Food Sci Biotechnol (2020) 29(10):1433–1438
https://doi.org/10.1007/s10068-020-00783-x

Influence of sodium reduction and storage temperature on the growth of total microbes and *Bacillus cereus* in naturally contaminated hamburger patty and loaf bread

Ho-Seok Chang[1] · Kwang-Young Song[1] · Young-Ji Kim[1] · Jung-Whan Chon[1] · Hyunsook Kim[2] · Keum-Il Jang[3] · Eui-Su Kim[4] · Kun-Ho Seo[1]

Received: 2 March 2020 / Revised: 22 May 2020 / Accepted: 3 June 2020 / Published online: 23 June 2020

Abstract This study aimed to investigate the influence of sodium reduction and storage temperature on the growth of total microbes and *Bacillus cereus* in naturally contaminated hamburger patty and loaf bread, respectively. The sodium reduction rate of hamburger patty and loaf bread was 20% and 30%, respectively, and experimental samples were kept at 4 °C, 25 °C, and 40 °C for 60 h. The microbiological analysis included the colony count of total microbes and *B. cereus.* The water activity (Aw), titratable acidity (TA), and pH were assessed as factors that inhibit microbial growth. In this study, Aw, TA, and pH of all samples were affected by the growth of total microbes and *B. cereus* during the storage period. Hence, these results suggested that sodium reduction in processed foods should be preferentially applied as a potent inhibition strategy after accurate assessment of inhibitors for different food types.

Keywords Sodium reduction · Microbial growth · *Bacillus cereus* · Processed food

✉ Kun-Ho Seo
bracstu3@konkuk.ac.kr

Ho-Seok Chang
argadis99@naver.com

Kwang-Young Song
drkysong@gmail.com

Jung-Whan Chon
alvarmar@naver.com

Hyunsook Kim
hyunsk15@hanyang.ac.kr

Keum-Il Jang
jangki@cbnu.ac.kr

[1] Department of Public Health and KU Center for Food Safety, College of Veterinary Medicine, Konkuk University, Hwayang-dong, Seoul 05029, Gwangjin-gu, Korea

[2] Department of Food Nutrition, College of Human Ecology, Hanyang University, Wangsimni-ro, Seoul 04710, Seongdong-gu, Korea

[3] Department of Food Science and Biotechnology, Chungbuk National University, Chungdae-ro, Seowon-gu, Cheongju 28644, Chungbuk, Korea

[4] Korea Advanced Food Research Institute, Korea Food Industry Association, Botdeul-ro, Ulwang-si 16001, Gyeonggi-do, Korea

Introduction

Salt improves the flavor of food products as well as their texture by enhancing the structural consistency (Drüeke, 2016; Kilcast and Angus, 2006). In addition, salt is available at low cost, can inhibit microbial growth, extend the shelf life of food products, and create fermentation conditions for the manufacture of fermented food products (Carbone et al., 2003; He et al., 2012; Kicast and Angus, 2006). On the contrary, excessive intake of sodium has been reported to cause hypertension and various chronic disorders including renal diseases and several cardiovascular diseases, such as heart attack and stroke (O'Donnell et al., 2015). The World Health Organization (WHO), thus, limits the recommended sodium intake at 2000 mg, and advanced countries including the USA, UK, and Japan, have continuously tried to reduce the dietary salt intake (Drüeke, 2016; Gibson et al., 2000; Micklegorough, 2005; Strazzullo et al., 2009). In Korea, the average daily salt intake was estimated to be 4546 mg in 2012, twice the amount recommended by WHO, resulting in country-wide efforts toward the promotion of low salt processed foods and consumer campaigns since March 2012 (MFDS, 2019).

However, reducing salt in processed foods increases the risk of food poisoning due to the growth of pathogenic or saprogenic microorganisms (Sofos, 1984; Stanojevic et al., 2009; Wijnker et al., 2006). Moreover, future research should be targeted toward establishing the relevant standards and regulations regarding the production of low salt processed foods. However, to the best of the authors' knowledge, no studies have performed to confirm the microbial safety of low sodium processed foods at different reduction levels and storage conditions.

Therefore, this present study investigated the influence of sodium reduction by 20–30% and storage temperature at 4 °C, 25 °C, and 40 °C on the growth of total microbes and *Bacillus cereus* in naturally contaminated processed foods—hamburger patty and loaf bread. For microbiological analysis, two classes of microbes were examined—total microbes, the sanitary-indicative microorganisms, and *B. cereus*, the food poisoning-causing bacterium widely distributed in nature. For physicochemical analysis, the water activity (Aw), titratable acidity (TA), and pH were also determined.

Materials and methods

Experimental conditions and sample production

The processed foods used in this study were commercially available hamburger patty and loaf bread that were easily found and commonly consumed in everyday life (Collins-Thompson et al., 1984; Collins et al., 1991; Choe et al., 2008; Gracia et al., 2009; O'Donell et al., 2015; MFDS, 2019; Smith et al., 2004). To prepare low salt samples, the salt content in these foods was reduced by 20 and 30%, respectively, compared to that in the original product during the salting stage. Hamburger patties and loaf breads used in the study were ordered and supplied by factories belonging to the Korea Food Industry Association that produce sodium-reduced products. The sodium content of the samples used in this study was based on the sodium reduction policy, which aims to reduce sodium intake by more than 20 percent by 2020 driven by Korea Ministry of Food and Drug Safety, and the unique characteristics of the food were also considered. A total of 240 samples per product were used in the study, and were categorized according to the storage temperature into the control (the original product) and low salt groups. For microbiological analysis, 10 samples from each group were tested. The storage temperature of the samples was set at 4 °C as cold, 25 °C as ambient, and 40 °C as stress condition. Owing to the shelf life, the hamburger patty and loaf bread were assessed for 60 h (2.5 days).

Quantitative culture of total microbes and *B. cereus*

Microbiological analysis of all the samples was performed according to the microbiological test methods described in Korean Food Standard Code by Korea Ministry of Food and Drug Safety (MFDS, 2019).

Firstly, each food sample was suspended in 225 ml of sterilized phosphorus buffer saline (PBS) (Difco, Sparks, MD, USA) in a sterilized stomacher bag and homogenized for 2 min using a BagMixer stomacher (Interscience, St. Nom, France). Subsequently, 1 ml of the homogenized solution was ten-fold diluted in physiological saline solution 0.85% (sterile) (Sigma, St. Louis, MO, USA), and inoculated onto the Petrifilm™ aerobic count plate (3 M, St. Paul, MN, USA) for quantitative culture of total microbes. After incubated for 48 h at 35 °C, total microbes was enumerated.

And for quantitative culture of *B. cereus,* 1 ml of each homogenate was serially diluted (tenfold) in phosphate-buffered saline (Sigma), and inoculated onto mannitol–egg yolk–polymyxin B agar (MYP) (Oxoid, Basingstoke, Hampshire, UK) followed by incubation at 30 °C for 24 h. After enumerated suspicious colonies on each plate, five typical colonies were subcultured and confirmed as *B. cereus* using the VITEK® 2 GP ID kit (bioMerieux, Marcy l'Etoile, France).

Measurement of Aw, TA and pH

One to five gram of each sample was placed in Aw measuring containers and the Aw meter (AquaLab 4TE, Pullman, WA, USA) was used for the measurement of Aw. For measurement of TA, 10 g sample was added to 100 ml distilled water and homogenized. The homogenized sample was subjected to agitated extraction at 25 °C for 30 min, and the resulting solution was filtered using Whatman™ Grade 4 Qualitative Filter Paper (GE Healthcare, Maidston, UK). The extract was diluted and titrated using 0.1 N NaOH solution (Sigma), and the equation below was used for the calculation.

$$\text{TA\%} = 0.0090 \times \text{volume of 0.1 N NaOH used} \times 100/\text{weight of the sample.}$$

And for measurement of pH, 10 g sample was diluted ten-fold in distilled water, homogenized, and subjected to agitated extraction at 25 °C for 30 min, followed by filtration using Whatman™ qualitative filter paper: grade 4 circles (GE Healthcare). The pH of the extract was measured at room temperature (25 °C) using a pH meter (Thermo Electron Corp., Milford, MA, USA).

Statistical analysis

All the results were obtained after repeating the procedures three times, and the SPSS Program (ver 18.0, SPSS Inc., Chicago, IL, USA) was used to calculate the mean and standard deviation. Depending on the analytical method, the mean values were compared by one-way ANOVA test (Duncan method). For all the analyses, the significance level was set at p = 0.05.

Results and discussion

Hamburger patty

The low salt group of hamburger patty contained samples with the salt content reduced by 20% compared to that in the original product. The sample was stored at 4 °C, 25 °C, and 40 °C for 60 h, during which the counts of total microbes and *B. cereus* were determined respectively (Tables 1, 2). On day 0, the count of total microbes in the low salt group was 2.91–2.93 log CFU/*g*, slightly higher than that in the control group, while *B. cereus* was not detected in both groups. The results according to the storage temperature showed that during 0–36 h of storage at 4 °C, no change in the total microbial count was noted in both groups. However, at 48 h of storage, the counts of total microbes in both groups increased to 3.65 log CFU/*g* and 2.87 log CFU/*g*, respectively, with significantly higher counts in the low salt group than in the control group ($p < 0.05$). At 60 h, close to the end of experiment, both groups exhibited around 3 log CFU/*g* without significant difference. During 0–48 h storage at 25 °C, the counts from both groups were maintained at a level around 2–3 log CFU/*g*, which increased to around 5 log CFU/*g* after 60 h. The count of total microbes in the low salt group stored at 40 °C was maintained at around 3 log CFU/*g* during 0–12 h, when *B. cereus* was not detected. However, after 24 h, the count of the total microbes increased to a level above 5 log CFU/*g* in both groups, and *B. cereus* began to appear in the low salt group. After 48 h, *B. cereus* was detected in both groups, with significantly higher count in the low salt group ($p < 0.05$). And after 60 h, the count of total microbes in the low salt group exceeded 6 log CFU/*g*. This level of microbial contamination begins to decompose and spoil foods (Nottingham, 1982; Solberg et al., 1990; Stewart, 1987). The level was also significantly higher than that in the control group ($p < 0.05$). The count of *B. cereus* was around 3 log CFU/*g* in both groups. The initial pH of the hamburger patty was reported to be

Table 1 The total microbial count in the hamburger patty stored at 4 °C, 25 °C, and 40 °C

Storage temperature (°C)	Storage period (h)	The total microbial count (log CFU/*g*)[1)]	
		Control samples	Sodium reduction samples
4	0	2.09 ± 0.12^{A}	2.91 ± 0.08^{B}
	12	2.08 ± 0.15^{A}	2.89 ± 0.27^{B}
	24	2.11 ± 0.23^{A}	3.05 ± 0.38^{B}
	36	2.16 ± 0.49^{A}	3.02 ± 0.36^{B}
	48	2.87 ± 0.40^{A}	3.65 ± 0.31^{B}
	60	3.49 ± 0.37^{A}	3.58 ± 0.54^{A}
25	0	2.16 ± 0.19^{A}	2.92 ± 0.23^{B}
	12	2.20 ± 0.35^{A}	2.95 ± 0.21^{B}
	24	2.17 ± 0.26^{A}	2.97 ± 0.23^{B}
	36	2.97 ± 0.39^{A}	3.15 ± 0.15^{A}
	48	3.73 ± 0.65^{A}	3.33 ± 0.56^{A}
	60	4.97 ± 0.59^{A}	5.32 ± 0.62^{A}
40	0	2.36 ± 0.18^{A}	2.93 ± 0.13^{B}
	12	2.99 ± 0.21^{A}	3.17 ± 0.33^{A}
	24	5.06 ± 0.62^{A}	5.28 ± 0.51^{A}
	36	5.08 ± 0.44^{A}	5.50 ± 0.38^{B}
	48	5.14 ± 0.57^{A}	5.77 ± 0.81^{B}
	60	5.54 ± 0.48^{A}	6.13 ± 0.65^{B}

A total of 360 samples were used in the experiment and 10 samples per group were measured every 12 h. All data are expressed as mean ± standard deviation. The low salt group of hamburger patty contained samples with the salt content reduced by 20% compared to that in the original product

Different letters within a column indicate a significant difference ($p < 0.05$)

Table 2 The total *Bacillus cereus* count in the hamburger patty stored at 4 °C, 25 °C, and 40 °C

Storage temperature (°C)	Storage period (h)	The total *Bacillus cereus* count (log CFU/*g*)[1)]	
		Control samples	Sodium reduction samples
4	0	ND	ND
	12	ND	ND
	24	ND	ND
	36	ND	ND
	48	ND	ND
	60	ND	ND
25	0	ND	ND
	12	ND	ND
	24	ND	ND
	36	ND	ND
	48	ND	ND
	60	ND	ND
40	0	ND	ND
	12	ND	ND
	24	ND	0.70 ± 0.14
	36	ND	0.81 ± 0.46
	48	2.22 ± 0.45^{A}	3.10 ± 0.28^{B}
	60	3.00 ± 0.62^{A}	3.22 ± 0.51^{A}

A total of 360 samples were used in the experiment and 10 samples per group were measured every 12 h. All the data are expressed as mean ± standard deviation. The low salt group of hamburger patty contained samples with the salt content reduced by 20% compared to that in the original product

ND not detected

Different letters within a column indicate a significant difference ($p < 0.05$)

5.89–5.99 in previous studies (Choe et al., 2008; Garcia et al., 2009). In this study, the initial pH was 5.87 and 5.90, respectively. In the low salt and control groups, consistent with that reported in the previous study (Garcia et al., 2009; Labots, 1981). The pH variation according to the storage temperature showed that when stored at 4 °C, the pHs of both groups were maintained within a stable range of 5.8–6.0 without significant difference between the groups ($p > 0.05$). The pH of the low salt and control groups that were stored at 25 °C and 40 °C decreased after 24 and 48 h, respectively, without significant difference throughout the experiment. TA of the hamburger patty in both groups was extremely low within the range of 0.08–0.11%. The growth of pathogenic microorganisms is prohibited at a pH lower than 4.0 (Baird-Parker and Freame, 1967; Collins-Thompson et al., 1984; Hamad, 2012). In other words, the pH of hamburger patty is not effective as a factor that inhibits the growth of microorganisms, and a drastic reduction in salt may have a negative effect on microbiological safety of various foods including hamburger patty. This was consistent with report of Taormina (2010). Sodium chloride could inhibit the growth and toxin production by *Clostridium botulinum* in processed meats and cheeses, and hence, sodium salts was contributed to prevent the spoilage and/or growth of microorganisms in various foods. Therefore, salt reduction should be considered according to the characteristics of each food.

Loaf bread

The low salt group of loaf bread contained samples with the salt content reduced by 30%. The samples were stored at 4 °C, 25 °C, and 40 °C for 60 h, and the count of total microbes was determined (Table 3). *B. cereus* was not detected in either of the loaf bread groups at any of the storage temperatures throughout the experiment. According to the storage temperature, the results showed that total microbes were not detected in either of the groups when stored at 4 °C. Whereas at 48 h of storage at 25 °C, the count of total microbes in the low salt group was 1.9 log CFU/*g*. After 60 h, the counts increased to 3 and 1.62 log CFU/*g* in the low salt and the control groups. In the samples stored at 40 °C, the count of total microbes in the low salt group after 24 h was 3.7 log CFU/*g*, which was significantly higher than that in the control group ($p < 0.05$), and the counts after 48 h increased to a level above 5 log CFU/*g* in both groups. After 60 h, the count of total microbes in the low salt group exceeded 6 log CFU/*g*,

Table 3 The total microbial count in the loaf bread stored at 4 °C, 25 °C, and 40 °C

Storage temperature (°C)	Storage period (h)	The total microbial count (log CFU/g)[1)]	
		Control samples	Sodium reduction samples
4	0	ND	ND
	12	ND	ND
	24	ND	ND
	36	ND	ND
	48	ND	ND
	60	ND	ND
25	0	ND	ND
	12	ND	ND
	24	ND	ND
	36	ND	ND
	48	ND	1.90 ± 0.21
	60	1.62 ± 0.1^{A}	3.07 ± 0.47^{B}
40	0	ND	ND
	12	ND	ND
	24	2.33 ± 0.54^{A}	3.70 ± 0.64^{B}
	36	4.56 ± 0.26^{A}	4.62 ± 0.82^{A}
	48	5.21 ± 0.49^{A}	5.44 ± 0.48^{A}
	60	5.68 ± 0.70^{A}	6.08 ± 0.42^{A}

A total of 360 samples were used in the experiment and 10 samples per group was measured every 12 h. All data are expressed as mean ± standard deviation. The low salt group of loaf bread contained samples with the salt content reduced by 30%

ND not detected

Different letters within a column indicate a significant difference ($p < 0.05$)

which means a high level of contamination. The influence of storage temperature on Aw of loaf bread was negligible and Aw of loaf bread tested in this study was 0.97, and Aw in both the low salt and control groups was maintained within a stable range of 0.96–0.97. Low Aw prevents microbial growth and decay, and Aw value that inhibits the growth of bacteria and fungi is below 0.60 and 0.80, respectively (Fontana, 2000; Labots, 1981). The microorganisms related to bread decomposition are *B. subtilis*, *B. licheniformis*, and *B. cereus* (Collins et al., 1991). Among them, *B. cereus* has been reported to be detected in sandwiches (Kim et al., 2011). Furthermore, the spores of *Bacillus* strains are able to survive through the process of baking as they are thermostable; their spores can germinate at the temperature of 25–30 °C and above 0.95 Aw (Thompson et al., 1993), emphasizing the importance of contamination control against *Bacillus* spp. in bread. Therefore, although *B. cereus* was not detected in this study, the effects of sodium reduction on microbiological safety should be considered because loaf bread has high moisture and Aw that facilitate microbial growth (Smith et al., 2004).

Conclusively, physicochemical properties such as Aw, TA, and pH could act as the major parameters in microbial growth (Presser et al., 1997; Wareing and Fernandes, 2007). In this study, the lack of an inhibitor that can replace salt and inhibit microbial growth in hamburger patty and loaf bread facilitated microbial growth upon sodium reduction. Thus, sodium reduction should be first applied to processed food products that have a physicochemical factor for inhibiting microbial growth, while research should continue to develop inhibition strategies for the advancement of the food processing industry.

Acknowledgements The authors would like to thank the Korea Food Industry Association for supporting this work through the 2015–2016 research project fund. The authors thank the technical support of all members of KU Center for One Health.

Compliance with ethical standard

Conflict of interest None of the authors of this study has any financial interest or conflict with industries or parties.

References

Baird-Parker J, Freame B. Combined effect of water activity, pH and temperature on growth of *Clostridium botulinum* from spore and vegetative cell inocula. J. Appl. Bacteriol. 30: 420-429 (1967)

Carbone LD, Bush AJ, Barrow KD, Kang AH. The relationship of sodium intake to calcium and sodium excretion and bone mineral density of the hip in postmenopausal African-American and Caucasian women. J. Bone Miner. Metab. 21: 415-420 (2003)

Choe JH, Jeong JY, Kim CJ. Effect of hot air dried kimchi powder on the quality characteristics of pork patties. Korean J. Food Cook. Sci. 24: 466-472 (2008)

Collins NF, Kirshner LAM, Von Holy A. A characterization of *Bacillus* isolates from ropy bread, bakery equipment and raw material. S. Afr. J. Sci. 87: 62-66 (1991)

Collins-Thompson DL, Krusky B, Usborne WR. The effect of nitrite on the growth of pathogens during the manufacture of dry and semi-dry sausages. Can. Inst. Food Technol. J. 17: 102 (1984)

Drüeke TB. Salt and health: time to revisit the recommendations. Kidney Int. 89: 259-260 (2016).

Fontana AJ. Understanding the importance of water activity in food. Cereal Food World 45: 7-10 (2000)

Garcia LM, Calvo MM, Dolores Selgas M. Beef hamburgers enriched in lycopene using dry tomato peel as an ingredient. Meat Sci. 83: 45-49 (2009)

Gibson J, Armstrong G, McIlveen H. A case for reducing salt in processed foods. Nutr. Food Sci. 30: 167-173 (2000)

Hamad SH. Factors Affecting the Growth of Microorganisms in Food. Vol. I, pp. 405-427. In: Progress in Food Preservation. Bhat R (ed), Alias AK (ed), Paliyath G (ed). John Wiley & Sons Ltd., New York City, NY, USA (2012)

He FJ, Campbell NR, MacGregor GA. Reducing salt intake to prevent hypertension and cardiovascular disease. Rev. Panam. Salud. Publica. 32:293-300 (2012)

Kilcast D, Angus F. Reducing salt in foods. CRC Press, Inc., Boca Raton, FL. USA. pp. 18-54 (2006)

Kim HY, Oh SW, Chung SY, Choi SH, Lee JW, Yang JY, Seo Kim YH, Park HE, Yang CY, Ha SH, Shin IS. An investigation of microbial contamination of ready-to-eat products in Seoul, Korea. Korean J. Food Sci. Technol. 43: 39-44 (2011)

Labots H. Aw und pH-wert konzept für die enteilung von fleischerzengnissen in verberbliche und lagerfahige produkte. Fleischwirtschaft 61: 1 (1981)

Mickleborough TD. Dietary salt, airway inflammation, and diffusion capacity in exercise-induced asthma. Med. Sci. Sports Exerc. 37: 904-914 (2005)

Ministry of Food and Drug Safety (MFDS). Korean Food Standards Codex. Available from: https://www.foodsafetykorea.go.kr/foodcode/01_03.jsp?idx=12. Accessed Dec. 31, 2019

Nottingham PM. Microbiology of carcass meats. pp. 13-65. In: Meat Microbiology. Brown MH (ed.). Applied Science Publishers, London, UK (1982)

O'Donnell M, Mente A, Yusuf S. Sodium intake and cardiovascular health. Circ. Res. 116:1046-57 (2015)

Presser KA, Ratkowsky DA, Ross T. Modelling the growth rate of *Escherichia coli* as a function of pH and lactic acid concentration. Appl. Environ. Microbiol. 63:2355-2360 (1997)

Smith JP, Daifas DP, El-Khoury W, Koukoutsis J, El-Khoury A. Shelf life and safety concerns of bakery products: A review. Crit. Rev. Food Sci. Nutr. 44: 19-55 (2004)

Sofos JN. Antimicrobial effects of sodium and other ions in foods: a review. J. Food Saf. 6: 45-78 (1984)

Solberg M, Buchalew JJ, Chen CM, Schaffner DW, O'Neill K, McDowell J, Post LS, Boderck M. Microbial safety assurance system for foodservice facilities. Food Technol. 44: 68-72 (1990)

Stanojevic D, Comic L, Stefanovic O, Solujic-sukdolak S. Antimicrobial effects of sodium benzoate, sodium nitrite and potassium sorbate and their synergistic action in vitro. Bulg. J. Agri. Sci. 15: 307-311 (2009)

Stewart GS. Micro-organisms in food-2. Sampling for microbiological analysis: principles and specific applications ICMSF, Blackwell Scientific Publications, Oxford, 1986. 310 pp. Price: £19·50 (cloth). Meat Sci. 19: 315 (1987). https://doi.org/10.1016/0309-1740(87)90078-7

Strazzullo P, D'Elia L, Kandala NB, Cappuccio FP. Salt intakes, stroke and cardiovascular disease: meta-analysis of prospective studies. Br. Med. J. 339: b4567 (2009)

Taormina PJ. Implications of Salt and Sodium Reduction on Microbial Food Safety. Crit. Rev. Food Sci. Nutr. 50: 209-227 (2010)

Thompson JM, Dodd CER, Waites WM. Spoilage of bread by *Bacillus*. Int. Biodeterior. Biodegrad. 32: 55-66 (1993)

Wareing P, Fernandes R. Micro-facts: The **w**orking companion for food microbiologists. 6 ed. RSC publishing, York, UK. pp.188-253 (2007)

Wijnker JJ, Koop G, Lipmam LJA. Antimicrobial properties of salt (NaCl) used for the preservation of natural casings. Food Microbiol. 23: 657-662 (2006)