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Abstract

Background and objectives: Refrigerated dough products have the potential to be a safety hazard to consumers because they could be consumed raw or undercooked. The objectives of this study were designed to evaluate the microbial and functionality changes in high pressured sugar-cookie dough as a function of a_w (0.80–0.87), pressure level (100–600 MPa), and holding time (1–6 min).

Findings: Endogenous microbial populations were marginally reduced (0.2–0.5 log CFU/g) by pressure treatments. However, treating the dough at 600 MPa for 6 min significantly reduced counts of inoculated *Escherichia coli* by as much as 2.0 log CFU/g. Increasing the a_w of cookie dough from 0.80 to 0.87 did not play a significant role in the reduction of microbial counts; however, it yielded a softer and thicker cookie when baked. Dough and cookie physical characteristics did not differ significantly among HPP-treated and control doughs within the same a_w level.

Conclusions: The results of this study suggest that pressure treatment has the potential to improve the microbiological quality of wheat-based cookie doughs. However, variations in food matrix composition must be considered because some food constituents, such as sugar and fat, may protect microorganisms against pressure-induced inactivation.

Significance and novelty: The results reported here have practical implications for the food industry and contributes to understand the effects of high-pressure processing on wheat-based cookie doughs and their microbial loads.

Keywords: cookie dough, dough functionality, *Escherichia coli*, food safety, high-pressure processing

1 Introduction

The potential of commercial ready-to-bake dough products to serve as vehicles of serious and life-threatening foodborne illnesses was illustrated by the outbreak of Shiga toxin-producing *Escherichia coli* O157:H7 infections linked to the consumption of raw refrigerated, prepackaged cookie dough that occurred in the United States in 2009 (Neil et al., 2012). Commercial ready-to-bake cookie dough is a premixed product of various ingredients in which wheat flour, sugar, egg, and fat are the major constituents. Many of these ingredients are known for their potential to carry disease-causing bacteria. Shelled eggs and wheat flour, for instance, have been implicated in several foodborne disease outbreaks (CDC, 2018; Reynolds et al., 2010; US-FDA, 2017). Premixed cookie dough usually has a moisture level that does not favor microbial growth (Pareyt & Delcour, 2008); however,

pathogenic microorganisms potentially present in their ingredients may survive for lengthy periods of time (Neil et al., 2012).

Commercial, refrigerated cookie dough is usually sold without undergoing a pathogen-reduction treatment during processing, because the product is intended to be cooked by the consumer before consumption. However, nationwide surveys on risky eating behaviors conducted in the U.S. revealed that eating raw or partially cooked bakery goods is a popular practice among consumers. In a survey conducted by Byrd-Bredbenner et al. (2008), 53% of 4,343 young adults surveyed admitted that they regularly consume homemade cookie dough. In another survey, 65 and 73% of the 1,032 consumers surveyed, respectively, indicated that they have tasted refrigerated store-bought cookie dough and raw homemade dough before baking, and another 55 and 14% admitted to having eaten cake mixes and biscuit dough before it was fully cooked (Ardent Mills, 2019). The investigation of the *E. coli* O157:H7 outbreak linked to commercial cookie dough also concluded that the product was consumed directly from refrigeration, without the required baking step (Neil et al., 2012). Relying only on consumers' education about the health risks associated with eating raw dough may not guarantee the absence of food safety incidents. Therefore, further measures should be implemented by manufacturers to eliminate or reduce the risk of pathogen contamination in ready-to-bake dough products prior to their distribution.

Postpackaging interventions, such as high-pressure processing (HPP), can be an alternative to improve the microbiological quality and technological properties of ready-to-bake dough products. HPP consists in treating foods with hydrostatic pressure for a determined period. HPP may be conducted commercially for the purposes of processing, to improve product shelf life or product safety, by inactivating enzymes or killing microorganisms. A study carried out by Barcenas et al. (2010) showed that 1 min of HPP at 250 MPa reduced the aerobic mesophilic bacteria and yeast/mold counts in wheat-bread dough from 4.2 to 2.0 log CFU/g. Similarly, Barcenilla et al. (2016) reported reductions of 0.5 and 0.7 log CFU/g in total aerobic bacteria and yeast/mold counts, respectively, after treating cake batter at 600 MPa for 6 min. To date, there has been no published research on the application of HPP in wheat flour-based mixtures to inactivate pathogenic microorganisms. Therefore, the objectives of this study were to

evaluate (a) the effectiveness of HPP treatments to reduce populations of endogenous microorganisms and a nonpathogenic *E. coli* strain inoculated into sugar-cookie dough as model organism; (b) the impact of dough water activity on the inactivation of microorganisms during HPP; and (c) the impact of HPP on dough functionality and the physical characteristics of the baked cookies.

2 Materials and methods

2.1 Bacterial strain and inoculum preparation

A nonpathogenic *E. coli* strain (ATCC 25922) was used in this study as model organism. This generic strain of *E. coli* is commonly used as quality control strain and has previously been used in HPP inactivation studies (Koseki & Yamamoto, 2006; Lavinas et al., 2008). The strain was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and propagated according to manufacturer's instructions. After propagation, the culture was aseptically transferred into cryogenic vials containing 20% sterile glycerol and stored at −80°C. The culture was reactivated by transferring a small portion of the frozen broth, taken with a sterile loop, into 9 ml Tryptic Soy Broth (TSB; Acumedia, Lansing, MI, USA) followed by incubation at 37°C for 24 hr. After incubation, bacterial cells were aseptically transferred into a 50-mL sterile conical tube and harvested by centrifugation at 4,000 *g*/4°C for 8 min (Sorvall™ ST 16R Centrifuge, Thermo Fisher Scientific Inc., Waltham, MA, USA). The pellet was then washed with 25 ml of 0.1% sterile peptone solution, harvested once again and subsequently re-suspended in 50 ml 0.1% sterile peptone solution.

2.2 Preparation and inoculation of sugar-cookie dough

The ingredients and quantities used to prepare the sugar-cookie dough are listed in **Table 1**. All ingredients were purchased at a local supermarket. To determine the impact of moisture content on microbial reduction during high-pressure processing, the water content of the cookie formulation was adjusted to yield doughs with average water activity (*a*w) of 0.80 (Recipe 1), 0.83 (Recipe 2), and 0.87 (Recipe 3).

Table 1 Ingredients and recipes used to prepare sugar-cookie dough

Ingredients	*Recipe 1 Weight (g)*	*Recipe 2 Weight (g)*	*Recipe 3 Weight (g)*
All-purpose wheat flour	750.0	750.0	750.0
Granulated sugar	651.0	651.0	651.0
Salt	12.0	12.0	12.0
Whole egg powder	4.9	4.9	4.9
Baking soda	12.0	12.0	12.0
Nonfat dry milk	13.0	13.0	13.0
All-purpose vegetable shortening	300.0	300.0	300.0
Deionized water (chilled in ice)	204.0	355.0	505.0
Pure vanilla extract	5.5	5.5	5.5
Total weight	1,952.4	2,103.4	2,253.4

Formulations varied only in the amount of water included in the recipe. Recipe 1 represents the water content of the original sugar-cookie dough formulation; while Recipe 3 contains the maximum water amount the formulation could hold without considerably affecting its handling and baking performance.

Ingredients were mixed using a 5-quart bowl lift stand mixer (KitchenAid, Model KV25G0XER, Benton Harbor, MI, USA) with a flat beater. To prepare the dough, all dry ingredients, except flour, were weighed into the bowl and mixed at low speed (KitchenAid speed 1) for 1 min. Shortening was then added to the bowl and mixed at low speed for 30 s, scraped down, and creamed for 1 min at high speed (KitchenAid speed 4). After creaming, the water and vanilla extract were added and mixed at low speed for 30 s, scraped down, and mixed for 1 min at high speed. Flour was then added to the mixture and mixed for 30 s at low speed and then for 1 min at high speed, with intermittent bowl wall cleaning every 30 s. Three independent batches of sugar-cookie dough were prepared for each water activity level, representing the whole-plot experimental replicates.

To inoculate the dough, the water contained in the formulation was partially replaced by the inoculum to achieve a concentration of approximately 7.0 log CFU/g. To minimize contamination, the mixer was placed inside a biosafety hood throughout the sample preparation process and the bowl was covered with a plastic lid while mixing. The mixer bowl and paddle were autoclaved after each use to ensure sterility. Endogenous microbiota, functionality, and baking performance tests were carried out using noninoculated dough.

2.3 Packaging and high-pressure processing

Each batch of dough was aseptically divided into 10–200 g samples, placed in 3-mil sterile nylon-polyethylene bags and vacuum sealed at 100 mbar (Model C200, Multivac Inc., Kansas City, MO, USA). Packaged samples were further placed in another vacuum pouch and sealed to avoid any contamination or leakage during high-pressure processing. One double-bagged sample (split-plot unit) was randomly set aside as a control and the rest were randomly subjected to either 100, 300, or 600 MPa for 1, 3, or 6 min (split-plot factors) in a complete 3 × 3 factorial design. High-pressure treatments were carried out in a Hiperbaric 55 equipment (Hiperbaric, Miami, FL, USA) at 22°C using water as the pressure-transmitting fluid. The temperature increase due to the adiabatic heating effect during processing was approximately 3°C per 100 MPa.

2.4 Microbiological analysis

To determine the impact of high-pressure processing on the endogenous microbial load, noninoculated cookie dough was analyzed for aerobic mesophilic bacteria (aerobic plate count [APC]), coliform, yeasts, and molds. To determine the number of surviving microbial cells, 25 g of pressure-treated and untreated control doughs were placed in a sterile plastic bag along with 225 ml of 0.1% sterile peptone solution and mechanically mixed with a stomacher blender (Stomacher 400, Seward Ltd, Bohemia, NY, USA) for 2 min. Original sample dilutions were further diluted (1:10) using 0.1% sterile peptone solution. For APC, dilutions were spread plated in duplicate on Standard Methods Agar (SMA; Acumedia) and incubated at 35°C for 48 hr, according to standard procedures (Maturin & Peeler, 1995). For yeast and mold counts, dilutions were spread plated in duplicate on Dichloran Rose Begal Chloramphenicol agar (DRBC; Acumedia) and incubated at 25°C in the dark for 5 days, according to standard procedures (Tournas et al., 1998). Coliform counts were determined using appropriate Petrifilm plates (3M Microbiology), according to official method 991.14 (AOAC, 2013). Dilutions were evaluated in duplicate on Petrifilm plates and incubated at 37°C for 24 hr.

Surviving *E. coli* populations after high-pressure processing were determined using injury-recovery media to account for sublethally injured cells. Twenty-five grams of inoculated pressure-treated and untreated control doughs were serially diluted as described before and appropriate dilutions were surface plated in duplicate on Tryptic Soy Agar (TSA; Acumedia) plates and incubated at 37°C for 3 hr to allow the recovery of stressed, injured cells. To minimize the growth of the endogenous microbiota potentially present on cookie dough, TSA plates were overlaid with approximately 9 ml of MacConkey Agar (MAC; Acumedia) after the cells-recovery period. All plates were then incubated at 37°C for 48 hr prior to enumeration. The limit of detection for all microbial enumeration methods was 10 CFU/g. The reduction in microbial load was calculated by subtracting the logarithms of the colony counts obtained from the control and high-pressure treated samples.

2.5 Dough functionality tests

2.5.1 Dough water activity and textural characteristics

High-pressure treated and nontreated (control) dough samples were allowed to reach room temperature before performing the functionality tests. The water activity of the dough was measured at 24°C with an Aqualab 4TE (Decagon Devices, Inc., Pullman, WA, USA). Two water activity measurements were taken for each sample. The stickiness and strength (cohesiveness) of the dough were evaluated with a TA-TX2 texture analyzer (Texture Technologies; Scarsdale, NY, USA) equipped with a Chen-Hoseney dough stickiness rig. Approximately, a 2 g piece was cut from the dough mass and placed in the apparatus. A small amount of dough was extruded through the holes and carefully removed from the lid surface using a spatula. The dough was extruded once again to a length of 1 mm, covered with a plastic cap and rested for 30 s to release the stress produced by extrusion. The dough was then compressed with a 25 mm acrylic cylinder probe to conduct the adhesive test. The test was conducted twice for each dough sample.

2.5.2 Baking performance

Baking quality of high-pressure treated and nontreated (control) dough samples was assessed according to AACC method 10.50.05 (AACCI, 2018). Dough samples were rolled out to a thickness of 0.7 cm and cut into circular shapes of 6 cm diameter using a cookie cutter mold. However, due to an excessive stickiness, dough samples with a_w level of 0.87 were weighed and hand-molded to approximately the same dimensions as those obtained with the cutting mold. The cut-out dough pieces were placed at well-spaced points on lightly greased cookie sheets and baked in an electrically preheated rotary oven (National Manufacturing Corporation; Lincoln, NE, USA) at 205°C (400°F) for 6.5 min. Each prepackaged dough sample yielded six cookies. After baking, cookies were allowed to cool down at room temperature for 30 min and then packed in high-density polyethylene bags until analysis, which was conducted within 7 hr. Dough production and baking experiments were replicated on three different days, and the results presented are the average of three trials.

2.5.3 Cookie dimensional and textural characteristics

Dimensions of baked cookies (i.e., diameter, thickness, and spread ratio) were measured according to AACC method 10.50.05 (AACCI, 2018). Briefly, to measure the thickness, 6 cookies were stacked on top of one another, the height (thickness) measured to nearest ½ mm using a caliper, and then restacked in different order and remeasured. In order to measure diameter, six cookies were laid edge to edge, the width (diameter) measured, rotated 90° angle, and measured again. The average thickness and diameter of cookies were calculated, separately, by averaging the two readings and dividing by six. Spread ratio was calculated as cookie diameter divided by thickness.

The texture of baked cookies was assessed with a TA-TX2 texture analyzer equipped with a 25-kg load cell and a 3-point bending rig, following the American Institute of Baking standard procedure for cookie hardness (AIB, 2012). The gap between the support beams was set to 40 mm to be half the diameter of the cookies. The support rig distance was kept constant throughout the analysis to ensure comparability of results. Each cookie was precisely centered on the support beams to

measure the maximum peak force, an index of cookie hardness, and the distance between the trigger force and the maximum peak force, an index of flexibility. Texture experiments were replicated on three different days and the results presented are the average of three trials. Six cookie samples were assessed in each replication.

2.6 Experimental design and data analysis

A split-plot analysis of variance (ANOVA) was used to determine the effect of water activity, pressure, and exposure time on inactivation of microbial cells and functional properties of dough. The experiment was designed with water activity as the whole-plot factor and, pressure and time as the split-plot factors in a randomized 3 × 3 factorial combination with three batches of sugar-cookie dough per water activity treatment as the whole-plot replicates. Data were analyzed with SAS software version 9.3 (SAS Institute, Cary, NC, USA). The ANOVAs were performed using the GLIMMIX procedure of SAS. Significant differences ($p \leq .05$) between means were separated by Tukey's test. All statistical analyses were performed with a significance level of $p \leq .05$.

3 Results and discussion

3.1 Effect of dough water activity on microbial reduction by high-pressure processing

High pressure requires the presence of a transmitting medium in order to be efficient. The presence of solutes (e.g., sugar, salt) in high concentrations can reduce the amount of the pressure-transmitting fluid (i.e., water) available in the food matrix to cause microbial injury (Georget et al., 2015). Wheat flour, sugar, and vegetable fats are the major constituents in sugar-cookie dough, which contributes to its low moisture content. To elucidate the influence that the a_w of dough had on the effectiveness of HPP treatments to reduce microbial load, the water content of the cookie formulation was increased to yield doughs with average a_w values of 0.80, 0.83, and 0.87 (Table 1).

The analysis of variance revealed that the reduction in counts of the endogenous microbiota (i.e., APC, coliform, yeasts, and molds)

present in cookie dough was primarily driven by the level of pressure applied ($p \leq .05$). In contrast, the population of *E. coli* inoculated into cookie dough was significantly impacted not only by pressure but also by the holding time, as well as the a_w—pressure interaction. To better understand the impact of this interaction on *E. coli* counts, the contributions of each variable or their combinations to explain the *E. coli* load were calculated through a variance component analysis (data not shown). Pressure level made the largest contribution to explaining the total variance (> 72%) in the data set, while the interaction effect a_w—pressure accounted for only 2.0%, a value smaller than the contribution made by the error term (12.8%). Likewise, a_w and time had a very small level of contribution in the total variance, accounting for 4.9 and 8.2%, respectively. Due to the low contribution of the interaction, it could be assumed that a_w and pressure may be acting independently of each other.

The analysis of variance also showed that, unlike pressure, the a_w of the dough analyzed in this study did not play a significant role at reducing microbial counts ($p > .05$). In general, increasing the a_w did not cause substantial further reductions in microbial load. For instance, no significant changes in the reduction of APC, coliform, and yeasts were observed when the a_w was increased from 0.80 to 0.87 (**Table S1**). However, reduction in *E. coli* counts decreased significantly from 1.4 to 1.1 log CFU/g after increasing the a_w from 0.83 to 0.87. Similarly, the increase in a_w resulted in lower reduction levels of mold counts, ranging from 0.2 log CFU/g at a_w = 0.83 to 0.1 log CFU/g at a_w = 0.87 (Table S1). Nevertheless, even though some

Table S1. Effect of dough water activity on the reduction of microbial load in sugar-cookie dough.

Dough	*Microbial Reduction (log CFU/g)**				
a_w	*E. coli* †	*APC* †	*Coliform*	*Yeasts*	*Molds*
0.80	1.4 ± 0.4 a	0.4 ± 0.1 a	0.3 ± 0.1 a	0.2 ± 0.1 a	0.1 ± 0.1 ab
0.83	1.4 ± 0.6 a	0.4 ± 0.1 a	0.3 ± 0.1 a	0.2 ± 0.1 a	0.2 ± 0.1 a
0.87	1.1 ± 0.5 b	0.4 ± 0.1 a	0.3 ± 0.1 a	0.1 ± 0.1 a	0.1 ± 0.1 b

* Reduction on microbial populations is expressed in log CFU/g and ± denotes standard deviation. Mean values within the same microorganism followed by different letters are significantly different (P ≤ 0.05).

† Non-pathogenic E. coli strain (ATCC 25922); APC, Aerobic Plate Count.

of these a_w levels were statistically significant, the reduction values were so close to one another that they may not be practically relevant from a microbiological standpoint.

Several research studies have reported that inactivation of microorganisms by HPP may be dependent on the level of a_w (Butz et al., 1994; Hayman et al., 2008; Morales et al., 2006); however, in complex food matrices, such as cookie dough, the high concentration of solute (i.e., sucrose) and fat may exert a baroprotective role against pressure-induced microbial inactivation, thus hampering the effect of a_w. Van Opstal et al. (2003) showed that *E. coli* strain MG1655 was pressure- sensitive in the absence of sucrose, but became highly pressure resistant in the presence of 10% to 50% (w/v) sucrose. Numerous research studies have, in fact, indicated that disaccharides (e.g., sucrose) and ionic solutes (e.g., NaCl) protect microorganisms against pressure-induced inactivation of vital cellular components by promoting the accumulation of intracellular compatible solutes (Goh et al., 2007; Koseki & Yamamoto, 2007; Molina-Gutierrez et al., 2002; Molina-Hoppner et al., 2004).

Similarly, fat/oil containing matrices have shown to increase the resistance of microorganisms to HPP destruction due to the formation of local (or global) low a_w refuges. Early work carried out by Simpson and Gilmour (1997) showed that inactivation rates of *Listeria monocytogenes* caused by high pressure were substantially lower when the bacterium was suspended in an olive oil/PBS emulsion (30% v/v oil) than when inoculated into PBS buffer alone. Therefore, due to the presence of high concentrations of sucrose and fat in the cookie dough, the net effect of a_w on microbial inactivation after pressure treatment may be difficult to assess. Since a_w and interaction effects did not play a significant role in microbial reduction, they were disregarded for further analysis and the average microbial reduction for each HPP treatment (main effects) was taken as the best estimate to evaluate for differences in treatments.

3.2 Effect of high-pressure processing on the endogenous microbial population of cookie dough

The increase of pressure in the environment surrounding microbial cells may affect not only their morphology but also may inhibit

Table 2 Concentration of endogenous and inoculated microorganisms in sugar-cookie dough after high-pressure processing treatment

Treatments		*Endogenous microorganisms*[a]				*Inoculated microorganisms*[a]
Pressure (MPa)	*Holding time (min)*	*APC*[b]	*Coliform*	*Yeasts*	*Molds*	*Escherichia coli*[b]
0	0	3.7±0.1 a	3.1±0.4 a	2.5±0.3 a	2.3±0.1 a	7.5±0.1 a
100	1	3.4±0.1 b	2.8±0.1 ab	2.4±0.1 a	2.3±0.1 ab	6.9±0.2 b
	3	3.3±0.1 bc	2.8±0.1 ab	2.4±0.1 a	2.3±0.1 abc	6.8±0.3 b
	6	3.3±0.1 bcd	2.8±0.1 b	2.4±0.1 a	2.2±0.1 abcd	6.6±0.3 bc
300	1	3.3±0.1 bc	2.8±0.1 b	2.4±0.1 a	2.2±0.1 abcd	6.4±0.2 cd
	3	3.3±0.1 bc	2.8±0.1 b	2.3±0.1 a	2.2±0.1 abcd	6.1±0.4 de
	6	3.2±0.1 cd	2.7±0.1 b	2.3±0.1 a	2.2±0.1 abcd	5.9±0.3 ef
600	1	3.3±0.1 bcd	2.8±0.1 b	2.3±0.1 a	2.1±0.1 bcd	5.9±0.3 ef
	3	3.2±0.1 cd	2.7±0.1 b	2.3±0.1 a	2.1±0.1 cd	5.7±0.2 fg
	6	3.2±0.1 d	2.7±0.1 b	2.3±0.1 a	2.1±0.1 d	5.5±0.2 g

a. Concentration of microbial populations is expressed in log CFU/g and±denotes *SD*. Mean values, within the same microorganism, that share the same letter(s) are not significantly different from one another ($p > .05$).
b. APC, Aerobic Plate Count; *E. coli*, nonpathogenic strain (ATCC 25922).

metabolic reactions and cause changes to genetic mechanisms, resulting in a cascade of events that may be catastrophic to microbial cell viability (Abe, 2007; Huang et al., 2014). Small but significant reductions in the endogenous microbial population of cookie dough were observed after the application of treatments (**Table 2**). The initial population of aerobic mesophilic bacteria was reduced significantly by all HPP treatments ($p \leq .05$). Treating dough at 100, 300, or 600 MPa for 1, 3, or 6 min reduced the APC counts by an average of 0.4 log CFU/g. Reduction trends of coliform counts in HPP-treated dough were similar to those observed for APC counts. Regardless of the pressure-holding time, HPP at 300 and 600 MPa resulted in about 0.3 log CFU/g reduction in the population of coliform bacteria (Table 2).

Regarding mold counts, treatment with 600 MPa was required to cause a significant reduction of 0.2 log CFU/g when compared with the initial counts in the control dough (Table 2). As observed in APC and coliform, no further significant decrease in mold counts was obtained by extending the treatment time. For yeast, no significant changes in counts were observed after the application of treatments when compared with the control (Table 2). Although usually an increase in pressure is associated with higher microbial inactivation rates, this

relationship was not observed in the present study. In addition, increasing the duration of the HPP treatments did not cause significant further reductions in microbial counts. These findings are in agreement with those of previous research studies on microbial inactivation in high-pressure treated flour-based foods (Aguirre et al., 2018; Barcenas et al., 2010; Barcenilla et al., 2016).

For instance, a study carried out by Barcenas et al. (2010) showed that 1 min of HPP at 250 MPa reduced the aerobic mesophilic bacteria and yeast/mold counts in wheat-bread dough from 4.2 to 2.0 log CFU/g. Aguirre et al. (2018) reported more than 4.0 and 2.5 log CFU/g reduction in aerobic mesophilic bacteria and yeast/mold counts, respectively, in sugar-snap cookie dough after HPP at 100 or 200 MPa for 2 min. The microbial reductions obtained in the present study were substantially lower than those reported by these authors, most likely due to the composition of the sugar- cookie dough.

As mentioned, food constituents such as proteins, sugars, and lipids could confer a certain degree of protection to microorganisms against high-pressure treatments, which would hinder the effectiveness of HPP (Georget et al., 2015). For instance, considerably lower microbial reductions than those reported by other authors were obtained by Barcenilla et al. (2016) using a more complex flour-based matrix. Their results showed reductions of 0.5 and 0.7 log CFU/g in total aerobic bacteria and yeast/mold counts, respectively, after treating layer cake batter at 600 MPa for 6 min. In the same study, Barcenilla et al. (2016) reported that lactic acid bacteria and total anaerobic bacteria counts remained unchanged after the application of HPP treatments when compared with the control cake batter. The microbial reduction levels obtained in the present study are similar to those reported by Barcenilla et al. (2016), perhaps due to similar composition of the food matrices evaluated.

3.3 Survival of E. coli inoculated into cookie dough after high-pressure treatment

The numbers of surviving *E. coli* (ATCC 25922) cells in cookie dough after HPP at various pressure levels and holding times are shown in Table 2. The initial population of *E. coli* was reduced significantly by all HPP treatments ($p \leq .05$). After treatment at 100 MPa, regardless

of the pressure-holding time, the *E. coli* population decreased from 7.5 to 6.8 log CFU/g. Increasing the pressure level to 300 or 600 MPa resulted in significant further reductions in *E. coli* counts. For instance, a reduction of 1.4 log CFU/g was achieved after treatment at 300 MPa, while at 600 MPa the *E. coli* population declined, on average, by 1.8 log CFU/g when compared with the control dough (Table 2). Increasing the time of treatment from 1 to 3 min, resulted in no or only negligible further inactivation of *E. coli* cells; however, by extending the exposure time to 6 min, significant further reductions in the order of 0.4 log CFU/g, on average, were observed. HPP of cookie dough at 600 MPa for 6 min decreased the *E. coli* counts by as much as 2.0 log CFU/g when compared with the initial counts in the control dough.

Although numerous studies on pressure-induced inactivation of *E. coli* have been published, direct comparisons between the pressure-inactivation data obtained in the present study and the data reported by other authors (e.g., Alpas et al., 1999; Liu et al., 2015) may not be appropriate due to remarkable differences in the complexity of the matrices harboring the microbial cells (e.g., phosphate buffer, milk, juice, and meat vs. cookie dough), as well as substantial differences between *E. coli* strains with respect to pressure resistance. However, HPP inactivation studies that have been carried out using complex food matrices and have used the same or similar model microorganisms may provide the most relevant comparison to the present study.

As mentioned, HPP-induced microbial inactivation may be influenced by both food matrix composition and inherent microbial characteristics. For instance, Garcia-Graells et al. (1999) compared the pressure resistance of *E. coli* MG1655 cells inoculated into phosphate buffer and milk. The results showed that *E. coli* inactivation in milk reached 2.3 log CFU/ml after treatment at 700MPa for 15 min, whereas in phosphate buffer, a pressure of 400 MPa for the same period of time was enough to achieve a reduction in counts of approximately 7.0 log CFU/ml. Similarly, Viazis et al. (2008) reported that *E. coli* ATCC 25922 survival was significantly higher in pressure-treated samples of human milk than in 0.1% peptone solution, with the former requiring more than 46 min at 400 MPa to achieve complete inactivation (9.0 log CFU/ml), while the later required only 10 min at the same pressure level in peptone solution for total inactivation.

Researchers have proposed that disaccharides, such as sucrose, may have the potential to interact with the cytoplasmic membrane and maintain its fluidity by (a) promoting the accumulation of compatible solutes (i.e., osmoprotectants) and (b) retarding the shift from the liquid phase to the gel phase under high-pressure environments, thus protecting vital cellular components and metabolic mechanisms (Molina-Hoppner et al., 2004). Moreover, research studies have suggested that the baroprotective effect of foods with high sucrose concentration is not solely due to the sugar content but also due to the lack of compressibility caused by the excess of solutes (Fauzi et al., 2017; Min et al., 2010). The high sucrose concentration in the cookie dough used in the present study may have, therefore, conferred a certain degree of protection to both the endogenous flour microorganisms and the inoculated *E. coli* cells against pressure-induced inactivation.

Other food constituents such as fats and oils may exert a baroprotective role against pressure-induced microbial inactivation. For instance, Garcia-Graells et al. (1999) reported that inactivation of *E. coli* cells was significantly higher in skim milk (0.05% fat, 3.0 log CFU/ml reduction) than in whole milk (3.6% fat, 1.6 log CFU/ml reduction), thus suggesting a baroprotective effect of fat. The formation of low water activity refuges within the food matrix due to the presence of fat/oil, it is believed to play a role in the observed protection against pressure. In addition, bacteria with hydrophobic surfaces may be attracted to these refuges where there is much lower water content and, therefore, lower HPP-induced inactivation (Georget et al., 2015).

In addition to the baroprotective effect exerted by some food constituents, an inherent variation in pressure resistance exists between strains of *E. coli*. For instance, Liu et al. (2015) compared the pressure sensitivity of several *E. coli* strains inoculated in ground beef. They reported that, after treating samples at 600 MPa and 25°C for 3 min, cell counts of the most resistant strain (*E. coli* O26-05-6544) decreased by only 2.0 log CFU/g, while the most sensitive strain (*E. coli* O157-C0283) decreased by more than 5.5 log CFU/g. Similarly, Alpas et al. (1999) reported that the viability loss of six *E. coli* O157:H7 strains inoculated in 1% peptone solution ranged from 2.8 to 5.6 log cycles after pressurization at 345 MPa and 25°C for 5 min, with about five-hundred fold difference between the most resistant and most sensitive strains. Therefore, it is important to highlight that the 2-log

reduction achieved in the present study with a model organism (*E. coli* – ATCC 25922) may translate to a lower reduction of a cocktail of pathogenic strains.

3.4 Effect of water activity and high-pressure processing on dough characteristics and baking performance

Sugar-cookie dough texture-related parameters were not significantly affected by any of the HPP treatments applied (**Table 3**). Increasing the pressure level and the pressure-holding time did not cause significant changes in the dough physical characteristics analyzed in the present study. In general, the stickiness and cohesiveness did not differ significantly between HPP-treated and control dough samples ($p > .05$). Increasing the water activity of the dough, however, resulted in a more sticky and cohesive dough.

Slightly contradictory results on the impact of HPP on quality characteristics of flour-based foods have been reported by other

Table 3 Effect of different levels of dough water activity, pressure and holding times on sugar-cookie dough physical characteristics

Treatments[a]			*Dough characteristics*[b]	
a_w[c]	*Pressure (MPa)*	*Holding time (min)*	*Stickiness (N)*	*Cohesiveness*
0.80	0	0	0.18±0.02 a	0.31±0.04 a
	100	1, 3, 6	0.19±0.01 a	0.32±0.03 a
	300	1, 3, 6	0.19±0.02 a	0.32±0.04 a
	600	1, 3, 6	0.19±0.01 a	0.31±0.03 a
0.83	0	0	0.49±0.02 a	1.47±0.08 a
	100	1, 3, 6	0.49±0.01 a	1.41±0.06 a
	300	1, 3, 6	0.49±0.02 a	1.47±0.16 a
	600	1, 3, 6	0.48±0.02 a	1.43±0.19 a
0.87	0	0	1.08±0.05 a	2.63±0.38 a
	100	1, 3, 6	1.07±0.07 a	2.69±0.22 a
	300	1, 3, 6	1.07±0.08 a	2.69±0.19 a
	600	1, 3, 6	1.08±0.10 a	2.73±0.25 a

a. No significant differences among HPP holding times were observed; therefore, to simplify the presentation of data, results from those dough quality tests were averaged.

b. Data reported as mean±*SD*. Mean values, within the same dough quality parameter and a_w level, that share the same letter are not significantly different from one another ($p > .05$).

c. a_w: water activity of the dough.

researchers. For instance, Barcenas et al. (2010) observed a decrease in bread dough stickiness when increasing the pressure-holding time beyond 1 min, while Aguirre et al. (2018) reported an increase in stickiness after treating sugar-snap cookie dough at 200 and 400 MPa for 4 and 15 min, respectively. In the present study, the uniform cohesiveness between HPP-treated and untreated doughs is in agreement with observations made by Barcenas et al. (2010) who detected no significant differences in the cohesiveness of HPP-treated and untreated wheat-bread doughs, and with Barcenilla et al. (2016) who reported that cohesiveness of cakes made of either control or HPP-treated batters did not differ significantly.

Research studies have reported that high-pressure treatments may alter the chemical and functional properties of wheat starch and gluten. For instance, Kieffer et al. (2007) reported that mild pressure treatments (e.g., 200 MPa) induced a significant strengthening of gluten; however, under extreme pressure (e.g., 800 MPa), gluten cohesivity was lost. The authors argued that cleavage and rearrangement of disulfide bonds were involved in the observed changes in gluten functionality. Studies carried out on wheat starch suspensions have indicated that high-pressure treatments (0.1–600 MPa for 15 min) does induce gelatinization of starch and thus alter dough hydration (Douzals et al., 2001).

The dimensional and textural characteristics of cookies made with HPP-treated dough were not significantly different than those observed in control samples (**Table 4**). Increasing the a_w of dough yielded thicker cookies when baked. Depending on the dough a_w, the diameter of cookies varied from 8.19 to 8.44 cm, while the thickness and spread ratio ranged from 0.82 to 1.19 and from 6.98 to 9.99, respectively. Previous studies have, however, reported an increase in diameter and a decrease in thickness in cookies made with HPP-treated dough when compared with control cookies, although such differences were not directly related to the intensity or the duration of the HPP treatment (Aguirre et al., 2018).

The spread ratio, a relationship between diameter and thickness of cookies, was not significantly altered by the application of HPP treatments, although it decreased considerably with the increase in water activity of the dough (Table 4). Aguirre et al. (2018) reported, instead, an increase in spread ratio when treating dough at high pressures;

Table 4 Effect of different levels of dough water activity, pressure, and holding times on cookie dimensional and textural characteristics

Treatments [a]			*Cookie dimensional characteristics* [b]			*Cookie textural characteristics* [b]	
a_w [c]	*Pressure (MPa)*	*Holding time (min)*	*Diameter (cm)*	*Thickness (cm)*	*Spread ratio* [d]	*Hardness (N)*	*Flexibility (cm)*
0.80	0	0	8.20±0.04 a	0.82±0.01 a	9.98±0.13 a	22.09±0.88 a	4.52±0.04 a
	100	1, 3, 6	8.20±0.03 a	0.82±0.01 a	10.04±0.10 a	22.72±2.37 a	4.52±0.06 a
	300	1, 3, 6	8.15±0.04 a	0.82±0.01 a	9.96±0.09 a	22.12±1.48 a	4.53±0.03 a
	600	1, 3, 6	8.19±0.02 a	0.82±0.01 a	10.01±0.08 a	22.94±1.79 a	4.56±0.05 a
0.83	0	0	8.49±0.14 a	0.93±0.03 a	9.17±0.13 a	3.68±0.48 a	4.33±0.07 a
	100	1, 3, 6	8.48±0.13 a	0.93±0.02 a	9.17±0.28 a	3.52±0.43 a	4.36±0.05 a
	300	1, 3, 6	8.46±0.13 a	0.92±0.02 a	9.18±0.27 a	3.46±0.41 a	4.36±0.07 a
	600	1, 3, 6	8.34±0.10 a	0.93±0.02 a	9.00±0.14 a	3.52±0.40 a	4.37±0.04 a
0.87	0	0	8.27±0.07 a	1.20±0.02 a	6.92±0.18 a	1.92±0.52 a	4.42±0.08 a
	100	1, 3, 6	8.32±0.17 a	1.18±0.02 a	7.06±0.18 a	1.98±0.49 a	4.39±0.11 a
	300	1, 3, 6	8.28±0.11 a	1.19±0.03 a	6.96±0.21 a	1.99±0.36 a	4.48±0.11 a
	600	1, 3, 6	8.27±0.05 a	1.19±0.03 a	6.98±0.19 a	2.17±0.27 a	4.48±0.06 a

a. No significant differences among HPP holding times were observed; therefore, to simplify the presentation of data, results from those cookie quality tests were averaged.

b. Data reported as mean±*SD*. Mean values, within the same cookie quality parameter and a_w level, that share the same letter are not significantly different from one another ($p > .05$).

c. a_w: water activity of the dough.

d. Spread ratio = Diameter/Thickness.

however, no significant differences were observed among HPP treatments (100–400 MPa, 2–15 min). Dough viscosity plays a key role in defining the degree of spread of the dough during baking as well as the final thickness of cookies (Hoseney & Rogers, 1994). No significant changes in the dough physical characteristics were observed among the different HPP treatments (Table 3), which could explain the observed uniformity in diameter and thickness of baked cookies.

Water plays an important role during dough and cookie preparation. The amount of water present in the dough influence its viscosity, which in turn may affect the degree of spread during baking and the final textural characteristics (Hoseney & Rogers, 1994). Miller et al. (1997) studied the effect of different water content on the spread of sugar-snap cookies. The results of their investigation concluded that varying the amount of water content in the cookie dough formulation has little, if any impact, on final cookie diameter; however, it did affect cookie dough spread rate during baking. These researchers reported that increasing the amount of water caused the spread

rate to increase, presumably by lowering dough viscosity due to a higher water absorption from soluble components like sugar, but at the same time shortened the set time (Miller et al., 1997). In the present study, the increase in water content in the formulation caused minimal changes to the cookie diameter but substantially decreased the spread factor, which differs with the results previously reported by Miller et al. (1997).

The variation in cookie thickness observed in the present study may be related to the so-called collapse phenomenon, which is associated with the water content of the formulation. During baking, the period of dough expansion is followed by a marked structural collapse, which is more pronounced in doughs with low water content because the proteins are not hydrated enough to form a gluten network and the water amount is insufficient to cause enough starch gelatinization (Chevallier et al., 2002). Therefore, the high thickness level observed in the present study for cookies made with dough containing high water activity may be related to a well-developed protein network and starch gelatinization, leading to a minimal structural collapse after baking.

The flexibility and the maximum force (hardness) to snap the cookies made of HPP-treated and control doughs did not vary significantly (Table 4). However, increasing the a_w of the dough considerably decreased the hardness of baked cookies. Previous research studies have reported detrimental effects of HPP treatments on quality attributes of flour-based foods, such as hardness. Aguirre et al. (2018), for instance, reported a significant increase in hardness of baked cookies when treating dough at 200–400 MPa, although no detrimental effects were observed at mild pressure levels (100 MPa). The diverse composition of food matrices evaluated across different studies may explain why results on HPP-treated dough quality parameters are somewhat contradictory.

4 Conclusions

Sugar-cookie dough offers a challenging environment for microbial inactivation by high pressure due to the presence of high concentrations of sugar (sucrose) and fat (shortening). The results obtained in

the present study nonetheless suggest that the microbial load of sugar-cookie dough could be reduced by the application of HPP treatments. HPP-treated doughs showed reduced levels of endogenous microbial populations (i.e., APC, coliform, and molds), although the reduction values may not be practically relevant from a microbiological standpoint. However, the population of *E. coli* inoculated in the dough was significantly reduced by as much as 2.0 log CFU/g when treated at 600 MPa for 6 min. It is important to highlight that strains of *E. coli*, including pathogenic ones, may exhibit wide differences with respect to pressure resistance. A lower reduction in microbial cell counts than that obtained in this study may, therefore, be observed in populations of pathogenic *E. coli* strains. Consequently, on that scenario, HPP may only contribute modestly to improving the microbiological safety of cookie dough.

The increase in the water activity of the dough did not play a significant role in microbial reduction. Furthermore, HPP treatments did not negatively affect the quality characteristics of doughs and cookies. Therefore, HPP could be used as a postpackaging intervention to reduce the levels of microbial contamination in ready-to-bake sugar-cookie dough. However, in adopting this technology, special attention should also be given to the food matrix composition because some food constituents, such as sugar and fat, can induce an increased pressure resistance in microbial cells. Further research is warranted to evaluate the efficacy of HPP treatments on reducing the load of stress-resistant strains of pathogenic *E. coli* in flour-based products and the impact of this technology on the sensory characteristics of baked goods.

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