pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 35, No. 3, pp. 279~283 (2020)
https://doi.org/10.13103/JFHS.2020.35.3.279

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Antimicrobial Effects of EcoCal® and GF Bactostop® Formulated in Emulsified Sausages against Lactic Acid Bacteria

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(Received April 1, 2020/Revised April 27, 2020/Accepted May 12, 2020)

ABSTRACT - In this study we evaluated the antimicrobial effects of EcoCal® (calcium oxide) and GF Bactostop® (organic acids mix) in sausages during storage at 10°C. The sausages were formulated with 0.1% EcoCal® (0.1ECO), 0.1% EcoCal®+0.5% GF Bactostop® (0.1ECO+0.5GF), 0.2% EcoCal® (0.2ECO), and 0.2% EcoCal®+0.5% GF Bactostop® (0.2ECO+0.5GF). Total aerobic and lactic acid bacteria in the sausages were enumerated on tryptic soy agar and Lactobacilli MRS agar, respectively, during storage at 10°C for 10 weeks. The 0.1ECO+0.5GF showed the most effective antimicrobial effects on the sausages, and 0.1ECO showed the second most effective antimicrobial effect. Total aerobic bacterial cell counts gradually increased in the control, 0.2ECO, and 0.2ECO+0.5GF groups, but cell growth was generally inhibited in 0.1ECO by approximately day 42 ($P<0.05$) and 0.1ECO+0.5GF by approximately day 49 ($P<0.05$). Lactic acid bacterial cell counts gradually increased in the control, 0.2ECO, and 0.2ECO+0.5GF groups, but the lactic acid bacteria growth was inhibited in 0.1ECO by approximately day 49 ($P<0.05$) and in 0.1ECO+0.5GF by approximately day 64. These results suggest that using 0.1% EcoCal®+0.5% GF Bactostop® in sausage formulation is useful for inhibiting lactic acid bacteria growth, thereby extending the shelf-life of the sausage product.

Key words : Sausages, EcoCal®, GF Bactostop®, Lactic acid bacteria, Antimicrobial

Sausages are manufactured from fresh ground meat, such as beef, chicken, and pork[1]. Sausages are produced through a series of processes; raw meat and additives are chopped, mixed, and emulsified, followed by the mixture being stuffed into the casing for cooking[2,3]. During processing, sausages are at risk of being exposed to and contaminated by various bacteria, especially during the vacuum-packaging process[4,5]. During the storage of vacuum-packaged sausages, lactic acid bacteria can cause spoilage[6]. Holzapfel[7] reported that 68% and 16.9% of the total lactic acid bacteria examined in their study were *Lactobacillus sake* and *Lactobacillus curvatus*, respectively, for vacuum-packaged processed meats. Lactic acid bacteria in meat products contribute to organoleptic downgrading of the meat products, and produce souring and gas[8]. Therefore, lactic acid bacteria in sausages must be controlled to maintain high quality.

EcoCal® is a commercial antimicrobial composed primarily of calcium oxide. Calcium oxide, a primary component of shell powder, is a food preservative with excellent antimicrobial activity[9]. This component also makes it useful as an acid regulator and dough conditioner[10]. GF Bactostop® is an organic acid mix composed of salt, ascorbic acid, sodium acetate, calcium lactate, tri-sodium citrate, and citric acid, and it can be used for food formulation as an antimicrobial. Mani-López et al.[11] reported that acetic acid, diacetates, acetate, and dehydroacetic acid showed obvious antimicrobial activities against bacteria and yeast in meat products. Additionally, Gálvez et al.[12] showed that the antimicrobial activities of bacteriocins increased when combined with organic acids.

Therefore, the objective of this study was to evaluate the antimicrobial effects of EcoCal® and GF Bactostop® on lactic acid bacteria in sausages.

Materials and Methods

Sausage preparation

Lean pork and pork fat were purchased from Dodram

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Food Co., Ltd. in Seongnam, Korea. They were ground separately through a grinder with 5 mm hole plate, and common ingredients (ground meat and ice, some additives and spices) and two types of commercial antimicrobial preservatives [Calcium oxide (EcoCal®, Micro-Tech Foods Ingredients, Inc., New Taipei city, Taiwan) and/or organic acids mix (GF Bactostop®, Pacovis Co., Stetten, Switzerland)] were measured and finely chopped to the ratios presented in Table 1, and the manufacturing process is shown in Fig. 1. The pre-ground pork, fat, and ice with 1.2% salt, 0.2% nitrite pickle salt (5% nitrite and 95% salt), 0.25% sodium pyrophosphate, and other ingredients were finely chopped using a bowl chopper (Type TK 20L, 2000S, Kilia Fleischwarenfabrik, Kiel, Germany) according to the formulations (Table 1) and manufacturing process of the sausage (Fig. 1). The finely emulsified sausage batter was stuffed into collagen casing #26 (Devro, INC, Sandy Run, SC, USA). The stuffed sausages were placed in a smoke chamber (Type IMAGO F3000, Metatek Co., Ltd., Nonsan, Korea) at 55ºC for 20 min, dried at 55ºC for 10 min, and smoked at 60ºC for 15 min. The sausages were then primary-heated at 72ºC for 20 min, secondary-heated at 78ºC for 10 min, then cooled by washing with cold water. The sausages were then vacuum-packaged, post-pasteurized at 90ºC for 10 min, then cooled in an ice/water bath. Manufactured sausages were stored at 10ºC for 10 weeks for microbiological analysis.

Microbiological analysis

Overall, 25 g of the sausage samples were removed aseptically from the vacuum-packaged samples, placed in a filter bag (3M, St. Paul, MN, USA) containing 50 mL of 0.1% buffered peptone water (BPW; Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and pummeled in a pummeler (Interscience, St. Nom, France) for 1 min[13]. The homogenates were serially diluted with 0.1% BPW, and the diluents were plated on tryptic soy agar (TSA; Becton, Dickinson and Company) and Lactobacilli MRS agar (Becton, Dickinson and Company) for the isolation of total aerobic bacteria and lactic acid bacteria, respectively. All plates were incubated at 37ºC for 24 h. The number of typical colonies on the plates were manually counted.

Statistical analysis

Bacteria cell count data were analyzed using a general linear model procedure with SAS® version 9.3 (SAS Institute, Cary, NC, USA). Least square (LS) means were

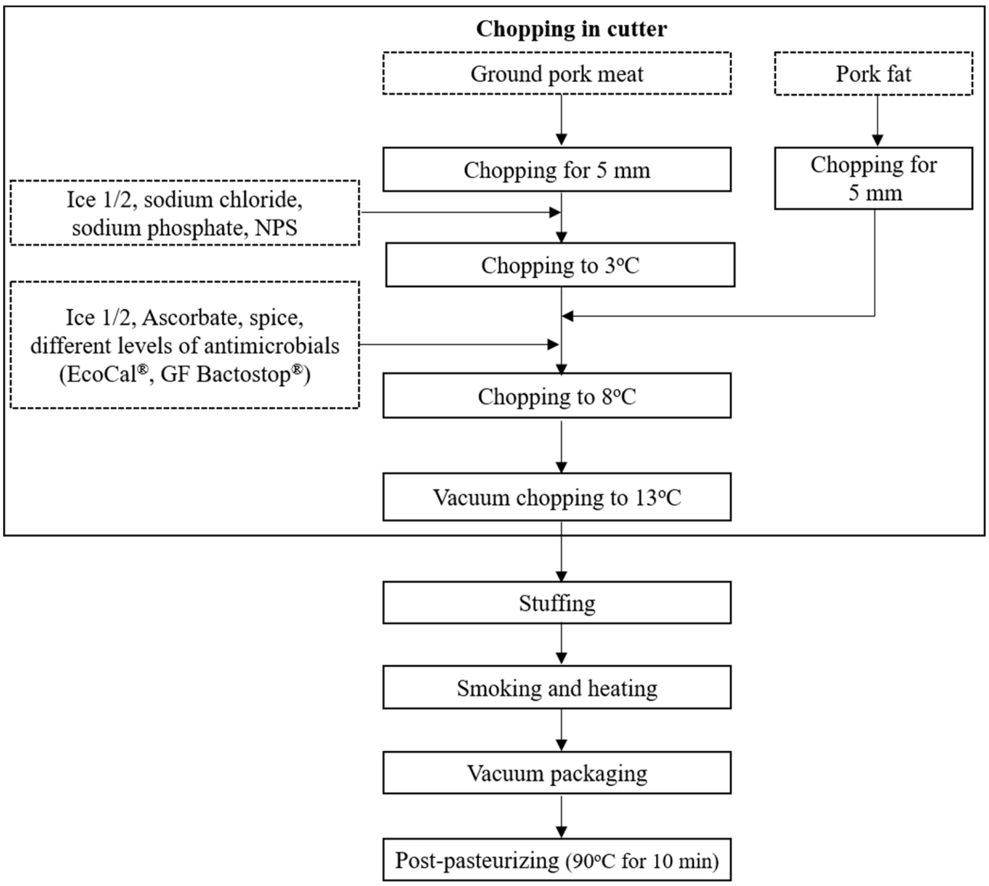

Fig. 1. Manufacturing process of emulsified sausages with different levels of Ecocal® and GF Bactostop®.

Table 1. Formula of experimental vacuum-packaged sausages (Unit: %)

Ingredients		Concentration				
		Control	0.1ECO	0.1ECO+0.5GF	0.2ECO	0.2ECO+0.5GF
Anti-microbial	EcoCal®[1)]	-	0.1	0.1	0.2	0.2
	GF Bactostop®[2)]	-	-	0.5	-	0.5
General ingredients	Ground lean pork (95% lean), 5 mm	57.5	57.5	57.5	57.5	57.5
	Fat (5 mm)	22.5	22.5	22.5	22.5	22.5
	Ice	16.75	16.65	16.15	16.55	16.05
	Salt	1.2	1.2	1.2	1.2	1.2
	Nitrite pickle salt (5% Nitrite+95% Salt)	0.2	0.2	0.2	0.2	0.2
	Sodium pyrophosphate	0.25	0.25	0.25	0.25	0.25
	Sodium ascorbate	0.05	0.05	0.05	0.05	0.05
	Sugar	0.75	0.75	0.75	0.75	0.75
	Sodium glutamate	0.2	0.2	0.2	0.2	0.2
	Fleischwurst spices	0.6	0.6	0.6	0.6	0.6
Total		100	100	100	100	100

1) This product is made by Micro-Tech Foods Ingredients, Inc. (Taiwan) and composed of shell calcium oxide.
2) This product (Product number: 1379) is made by Pacovis Co. (Switzerland). It contains sodium acetate, salt, calcium lactate, tri-sodium citrate, ascorbic acid, and citric acid, and its pH value is 5.5±0.5.

used for mean comparisons among the treatment groups by pairwise *t-test* at α=0.05.

Results and Discussion

Although there was some sample variation in total bacterial cell counts, the cell counts of total aerobic bacteria in the sausages gradually increased with increasing storage time (Table 2). These variations in cell counts were likely due to sample variations[14)]. In general, 0.1ECO and 0.1ECO+0.5GF groups had lower (P<0.05) cell counts for total aerobic bacteria compared to the other groups. Total bacterial cell counts gradually increased in control, 0.2ECO, and 0.2ECO+0.5GF groups, but bacterial growth was generally inhibited in 0.1ECO by approximately day 42 (P<0.05) and 0.ECO+0.5GF by approximately day 49 (P<0.05). The lactic acid bacterial cell counts in the sausages increased as storage time increased (Table 3). In addition, 0.1ECO and 0.1ECO+0.5GF groups had lower (P<0.05) cell counts in lactic acid bacteria than the other groups. In these treatments, the lactic acid bacterial cell counts were below the detection limit (0.5 Log CFU/g) by day 49. Therefore, 0.1ECO and 0.1ECO+0.5GF groups were analyzed for an additional three weeks. At the end of the storage period, there were significant differences in the cell counts of lactic acid bacteria between the two groups. The 0.1ECO+0.5GF group had cell counts of lactic acid bacteria below the detection limit by day 64, but the cell counts of lactic acid bacteria in the 0.1ECO group were below the detection limit by day 49, then increased to 4 Log CFU/g. These results showed that the addition 0.1% of EcoCal® and 0.5% of GF Bactostop® may be effective in inhibiting lactic acid bacteria growth in sausages. A study by El-Aziz et al.[15)] showed that CaO, a major component of EcoCal®, had antimicrobial effects on minced meat, and this result is similar to our result. The antimicrobial effects of CaO are caused by active oxygen and alkaline pH, which in turn cause the destruction of the bacterial cell membranes[16)]. However, the antimicrobial effect of CaO were not concentration dependent. According to Bodur et al.[17)] the antimicrobial effect of 0.05% CaO on *E. coli* was higher than that of 0.1% CaO, but antimicrobial effects on *Listeria monocytogenes* and *Salmonella* were showen at 0.1% CaO. This means that the antimicrobial effect of Ecocal may not be concentration dependent for total aerobic bacteria. In addition, Schirmer and Langsrud[18)] reported that the organic acids mix, which is similar to GF Bactostop®, showed greater antimicrobial activity when combined with 50% CO_2 modified atmosphere-packed (MAP) during storage. Therefore, the use of GF Bactostop® in vacuum-packaged sausages may increase the antimicrobial activity of vacuum-packaging, resulting in the extension of shelf-life. Insoluble CaO had been reported in several studies to increase the absorptivity as well as the solubility in acid through reactions with organic acid[19-21)].

Table 2. Total aerobic bacterial cell counts (mean±SD; Log CFU/g) in vacuum-packaged sausages during storage at 10ºC for 71 days

Time	Control	0.1ECO	0.1ECO+0.5GF	0.2ECO	0.2ECO+0.5GF
Day 0	0.6±0.2 Bb	2.2±1.2 ABbc	0.5±0.0 Bb	1.1±0.2 Bbc	3.4±3.1 Ab
Day 11	2.0±2.1 Ab	2.4±2.1 Abc	1.1±0.2 Ab	2.2±2.4 Abc	1.8±1.9 Ab
Day 21	2.5±0.1 Ab	5.0±1.7 Aab	2.0±0.4 Ab	2.8±0.5 ABb	2.9±0.2 ABb
Day 28	1.1±0.5 Ab	<0.5* Ac	<0.5 Ab	<0.5 Ac	1.9±0.4 Ab
Day 38	2.6±1.4 BCb	3.7±1.8 Bb	<0.5 Cb	6.5±0.1 Aa	5.5±0.4 ABab
Day 42	7.2±0.5 Aa	1.2±1.0 Bc	3.0±2.9 Bab	7.0±0.1 Aa	7.3±0.4 Aa
Day 49	6.7±0.1 Aa	4.2±0.5 Bb	1.6±0.1 Cb	7.4±0.2 Aa	6.8±0.4 Aa
Day 57	-**	6.4±0.3 Aab	3.0±1.2 Bab	-	-
Day 64	-	6.3±0.2 Aab	4.2±0.5 Ba	-	-
Day 71	-	6.4±0.5 Aa	4.9±0.8 Aa	-	-

* Below the detection limit (0.5 log CFU/g).
** Not tested.
A-C Means within the same column with different letters are significantly different ($P<0.05$).
a-c Means within the same row with different letters are significantly different ($P<0.05$).

Table 3. Lactic acid bacterial cell counts (mean±SD; Log CFU/g) in vacuum-packaged sausages during storage at 10ºC for 71 days

Time	Control	0.1ECO	0.1ECO+0.5GF	0.2ECO	0.2ECO+0.5GF
Day 0	0.7±0.3 Ac	0.9±0.6 Abc	<0.5 Aa	0.9±0.1 Ad	<0.5 Ac
Day 11	<0.5* Ac	<0.5 Ac	<0.5 Aa	<0.5 Ad	1.8±1.8 Ac
Day 21	<0.5 Ac	<0.5 Ac	<0.5 Aa	<0.5 Ad	<0.5 Ac
Day 28	<0.5 Bc	<0.5 Bc	<0.5 Ba	2.4±2.8 Ac	1.2±0.2 ABc
Day 38	1.0±0.7 Bc	<0.5 Bc	<0.5 Ba	4.5±0.1 Ab	4.3±0.0 Ab
Day 42	4.4±1.1 Bb	<0.5 Cc	<0.5 Ca	6.0±0.2 Aab	6.1±0.2 Aa
Day 49	6.5±0.2 Aa	<0.5 Cc	<0.5 Ca	7.4±0.1 Aa	3.5±0.1 Bb
Day 57	-**	2.3±2.5 Ab	<0.5 Ba	-	-
Day 64	-**	4.3±0.7 Aa	<0.5 Ba	-	-
Day 71	-	4.1±0.6 Aa	1.9±2.0 Ba	-	-

* Below the detection limit (0.5 log CFU/g).
** Not tested.
A-C Means within the same column with different letters are significantly different ($P<0.05$).
a-c Means within the same row with different letters are significantly different ($P<0.05$).

Therefore, a mixture of CaO and organic acids mix may increase the solubility and antimicrobial activity of lactic acid and total aerobic bacteria.

In conclusion, a mixture of 0.1% of EcoCal® with 0.5% of GF Bactostop® in sausages showed the highest antimicrobial effects on lactic acid bacteria, which are usually spoilage bacteria in sausages, and 0.1% of EcoCal® showed the second highest antimicrobial effect. Therefore, the use of 0.1% EcoCal® and a mixture of 0.1% of EcoCal® and 0.5% GF Bactostop® may be useful in inhibiting lactic acid bacteria growth in sausages, which can extend the shelf-life of the sausage.

Acknowledgement

This research was financially supported by Geo Food Tec Institute.

국문요약

본 연구는 EcoCal® (산화 칼슘) 및 GF Bactostop® (유기산염 믹스)을 소시지에 사용하였을 때 항균 효과를 확인하였다. 소시지 제조 시, 대조군과 0.1% EcoCal® (0.1ECO), 0.1% EcoCal®+0.5% GF Bactostop® (0.1ECO + 0.5GF), 0.2% EcoCal® (0.2ECO) 및 0.2% EcoCal®+0.5% GF GF Bactostop® (0.2ECO+0.5GF) 등 총 5개 군을 첨가

하여 소시지를 제조하였다. 제조가 완료된 소시지를 진공 포장하고 10주 동안 10°C에서 저장하며 7-10일 마다 시료를 균질화 하고 tryptic soy agar 및 Lactobacilli MRS agar에 도포하여 호기성 일반세균 및 젖산균을 각각 확인하였다. 그 결과, 0.1ECO+0.5GF 첨가한 소시지가 가장 효과적인 항균 효과를 나타냈으며, 0.1ECO 첨가 소시지가 두 번째로 효과적인 항균 효과를 나타냈다(P<0.05). 일반세균 수는 대조군, 0.2ECO 및 0.2ECO+0.5GF 첨가 소시지에서는 저장 후 42일까지 점차 증가했지만(P<0.05), 0.1ECO 및 0.1ECO+0.5GF 첨가 소시지에서는 저장 후 49일까지 일반세균의 생장이 억제되었다(P<0.05). 젖산균 수는 대조군, 0.2ECO 및 0.2ECO+0.5GF 첨가 소시지에서 약 49 일까지 증가하였으나(P<0.05), 0.1ECO 및 0.1ECO+0.5GF 첨가 소시지에서 젖산균 생장이 억제되었다. 본 결과는 소시지 제형에 0.1% EcoCal®+0.5% GF Bactostop®을 첨가하면 젖산균의 생장을 억제하여 소시지의 보존 기한을 연장시키는데 유용할 것으로 판단된다.

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