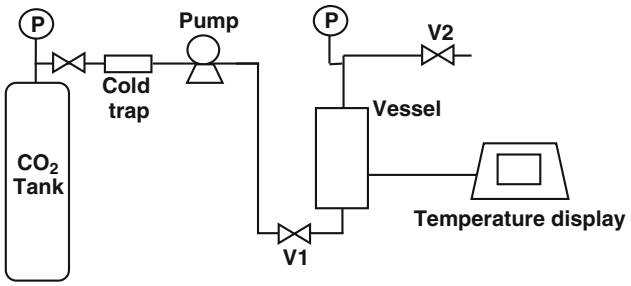

Fig. 1 Scheme of the dense phase carbon dioxide (DPCD) system. *V1* and *V2* are the CO_2 inlet valve and the CO_2 relief valve, respectively

DPCD Treatments

Sausages were subjected to different DPCD treatments in a HPR Series Reactor 1000 (Supercritical Fluid Technologies Inc., Denmark). The equipment consists of a vessel with a 1-L stainless steel cylinder (260 mm high, 92 mm in inner diameter), with a maximum pressure of 68.9 MPa and a maximum temperature of 200 °C, and an integrated control module. Commercial grade liquid CO_2 of 99.5 % purity (Beijing BeiWen Gas Factory, Beijing, China) was used. Figure 1 describes the DPCD system.

To evaluate the effects of CO_2 pressure, the temperature was fixed at 60 °C and the treatment time at 30 min. The CO_2 pressures evaluated were 10, 20 and 30 MPa. Sausages were loaded into the vessel at 60 °C, and then CO_2 was added into the closed vessel. The compression time was between 4 and 6 min. After 30 min, the pressure was released (decompression time 8–10 min). A control treatment was carried out keeping the sausages into the vessel at 60 °C, without CO_2, for 30 min.

To evaluate the effect of treatment time, the temperature was fixed at 55 °C and the CO_2 pressure at 10 MPa. Sausages were loaded into the vessel at 55 °C, and then CO_2 was added into the closed vessel. After reaching 10 MPa (compression time 4 min), three treatment times were evaluated: 2, 14 and 25 min. Then, the pressure was released (decompression time 8 min). A control treatment was carried out keeping the sausages into the vessel at 55 °C, without CO_2, for 25 min.

pH Measurement

The pH values were measured both in fresh sausages and after the DPCD treatments. The measurements were carried out in triplicate using a puncture pH meter (Testo 250, Testo Instruments Co., Ltd. Schwarzwälder, Germany).

Weight Loss

Weight loss (WL) was calculated by weighing sausages (Mettler Toledo, model PL 2002) before and after the DPCD treatments. After the DPCD treatments, sausages were dried with a paper towel to dry the released fluids. Then, WL was calculated using the following relationship:

$$\mathrm{WL} = \left[\frac{(m_1 - m_2)}{m_1}\right] \times 100$$

where m_1 is the mass of the sausage before the DPCD treatment and m_2 is the mass of the sausage after the DPCD treatment.

Total Expressible Fluid

Total expressible fluid (TEF) of sausages was determined following the method reported by Lee et al. [15], with slight modifications. For each treatment, this parameter was determined 24 h after the DPCD treatment on four sausage cores (25 mm in diameter, 20 mm high) at room temperature. Cores are compressed up to 70 % of height using a cylinder probe (50 mm in diameter) and held for 1 min with four filter papers under and three above of the sample, using a 50-kg load cell at a crosshead speed of 5 mm s^{-1}. This parameter was measured on a texture analyzer TA.XT*plus2* (Stable Micro Systems Ltd., UK). Samples were weighed before (m_1) and after (m_2) being compressed. Results are expressed as percentage of fluid released, as follows:

$$TEF = \left[\frac{(m_1 - m_2)}{m_1}\right] \times 100$$

Color Parameters

After the DPCD treatments, sausages were cut into 20-mm-high pieces. Color parameters were measured using a Minolta colorimeter model CR400 with illuminant C and 2° observer angle. Results were expressed as L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) in the CIELab system. Each color parameter was measured in the center and on the surface of each piece, with a total of 12 pieces for each treatment.

Warner–Bratzler Shear Force

For each treatment, Warner–Bratzler shear force (WBSF) values were measured 24 h after the DPCD treatments on four sausage cores (25 mm in diameter, 20 mm high) at room temperature (25 °C). Each piece was sheared with a Warner–Bratzler shear blade attached to a texture analyzer TA.XT*plus2* (Stable Micro Systems Ltd.) equipped with a 50-kg load cell and a crosshead speed of 5 mm s^{-1}. The Texture Expert computer software (Stable Micro Systems Ltd.) was used for data collection, and WBSF values were recorded as the maximum peak force of shearing (expressed in *N*).

Texture Profile Analysis

For each treatment, the texture profile was analyzed 24 h after the DPCD treatments on four sausage cores (25 mm in diameter, 20 mm high) at room temperature. The analysis was carried out at room temperature (25 °C). Texture parameters were determined with a double compression test, using a texture analyzer TA.XT*plus2* (Stable Micro Systems Ltd.) equipped with a 50-kg load cell. The compression assays were carried out using a cylindrical probe (50 mm in diameter). Samples were compressed up to 50 % of their original height with a rate of compression of 5 mm s^{-1}. The Texture Expert computer software (Stable Micro Systems Ltd.) was used for data collection, and the parameters evaluated were hardness, springiness, cohesiveness and chewiness.

Microbiological Analysis

The sausages were sampled aseptically (10 g) and stomached for 2 min in 0.1 % sterile peptone water. Serial dilutions were made and plated onto appropriate culture media to determine aerobic total count in Plate Count Agar (Beijing Land Bridge Technology Co., Ltd., China) at 37 °C for 48 h, enterobacteriaceae in Violet Red Bile Glucose Agar (Beijing Land Bridge Technology Co., Ltd.) at 37 °C for 48 h, lactic acid bacteria in MRS agar (Beijing Land Bridge Technology Co., Ltd.) at 30 °C for 48 h, and micrococci in Mannitol Salt Agar (Beijing Land Bridge Technology Co., Ltd.) incubated at 30 °C for 48 h. Microbiological counts were expressed as Log CFU g^{-1}.

Statistical Analysis

Differences between treatments were evaluated using one-way analysis of variance (ANOVA) followed by multiple comparison Tukey test ($p = 0.05$). Data were analyzed using Infostat software version 2011 [8].

Results and Discussion

Technological Properties

Tables 1 and 2 present the effects of the application of different CO_2 pressures and treatment times on pH, WL and TEF of lamb sausages.

Table 1 Effects of the application of different CO_2 pressures on pH values, weight loss (WL) and total expressible fluid (TEF) of lamb sausages (temperature 60 °C; treatment time 30 min)

Treatment	pH	WL (%)	TEF (%)
Fresh	6.07 ± 0.03 b	–	–
Control*	6.04 ± 0.04 b	3.53 ± 0.35 b	6.09 ± 0.77 b
10 MPa	6.12 ± 0.02 ab	7.86 ± 1.67 a	9.81 ± 0.13 a
20 MPa	6.12 ± 0.04 ab	6.74 ± 0.66 a	9.64 ± 0.41 a
30 MPa	6.19 ± 0.02 a	7.15 ± 0.40 a	10.26 ± 1.35 a

a–b Mean values with different letters in the same column are significantly different ($p < 0.05$)

* Sausages treated at 60 °C for 30 min without CO_2

Table 2 Effects of the application of different CO_2 treatment times on pH values, weight loss (WL) and total expressible fluid (TEF) of lamb sausages (CO_2 pressure 10 MPa; temperature 55 °C)

Treatment	pH	WL (%)	TEF (%)
Fresh	5.87 ± 0.02 c	–	–
Control*	5.89 ± 0.01 c	3.47 ± 0.18 b	4.59 ± 0.20 b
CO_2-2 min	5.91 ± 0.03 c	6.23 ± 1.10 a	6.76 ± 1.50 a
CO_2-14 min	6.05 ± 0.02 b	6.62 ± 1.31 a	10.07 ± 0.80 a
CO_2-25 min	6.12 ± 0.01 a	8.69 ± 1.00 a	11.12 ± 1.48 a

a–c Mean values with different letters in the same column are significantly different ($p < 0.05$)

* Sausages treated at 55 °C for 25 min without CO_2

Fresh and control sausages had no significant ($p > 0.05$) differences in pH values (Tables 1 and 2). These results indicate that the heating of the sausages (60 °C for 30 min or 55 °C for 25 min) caused no modifications in this parameter. No significant differences ($p > 0.05$) were observed in the pH of sausages treated at different CO_2 pressures (10, 20 or 30 MPa); however, sausages treated at 30 MPa showed significantly ($p < 0.05$) higher pH values than control ones (Table 1). Also, sausages treated for 25 min had significantly ($p < 0.05$) higher pH values than those treated for 2 or 14 min. In addition, sausages treated for 14 min had pH values significantly ($p < 0.05$) higher than those treated for 2 min (Table 2).

Thermal denaturation of meat proteins increases pH values as a consequence of the exposure and ionization of the buried groups that take place during heating [14, 21]. In meat of normal pH, the pH rises upon heating, at 40 °C by 0.1 units, at 45–65 °C by 0.2–0.3 units and at 70–80 °C by about 0.4 units [14]. In the present study, the heating of the sausages at 60 °C for 30 min or at 55 °C for 25 min showed no changes in the pH values compared with fresh sausages, which may indicate that the modifications in meat protein conformation were not important. Both the increase in CO_2 pressure (at 30 MPa) and treatment time increased the pH values of lamb sausages. In contrast, several studies have reported that DPCD treatments applied to different meat and meat products either cause no modifications or decrease the pH values. Yan et al. [22] observed no significant modifications in the pH values of fresh pork loin when CO_2 pressure increased from 0 to 14 MPa (50 °C for 30 min), but significant decreases when CO_2 pressure was 21 MPa (50 °C for 30 min). Qu et al. [18] reported that the pH values of minced mutton (added with salts—NaCl, sodium tripolyphosphate, hexametaphosphate and pyrophosphate-soy protein isolate and starch) were higher in samples treated at 10 MPa (55 °C for 30 min) than those only heated in the same conditions. In contrast, they observed that the treatment with CO_2 at 20 or 30 MPa decreased the pH values. When meat is exposed to CO_2, its absorption may modify the pH, depending on the buffering capacity of the meat. This may be explained by the fact that CO_2 dissolves in the aqueous part of a food by forming carbonic acid, which further dissociates to give bicarbonate, carbonate and H^+ ions, lowering the pH [6]. The pH reduction by dissolved CO_2 depends on the applied pressure at a constant temperature [16]. Pressure affects both the solubilization rate of CO_2 and its solubility in the medium. An increase in pressure level enhances the solubilization of CO_2. However, in the present study, the pH values were higher when the meat was treated with CO_2 (at 30 MPa). This change could be attributable to the modification of the myofibrillar protein conformation during the DPCD treatments, which may have induced an increase in pH values. Also, the presence of NaCl may have reduced the lowering of pH by the DPCD treatments [6].

Control sausages (60 °C for 30 min) had significantly ($p < 0.05$) lower WL than sausages treated at different CO_2 pressures (Table 1). Sausages treated at 10 MPa during different treatment times had significantly ($p < 0.05$) lower WL than control ones (55 °C–25 min). The WL of CO_2-treated sausages showed no significant differences ($p > 0.05$) between different CO_2 pressures or treatment times.

Control sausages (60 °C for 30 min or 55 °C for 25 min) had significantly ($p < 0.05$) lower TEF values than CO_2-treated ones (Tables 1, 2). Sausages treated at 30 MPa had higher TEF values than those treated at 10 or 20 MPa; however, this increase was not significant ($p > 0.05$). No significant ($p > 0.05$) differences were observed between TEF values of sausages treated with CO_2 at different times.

Both the increase in CO_2 pressure and treatment time increased WL and TEF. This indicates not only that fluids were lost during the DPCD treatments but also that the retained fluids were more easily released when an external force was applied. Choi et al. [5] found that the weight loss of pork loins increased with the increase in CO_2 pressure (from 7.4 to 15.2 MPa, 31.1 °C for 10 min), but observed

no significant differences. Chao et al. [2] reported that higher CO_2 pressures at constant temperature do not increase the water loss of ground beef when comparing 17.2 MPa at 35 °C versus 31 MPa at 35 °C and 17.2 MPa at 50 °C versus 31 MPa at 50 °C. However, these authors also reported that water loss increases when temperature increases from 35 to 50 °C at both 17.2 and 31 MPa. Their results indicate that the effect of temperature on water loss is more important than that of CO_2 pressure. These authors also found that lipid extraction was higher for ground beef treated with CO_2 at 31 MPa at 35 or 50 °C than at 17.2 MPa at 35 or 50 °C. Pressure was the most important factor for extracting lipids from ground beef. Concerning water-holding capacity, Yan et al. [22] observed lower values of this parameter when pork meat was treated at higher CO_2 pressures (>14 MPa), but the differences were not significant. Qu et al. [18] found that temperatures higher than 60 °C were needed to achieve a higher water-holding capacity in minced mutton. These authors also reported that the increase in CO_2 pressure at temperatures lower than 60 °C does not form a gel structure able to retain water.

According to these results, the WL obtained during the treatment with CO_2, which showed a tendency to increase when pressure or treatment time increased, may be caused by both water loss and lipid extraction. Regarding TEF, it increased when CO_2 pressure increased, although not significantly. Also, fluid retention showed a tendency to decrease with treatment time, which may also be associated with a longer exposure time at moderate temperatures. The formation of a matrix capable of retaining liquids from meat proteins is dependent on certain conditions. In our work, in agreement with that indicated by Qu et al. [18], the application of CO_2 pressures lower than 50 MPa in combination of moderate temperatures (<60 °C) would not be enough to form a strong gel. Nevertheless, it is possible that some weak interactions between proteins were established.

Color Parameters

The first impression that the consumer receives from a food is given by the sense of sight and among the properties observed, the color, shape and surface characteristics highlight. Thus, the color has a place of preference among the factors that define the quality of a food [20]. Tables 3 and 4 present the effects of the application of different CO_2 pressures and treatment times on internal and surface color parameters of lamb sausages.

Regarding internal color parameters, L^* (lightness) was significantly different between treatments. Control sausages (60 °C for 30 min) showed significantly ($p < 0.05$) lower L^* values than sausages treated at different CO_2 pressures, which in turn showed no differences between them (Table 3). Sausages treated at 10 MPa for 2 min had no significant differences ($p > 0.05$) with the control (55 °C for 25 min). However, sausages treated with CO_2 for 14 min or 25 min had significantly ($p < 0.05$) higher lightness values than those treated for 2 min or control (Table 4). A similar behavior was observed for L^* values measured on the surface of the sausages.

Neither a^* (redness) nor b^* (yellowness), measured at the center of the sausages, showed significant differences

Table 3 Effects of the application of different CO_2 pressures on CIELab color parameters evaluated at the center and surface of lamb sausages (temperature 60 °C; treatment time 30 min)

Treatment	L^*	a^*	b^*
Internal			
Control*	46.26 ± 1.28 b	15.51 ± 1.13	12.31 ± 0.74
10 MPa	56.13 ± 0.17 a	14.43 ± 0.52	12.15 ± 0.43
20 MPa	54.63 ± 0.68 a	14.78 ± 0.75	12.40 ± 0.19
30 MPa	54.26 ± 0.85 a	15.66 ± 0.12	13.02 ± 0.31
Surface			
Control*	45.24 ± 0.71 b	11.18 ± 0.39 a	10.59 ± 0.14 b
10 MPa	57.82 ± 0.26 a	6.72 ± 0.28 b	13.51 ± 0.13 a
20 MPa	56.50 ± 1.45 a	6.99 ± 0.17 b	13.26 ± 0.09 a
30 MPa	57.81 ± 0.80 a	6.64 ± 0.04 b	13.77 ± 0.16 a

a–b Mean values with different letters in the same column are significantly different ($p < 0.05$)

* Sausages treated at 60 °C for 30 min without CO_2

Table 4 Effects of the application of different CO_2 treatment times on CIELab color parameters evaluated at the center and surface of lamb sausages (CO_2 pressure 10 MPa; temperature 55 °C)

Treatment	L^*	a^*	b^*
Internal			
Control*	42.81 ± 0.55 b	16.50 ± 1.13 a	12.19 ± 0.26
CO_2 for 2 min	44.34 ± 0.77 b	13.74 ± 0.15 b	12.11 ± 0.17
CO_2 for 14 min	50.83 ± 1.20 a	13.52 ± 0.39 b	12.37 ± 0.31
CO_2 for 25 min	52.42 ± 1.04 a	14.37 ± 0.66 b	11.78 ± 0.28
Surface			
Control*	44.62 ± 0.27 b	8.02 ± 0.32 a	13.72 ± 0.34 a
CO_2 for 2 min	47.68 ± 3.96 b	7.62 ± 0.06 ab	11.23 ± 1.09 b
CO_2 for 14 min	54.47 ± 1.11 a	7.06 ± 0.09 b	12.46 ± 0.13 b
CO_2 for 25 min	55.48 ± 0.38 a	7.20 ± 0.28 b	12.56 ± 0.16 ab

a–b Mean values with different letters in the same column are significantly different ($p < 0.05$)

* Sausages treated at 55 °C for 25 min without CO_2

($p > 0.05$) between the sausages treated at different CO_2 pressures and the control (Table 3). However, control sausages presented significantly ($p < 0.05$) higher a^* and lower b^* values than sausages treated with CO_2 at 10, 20 or 30 MPa when these parameters were measured on the surface. Neither a^* (redness) nor b^* (yellowness) were affected by the different CO_2 pressures applied (Table 3). Sausages treated with CO_2 at 10 MPa for 2, 14 or 25 min had significantly ($p < 0.05$) lower a^* values than control ones (Table 4). Besides, control sausages had significantly ($p < 0.05$) higher surface a^* values than sausages treated with CO_2 for 14 or 25 min. Nevertheless, a^* values for sausages treated with CO_2 for 2 min showed no significant differences ($p > 0.05$) with those of the other treatments. Control sausages presented b^* values on the surface significantly ($p < 0.05$) higher than those treated with CO_2 for 2 or 14 min. Besides, the b^* values of sausages treated with CO_2 for 25 min had no significant differences in comparison with the other treatments.

Choi et al. [5] reported that pork loin chunks treated with CO_2 at 7.4 or 15.2 MPa (31.1 °C, 10 min) had higher L^* and b^* values and lower a^* values than the control samples. They also observed that L^* values significantly increased with the increase in pressure level. These authors found that the color change was correlated to the denaturation of proteins, mainly sarcoplasmics, which occurred during the treatment. The denaturation of sarcoplasmic proteins (phosphorylase b, creatine kinase, triosephosphate isomerase and one unknown protein) masked the red color of the sarcoplasm by precipitation and caused the muscle to become pale. Also, in fresh pork and in minced mutton, Yan et al. [22] and Qu et al. [18], respectively, observed that L^* increased slightly and that a^* decreased with the increase in CO_2 pressure. Moreover, Sirisee et al. [19] observed that the color of ground beef changed after CO_2 treatment and that it looked like cooked meat. The degree of water binding to the meat surface has a major effect on color, since the free water is responsible for giving a pale appearance and transmitting light to subsurface levels, where it can be scattered or absorbed [1]. In the present study, lightness increased with the increase in CO_2 pressure and treatment time. This result is associated with the higher TEF values obtained for those sausages. Modifications in a^* and b^* values may be associated with modifications in meat proteins structures.

Textural Properties

Figures 2 and 3 present the effects of the application of different CO_2 pressures and treatment times on the WBSF values of lamb sausages. Sausages treated at different CO_2 pressures or treatment times showed significantly ($p < 0.05$) higher WBSF values than their respective controls (60 °C for 30 min or 55 °C for 25 min). Also, sausages treated at different CO_2 pressures had no significant differences ($p > 0.05$) between them. However, sausages treated with CO_2 at 10 MPa for 14 min had significantly ($p < 0.05$) higher WBSF values than those treated for 2 min and similar to the ones treated for 25 min.

Figures 4 and 5 present the effects of the application of different CO_2 pressures and treatment times on hardness, springiness, cohesiveness and chewiness values of lamb sausages. Sausages treated at different CO_2 pressures had significantly ($p < 0.05$) higher values for all the textural parameters evaluated than their controls (60 °C for 30 min). No significant differences were observed between the CO_2 pressures applied. Sausages treated with CO_2 at 10 MPa for 14 or 25 min showed significantly ($p < 0.05$)

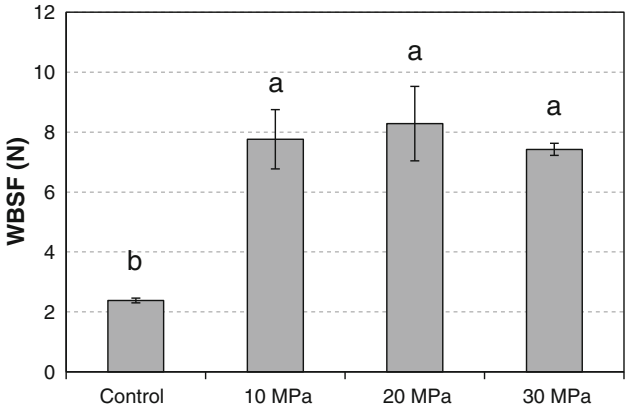

Fig. 2 Effects of the application of different CO_2 pressures on Warner–Bratzler shear force (WBSF) values of lamb sausages (temperature 60 °C; treatment time 30 min). *a–b* Mean values with *different letters* are significantly different ($p < 0.05$). Control sausages were treated at 60 °C for 30 min without CO_2

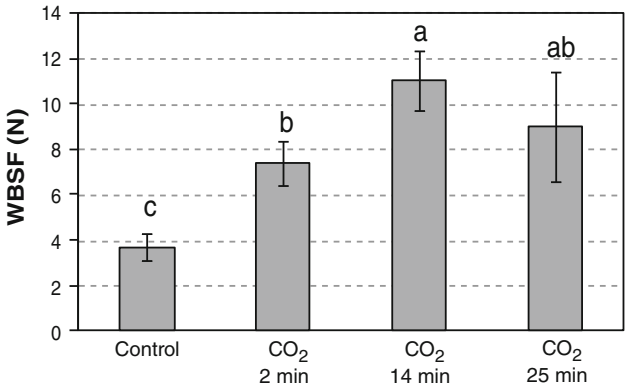

Fig. 3 Effects of the application of different CO_2 treatment times on Warner–Bratzler shear force (WBSF) values of lamb sausages (CO_2 pressure 10 MPa; temperature 55 °C). *a–b* Mean values with *different letters* are significantly different ($p < 0.05$). Control sausages were treated at 55 °C for 25 min without CO_2

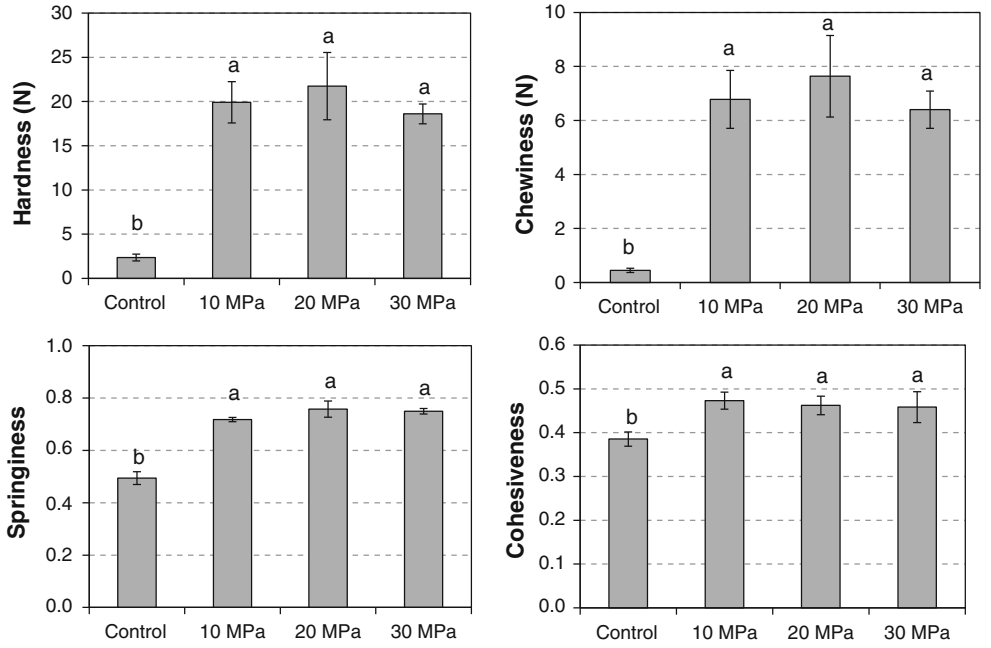

Fig. 4 Effects of the application of different CO_2 pressures on textural parameters of lamb sausages (temperature 60 °C; treatment time 30 min). *a–b* Mean values with *different letters* are significantly different ($p < 0.05$). Control sausages were treated at 60 °C for 30 min without CO_2

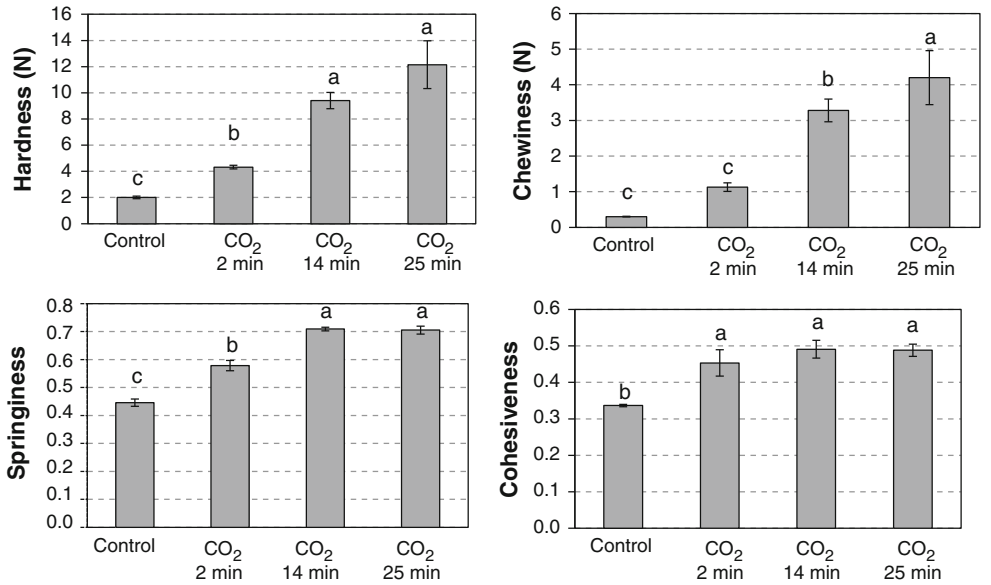

Fig. 5 Effects of the application of different CO_2 treatment times on textural parameters of lamb sausages (CO_2 pressure 10 MPa; temperature 55 °C). *a–b* Mean values with *different letters* are significantly different ($p < 0.05$). Control sausages were treated at 55 °C for 25 min without CO_2

higher hardness and springiness values than those treated for 2 min, which were also significantly ($p < 0.05$) higher than the control (55 °C for 25 min). For chewiness, control sausages and sausages treated with CO_2 for 2 min had similar values, but significantly ($p < 0.05$) lower than sausages treated with CO_2 for 14 or 25 min. Besides, sausages treated with CO_2 for 14 min had significantly ($p < 0.05$) lower values than those treated for 25 min. Regarding cohesiveness, control sausages had significantly ($p < 0.05$) lower values than those of the other treatments.

Treatments with CO_2 increased the values of WBSF and textural parameters. Pressure levels higher than 10 MPa showed no modifications in these parameters, whereas longer treatment times increased them. In pork loin treated

Table 5 Effects of the application of different CO_2 pressures on aerobic total counts (ATC), enterobacteriaceae (EB), lactic acid bacteria (LAB) and micrococci (MC) counts expressed as Log CFU g^{-1} (temperature 60 °C; treatment time 30 min)

Treatment	ATC	EB	LAB	MC
Fresh	6.73 ± 0.30 a	6.72 ± 0.05 a	4.09 ± 0.16 b	5.29 ± 0.13 a
Control*	5.38 ± 0.05 b	5.37 ± 0.17 b	4.35 ± 0.16 a	3.41 ± 0.10 b
10 MPa	4.62 ± 0.05 c	1.10 ± 0.17 c	<1	3.40 ± 0.15 b
20 MPa	3.86 ± 0.14 d	<1	<1	3.33 ± 0.19 b
30 MPa	4.21 ± 0.08 d	<1	<1	3.20 ± 0.25 b

a–b Mean values with different letters in the same column are significantly different ($p < 0.05$)

* Sausages treated at 60 °C for 30 min without CO_2

Table 6 Effects of the application of different CO_2 treatment times on aerobic total counts (ATC), enterobacteriaceae (EB), lactic acid bacteria (LAB) and micrococci (MC) counts expressed as Log CFU g^{-1} (CO_2 pressure 10 MPa; temperature 55 °C)

Treatment	ATC	EB	LAB	MC
Fresh	6.73 ± 0.30 a	6.72 ± 0.05 a	4.09 ± 0.16 ab	5.29 ± 0.13 a
Control*	5.57 ± 0.01 b	5.25 ± 0.01 c	4.26 ± 0.20 a	4.83 ± 0.16 b
CO_2 for 2 min	6.37 ± 0.03 a	5.71 ± 0.26 b	4.20 ± 0.08 a	3.23 ± 0.08 d
CO_2 for 14 min	5.42 ± 0.09 b	<1	4.04 ± 0.46 ab	3.26 ± 0.04 d
CO_2 for 25 min	4.73 ± 0.09 c	<1	3.46 ± 0.05 b	3.93 ± 0.16 c

a–b Mean values with different letters in the same column are significantly different ($p < 0.05$)

* Sausages treated at 55 °C for 25 min without CO_2

with CO_2 at 7.4 or 15.2 MPa (31.1 °C, 10 min), Choi et al. [5] observed that WBSF values were higher than those of control samples, but this increase was no significant. In contrast, Ferrentino et al. [9] found that cooked ham treated with CO_2 at 12 MPa (50 °C, 5 min) had lower resistance to the compression than control samples. However, their statistical analysis revealed no significant differences between fresh and treated samples. Regarding textural parameters, Qu et al. [18] reported that, in minced mutton treated with CO_2 at 60 °C for 30 min, the increase in CO_2 pressure from 10 to 50 MPa caused the increase in hardness and chewiness values but no modifications in cohesiveness or springiness.

In the present study, the treatment with CO_2 increased the values of WBSF and textural parameters, probably due to the denaturation of meat proteins, or some weak interactions between them, which formed a more resistant structure. The formation of that structure was favored by the treatment time at constant temperature (55 °C), but was not influenced by the increase in pressure level from 10 to 30 MPa.

Microbiological Analysis

Tables 5 and 6 present the effects of the application of different CO_2 pressures and treatment times on microbiological counts of lamb sausages.

Fresh sausages had significantly ($p < 0.05$) higher aerobic total counts (ATC), enterobacteriaceae (EB) and micrococci (MC) counts than control ones (60 °C for 30 min or 55 °C for 25 min). Lactic acid bacteria (LAB) counts were not significantly different between fresh and control sausages treated at 55 °C for 25 min but were significantly ($p < 0.05$) lower than those of control sausages treated at 60 °C for 30 min. The treatment with CO_2 at 10, 20 or 30 MPa for 30 min at 60 °C decreased ATC significantly in relation to control sausages; however, no significant ($p > 0.05$) differences were observed between treatments with CO_2 at 20 or 30 MPa. Regarding EB and LAB, the treatment with CO_2 at 10 MPa was enough to reduce the counts to $\leq$1 Log CFU g^{-1}. Control sausages showed no significant ($p > 0.05$) differences in MC counts in comparison with CO_2-treated ones. On the other hand, the treatment with CO_2 at 10 MPa and 55 °C for 2 min showed no significant ($p > 0.05$) modification in ATC; however, the increase in treatment time (14 or 25 min) allowed achieving a reduction in ATC up to 2 Log CFU g^{-1} in comparison with fresh sausages. Also, sausages treated with CO_2 at 10 MPa for 14 or 25 min showed a significant ($p < 0.05$) decrease in EB counts, achieving less than 1 Log CFU g^{-1}. LAB counts were significantly reduced only when sausages were treated with CO_2 at 10 MPa for 25 min. MC counts were significantly ($p < 0.05$) lower in sausages treated with CO_2 at 10 MPa

at 2, 14 or 25 min than the control ones. To achieve higher reductions in microbiological counts, the application of CO_2 treatments at 55 °C–10 MPa should be longer than 25 min. However, when the treatment temperature was 60 °C and the treatment time was 30 min, no significant ($p < 0.05$) differences were observed when pressure increased from 20 to 30 MPa. Among the bacteria evaluated, EB were the most sensitive, followed by LAB, whereas MC were the most resistant to CO_2 treatments.

Conclusion

DPCD treatments were effective in the reduction of bacterial counts. The highest reductions were obtained applying a CO_2 pressure of 20 MPa at 60 °C for 30 min. However, WL and TEF increased and color parameters and textural properties were affected. These changes may be related to modifications in the meat protein conformation. Thus, weak interactions between proteins may be established which lead to the formation of gel-like structures. Further research is needed to understand the molecular changes that take place in meat proteins during the application of DPCD treatments.

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