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ARTICLE

Minimum Inhibitory Concentration (MIC) of Propionic Acid, Sorbic Acid, and Benzoic Acid against Food Spoilage Microorganisms in Animal Products to Use MIC as Threshold for Natural Preservative Production

Yeongeun Seo[1,†], Miseon Sung[2,†], Jeongeun Hwang[2], and Yohan Yoon[1,2,*]

[1]Risk Analysis Research Center, Sookmyung Women's University, Seoul 04310, Korea

[2]Department of Food and Nutrition, Sookmyung Women's University, Seoul 04310, Korea

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***Corresponding author** : Yohan Yoon
Department of Food and Nutrition,
Sookmyung Women's University,
Seoul 04310, Korea
Tel: +82-2-2077-7585
Fax: +82-2-710-9479
E-mail: yyoon@sookmyung.ac.kr

***ORCID**
Yeongeun Seo
https://orcid.org/0000-0003-4986-9770
Miseon Sung
https://orcid.org/0000-0002-1430-692X
Jeongeun Hwang
https://orcid.org/0000-0001-9909-9490
Yohan Yoon
https://orcid.org/0000-0002-4561-6218

† These authors contributed equally to this work.

Abstract Some preservatives are naturally contained in raw food materials, while in some cases may have been introduced in food by careless handling or fermentation. However, it is difficult to distinguish between intentionally added preservatives and the preservatives naturally produced in food. The objective of this study was to evaluate the minimum inhibitory concentration (MIC) of propionic acid, sorbic acid, and benzoic acid for inhibiting food spoilage microorganisms in animal products, which can be useful in determining if the preservatives are natural or not. The broth microdilution method was used to determine the MIC of preservatives for 57 microorganisms. Five bacteria that were the most sensitive to propionic acid, benzoic acid, and sorbic acid were inoculated in unprocessed and processed animal products. A hundred microliters of the preservatives were then spiked in samples. After storage, the cells were counted to determine the MIC of the preservatives. The MIC of the preservatives in animal products ranged from 100 to 1,500 ppm for propionic acid, from 100 to >1,500 ppm for benzoic acid, and from 100 to >1,200 ppm for sorbic acid. Thus, if the concentrations of preservatives are below the MIC, the preservatives may not be added intentionally. Therefore, the MIC result will be useful in determining if preservatives are added intentionally in food.

Keywords natural production preservatives, minimum inhibitory concentration, animal products

Introduction

Benzoic acid, propionic acid, and sorbic acid are food preservatives that extend the shelf life of food by preventing the deterioration of quality by microorganisms (Silva and Lidon, 2016). Some preservatives are naturally contained in raw food materials or

may be introduced into the food by careless handling or fermentation (Jang et al., 2020; Kim et al., 2018; Lee et al., 2013; Lim et al., 2013; Park et al., 2008; Yun et al., 2017; Yun et al., 2019). However, it is difficult to distinguish between intentionally added preservatives in the food and the preservatives naturally produced in food (Park et al., 2008).

The World Health Organization (WHO) reported that benzoic acid is produced by many plants as an intermediate product in the formation of other compounds, and is detected in high concentrations in berries and in animals (WHO, 2000). Several studies have shown that benzoic acid is frequently detected in dairy products (Cakir and Cagri-Mehmetoglu, 2013; Qi et al., 2009). Benzoic acid in dairy products may be produced by lactic acid bacteria or an anaerobic metabolism of phenols in cheese (Sieber et al., 1995). Kurisaki et al. (1973) showed that benzoic acid can be produced from phenylalanine in yeast-ripened cheese. Another study has reported that yeast-mold counts affect the formation of benzoic acid (Yerlikaya et al., 2021).

Although propionic acid is not a component of fats or oils, it has been reported to occur as an intermediate metabolite by oxidation of fatty acids (FAO and WHO, 1974), and the Code of Federal Regulation specified that propionic acid is produced by chemical synthesis or bacterial fermentation (FDA, 2022). The Environmental Protection Agency (EPA) also reported that propionic acid is a common intermediate metabolite in the living body, and is one of the metabolites produced by the decomposition of several amino acids (EPA, 1991). Thus, the European Food Safety Authority (EFSA) published a scientific opinion reevaluating propionic acid as a naturally occurring substance (EFSA, 2014). Sorbic acid is naturally found in the oil of ash tree berries in 1859 (Sofos, 1989). Kim et al. (1999) reported the contents of benzoic acid and sorbic acid in 39 plants used as tea or spices in Korea, the content of benzoic acid in spices and the content of sorbic acid in teas or spices were less than 10 ppm. Yun et al. (2017) reported the levels of natural preservatives of sorbic acid in spices. Sorbic acid was found in 88 samples from a total of 493 samples with a concentration of not detected-57.70 mg/L.

Many countries have regulations to limit the concentrations of benzoic acid, sorbic acid, and propionic acid in food for intentional addition. However as described above, the natural production of these preservatives cannot be distinguished from the current technology. If the preservatives are added intentionally to food, their purpose is to inhibit microbial growth. Notably, preservative concentration below minimal inhibitory concentration (MIC) in food could be due to natural production. Various studies on MIC of preservatives against microorganisms have been conducted (Haque et al., 2009; Stanojevic et al., 2009; Warth, 1985; Warth, 1986). However, these studies usually used broth media rather than food matrices. In addition, the previous studies examined one microorganism. Because of the reasons, the results from the studies were not appropriate to be used for microbial standards. If MIC for preservatives are determined with a mixture of microorganisms, which are the most sensitive against the preservatives, in food matrices, the results could be used for establishing microbial standards. In this case, even though the food preservatives are detected in food, if the concentration is below the MIC, the food preservatives might be produced naturally rather than intentional addition, because people do not add the preservatives below the MIC determined with the most sensitive microorganism.

Therefore, the objective of this study was to determine the MIC of propionic acid, sorbic acid, and benzoic acid to the most sensitive microorganisms in animal products, to be used as a standard for determining if the preservatives in food are natural production or intended addition.

Materials and Methods

Sample preparation

Unprocessed animal products and processed animal products were selected based on following criteria; i) cases of research

on natural preservatives, ii) food items and raw materials with high consumption (MFDS, 2020), iii) fat content. For unprocessed animal products, eggs, chicken breast, chicken legs, pork ribs, pork sirloin, beef ribs, beef chuck, and milk samples were used. For processed animal products, processed butter, fermented milk, ground meat product, natural cheese, and smoked egg samples were used. These samples were purchased from local supermarkets and butcher shops.

Inoculum preparation

Considering the strain variation of microorganisms, a strain mixture for each microorganism was prepared as inoculum. Bacteria strains were cultured in 10 mL of culture media at optimal incubation temperature for 24 h. Aliquots (0.1 mL) of the cultures were inoculated in 10 mL fresh culture media and subcultured at optimal temperature for 24 h. Yeast and mold strains were cultured in 10 mL of culture media at optimal incubation temperature for 24–48 h. Aliquots (0.1 mL) of the cultures were inoculated in 10 mL fresh culture media and subcultured at optimal temperature for 24–48 h. The cultures of the strains for each microorganism species were mixed. Each mixture was then centrifuged at 1,912×g and 15 min for 4℃, and the cell pellets were washed twice with phosphate-buffered saline [PBS; KH_2PO_4 0.2 g, Na_2HPO_4 1.5 g, NaCl 8.0 g, KCl 0.2 g, 1 L of distilled water (DW), pH 7.4]. For the bacteria and yeast inocula, cell pellets were diluted with PBS to have 6 Log CFU/mL. For the mold inocula, the resulting suspensions of conidia were vigorously vortexed, and sterile DW was added to the suspension to have 5 Log CFU/mL. Mold cell counts were measured by a hemacytometer, which was confirmed by a serial dilution plate count. The microorganism strains and culture media used in this study were presented in Table 1.

Selection of microorganisms for food application

Minimum inhibitory concentrations of preservatives for microorganisms at pH 7.0

MIC were determined by a broth microdilution method according to the recommendation of the CLSI M07-A, M27-A, and M38-A (Balouiri et al., 2016; CLSI, 2002; CLSI, 2008; CLSI, 2012). Mueller Hinton Broth (MHB; Becton Dickinson, Franklin Lakes, NJ, USA) was used for bacterial cultures, and RPMI-1640 medium (Thermo Fisher Scientific, Waltham, MA, USA) was used for yeast and mold cultures. The pH of MHB was adjusted to pH 7.0 using HCl and NaOH, and the pH of RPMI-1640 medium was adjusted to pH 7.0 with 0.165M MOPS (M1254, Sigma-Aldrich, Gillingham, UK). Preservatives examined were extra pure grade propionic acid (Daejung, Siheung, Korea), food-grade benzoic acid (W213101, Sigma-Aldrich), sorbic acid (W392103, Sigma-Aldrich), calcium propionate (Niacet B.V., Tiel, Netherlands), sodium propionate (Niacet B.V.), sodium benzoate (Wuhan Youji Industries, Hubei, China), and potassium sorbate (Ningbo Wanglong Technology, Zhejiang, China). The stock solution of the preservative was dissolved in MHB and RPMI-1640 medium, and they were two-fold diluted serially with MHB and RPMI-1640 medium. The tests were performed in 96 well-microtiter plates, and 180 μL of diluted preservative solutions with different concentrations were placed in the wells. Each well was inoculated with 20 μL of the inocula at 4 Log CFU/mL. The 96 well microtiter plates were incubated at 35℃ for 24 h for the growth of the bacteria and yeast, and at 35℃ for more than 48 h for the growth of the fungi. Positive control was the media inoculated with bacteria without a preservative, and negative control was media only. Concentrations at which no optical turbidity was observed after incubation were considered MIC.

Minimum inhibitory concentrations of preservatives for microorganisms at pH 4.5, 5.5, and 6.0

To examine the antimicrobial effect of preservatives at low pH, five bacteria that were the most sensitive to the preservatives at pH 7.0 were subjected to propionic acid, benzoic acid, and sorbic acid in MHB at pH 4.5, 5.5, and 6.0. To

Table 1. Microorganisms examined in this study

Microorganism	Strain	Culture conditions	
		Media	Temp. (°C)
Bacteria			
Acetobacter aceti	KCTC12290	BHIB	25
Acetobacter pasteurianus	KCTC12289	BHIB	25
Acinetobacter calcoaceticus	NCCP16013	BHIB	25
Aeromonas salmonicida	KCCM40239	BHIB	25
Alcaligenes faecalis	KCTC2678	TSB	37
Alcaligenes xylosoxidans ssp. *xylosoxidans*	NCCP15702	TSB	30
Bacillus cereus	NCCP16296, 15910, 15909, 14796, 14043	TSB	30
Campylobacter coli	ATCC33559	CA	42
Campylobacter jejuni	ATCC33560	CA	42
Carnobacterium maltaromaticum	KCTC3602	TSBYE	30
Clostridium perfringens	NCCP15912, 15911	BHIB	37
Enterobacter aerogenes	NCCP16285	TSB	37
Enterobacter amnigenus	NCCP15837	TSB	30
Enterobacter cloacae	NCCP14672	TSB	37
Enterococcus casseliflavus	KCCM40712	BHIB	37
Enterococcus faecium	KCCM12118	BHIB	37
Erwinia carotovora subsp. *carotovora*	KCCM11319	BHIB	30
Escherichia coli	NCCP16186, 16185, 15663, 15651, 13588	TSB	37
Enterohemorrhagic *E. coli*	NCCP15961, 15957, 15739, 15656, 14541	TSB	37
Lactobacillus delbrueckii subsp. *lactis*	KCTC3636	MRSB	37
Listeria monocytogenes	ATCC BBA-839, 51774, 13932	TSBYE	30
Micrococcus luteus	KCCM11211	TSB	25
Moraxella catarrhalis	KCCM42707	BHIB	37
Proteus mirabilis	KCTC2566	TSB	37
Proteus vulgaris	KCTC2579	TSB	37
Pseudomonas fluorescens	KCTC42821	TSB	30
Pseudomonas putida	KCCM11348	TSB	25
Salmonella Enteritidis	NCCP14544, 13701, 12243, 12236	TSB	37
Salmonella Typhimurium	NCCP12441, 12219	TSB	37
Serratia liquefaciens	KCTC42170	TSB	30
Serratia marcescens	KCTC42171, 2516	TSB	30
Staphylococcus aureus	NCCP14400, 14401, 14402, 14403, 14404, 14405, 14406, 14407	TSB	37
Streptococcus pyogenes	KCCM40411	BHIB	37
Streptococcus salivarius subsp. *thermophilus*	KCTC3779	MRSB	37
Vibrio parahaemolyticus	ATCC43996, 33844, 27519, 17802	Marine broth	37

Table 1. Microorganisms examined in this study (continued)

Microorganism	Strain	Culture conditions	
		Media	Temp. (℃)
Yersinia enterocolitica	KVCC BA2100003, BA2100004, BA2100005, NCCP12713	BHIB	30
Yeast			
Brettanomyces bruxellensis	KCCM11490	YMB	25
Candida lipolytica	NCCP32688	PDB	30
Candida zeylanoides	KCTC27413	PDB	25
Debaryomyces hansenii	KCCM50192, 12084	PDB	25
Meyerozyma guilliermondii	KCTC27416	PDB	25
Ogataea polymorpha	KCTC17566	PDB	25
Saccharomyces cerevisiae	KCTC7296, 7107	PDB	25
Yarrowia lipolytica	KCTC17170, 7272	PDB	25
Zygosaccharomyces bailii	KCTC7539	PDB	25
Zygosaccharomyces rouxii	KCTC7880	PDB	25
Mold			
Alternaria alternata	NCCP32766	PDB	30
Aspergillus flavus	KCCM60330	PDB	25
Aspergillus niger	NCCP32627	PDB	37
Aspergillus oryzae	NCCP32629	PDB	30
Aspergillus versicolor	KCCM60336	PDB	25
Cladosporium cladosporioides	KCTC26745	PDB	25
Cladosporium sphaerospermum	KCTC26739	PDB	25
Geotrichum capitatum	NCCP32601	PDB	30
Mucor plumbeus	KCCM60265	PDB	25
Penicillium roqueforti	KCTC6080	PDB	25
Rhizopus oryzae	KCTC46312	PDB	25

BHIB, brain heart infusion broth; TSB, tryptic soy broth; CA, Columbia agar with 5% sheep blood; TSBYE, tryptic soy broth with 0.6% yeast extract; MRSB, lactobacilli-MRS broth; PDB, potato dextrose broth.

determine MIC according to the method described in the section of 'Minimum inhibitory concentrations of preservatives for microorganisms at pH 7.0', the pH of MHB was adjusted with HCl.

Determination of minimum inhibitory concentrations of selected microorganisms in animal products

Bacteria that were the most sensitive to propionic acid, benzoic acid, and sorbic acid were used to determine MIC of preservatives in unprocessed animal products (eggs, chicken breast, chicken legs, pork ribs, pork sirloin, beef ribs, beef chunk, and milk) and processed animal products (processed butter, ground meat product, natural cheese, and smoked eggs). The selected bacteria were *Campylobacter coli* ATCC33559, *Campylobacter jejuni* ATCC33560, *Erwinia carotovora* KCCM11319, *Micrococcus luteus* KCCM11211, and *Moraxella catarrhalis* KCCM42707. A mixture of the bacteria was

prepared according to the procedure described in the section of 'Inoculum preparation'. Inoculum 0.1 mL was inoculated to 25 g of food sample in a sample bag to obtain a concentration of 4 Log CFU/g. A hundred microliters of the preservatives were then spiked in samples to have 0, 100, 500, 1,000, and 1,500 (1,200 ppm for sorbic acid) ppm. Pork ribs, pork loin, beef ribs, beef chunks, milk, processed butter, fermented milk, and natural cheese were stored at 10℃. Poultry and processed meat products were stored at 5℃, and smoked eggs were stored at 25℃. The sample (25 g) was aseptically transferred to a sample bag containing 225 mL of buffered peptone water (BPW; Becton Dickinson, Sparks, MD, USA), and the sample was pummeled for 60 s in a pummeler (BagMixer® 400, Interscience, Saint Nom la Bretehe, France). One milliliter of the homogenate was serially diluted with BPW, and the homogenates were dispensed on an aerobic bacteria count plate (AC Petrifilm; 3M™ Petrifilm aerobic count plate, 3M, St. Paul, MN, USA) to quantify the total bacteria. The AC Petrifilms were incubated at 35℃ for 48 h, and the colonies were then manually counted. The end time of the storage was determined as the time when the bacterial cell counts in the 0-ppm sample increased to 6 Log CFU/g. This experiment was repeated three times. The bacterial cell counts for each concentration of preservatives at the end of the storage were compared to the cell counts on day 0. This comparison was conducted by pairwise t-test at α=0.05 with the general linear model procedure (proc glm) of SAS® (ver.9.4, SAS Institute, Cary, NC, USA). If the difference was not significant, the concentration was determined as MIC per each replication. Among the MIC of 3 replications, the lowest MIC was determined as a final MIC.

pH measurement

To measure pH of the samples, 18 mL of DW was added to 2 g of the sample, and it was homogenized for 60 s in a pummeler. The pH of homogenate was measured using a pH meter (Thermo Fisher Scientific).

Results and Discussion

Minimum inhibitory concentrations of preservatives to food spoilage microorganisms in broth media

Control of microorganism growth in raw food materials and products is important in ensuring product safety, shelf life, and consumers' health. In meat, *Pseudomonas*, *Acinetobacter*, and *Brochothrix* mainly affect the quality and may cause spoilage (Liang et al., 2021; Wei et al., 2021). Also, pathogenic bacteria such as *Escherichia coli*, *Salmonella*, *Campylobacter*, *Listeria monocytogenes*, and *Staphylococcus aureus* are frequently detected in meat (Kim et al., 2020; Lee and Yoon, 2021; Park et al., 2021; Yang et al., 2022). Spoilage yeasts mainly include *Zygosaccharomyces*, *Saccharomyces*, *Candida* and *Brettanomyces*, and spoilage molds include *Zygomycetes*, *Penicillium*, *Aspergillus*, etc. (Blackburn, 2006). Especially, spoiled meats and cheeses often have high cell counts of *Debaryomyces*, *Yarrowia,* and *Rhodotorula* (Blackburn, 2006). The MIC of propionic acid, sorbic acid, and benzoic acid to these microorganisms in broth media were determined at pH 7.0 (Table 2). To increase the solubility of preservatives, salts were combined with the preservatives. Calcium propionate, sodium propionate, sodium benzoate, and potassium sorbate were also examined, and they had higher MIC than acid-type preservatives (Table 2). *C. coli, C. jejuni*, *M. catarrhalis*, *E. carotovora*, and *M. luteus* had lower MIC for the preservatives (propionic acid, benzoic acid, and sorbic acid), compared to other microorganisms. The preservative used in this study is a weak-acid type, which increases the number of non-dissociated molecules, when the pH is lowered. Thus, the molecules easily penetrate the microbial cell membrane or protoplasm, which prevents microbial growth (Theron and Lues, 2007). Unlike the acidic-preservatives, salt preservatives are considered to have a high MIC, because their pH were close to neutral. To investigate the antibacterial activity of preservatives according to pH, MIC of the preservatives were investigated by adjusting the pH of the

Table 2. Minimum inhibitory concentration (MIC) of propionic acid, calcium propionate, sodium propionate, benzoic acid, sodium benzoate, sorbic acid, and potassium sorbate in broth media at pH 7.0

Microorganism	MIC (ppm)[1)]						
	Propionic acid	Benzoic acid	Sorbic acid	Calcium propionate	Sodium propionate	Sodium benzoate	Potassium sorbate
Acetobacter aceti	1,600	3,000	2,000	>51,200	51,200	25,600	25,600
Acetobacter pasteurianus	1,600	1,500	2,000	>51,200	51,200	25,600	25,600
Acinetobacter calcoaceticus	800	1,500	1,000	1,744	5,338	5,968	6,651
Aeromonas salmonicida	800	1,500	1,000	6,400	6,400	3,200	1,600
Alcaligenes faecalis	800	1,500	2,000	6,978	42,704	2,984	6,651
Alcaligenes xylosoxidans ssp. *xylosoxidans*	1,600	1,500	2,000	6,978	51,200	11,935	13,302
Bacillus cereus	1,600	3,000	2,000	>51,200	85,407	23,870	26,605
Campylobacter coli	800	750	250	1,744	2,669	746	104
Campylobacter jejuni	800	375	250	1,744	3,200	800	104
Carnobacterium maltaromaticum	1,600	3,000	>2,000	6,400	>51,200	12,800	25,600
Clostridium perfringens	1,600	1,500	1,000	>55,822	42,704	5,968	13,302
Enterobacter aerogenes	1,600	1,500	2,000	6,978	21,352	11,935	13,302
Enterobacter amnigenus	1,600	1,500	2,000	1,744	21,352	5,968	6,651
Enterobacter cloacae	1,600	3,000	2,000	13,956	85,407	11,935	13,302
Enterococcus casseliflavus	1,600	3,000	2,000	>51,200	85,407	47,741	53,210
Enterococcus faecium	1,600	3,000	2,000	>51,200	>51,200	51,200	51,200
Erwinia carotovora subsp. *carotovora*	400	750	1,000	1,600	400	3,200	1,600
Escherichia coli	1,600	1,500	2,000	13,956	85,407	11,935	13,302
Enterohemorrhagic *E. coli*	1,600	1,500	2,000	13,956	42,704	11,935	13,302
Lactobacillus delbrueckii subsp. *lactis*	3,200	>3,000	2,000	6,400	51,200	3,200	6,400
Listeria monocytogenes	1,600	1,500	2,000	>55,822	21,352	5,968	6,651
Micrococcus luteus	800	750	1,000	12,800	>51,200	1,600	25,600
Moraxella catarrhalis	400	750	500	6,400	800	1,600	800
Proteus mirabilis	1,600	3,000	2,000	27,911	85,407	23,870	26,605
Proteus vulgaris	1,600	1,500	2,000	>55,822	42,704	23,870	26,605
Pseudomonas fluorescens	1,600	1,500	2,000	12,800	12,800	5,968	6,651
Pseudomonas putida	1,600	1,500	1,000	436	2,669	5,968	6,651
Salmonella Enteritidis	1,600	1,500	2,000	6,978	42,704	11,935	13,302
Salmonella Typhimurium	1,600	1,500	2,000	6,978	42,704	11,935	6,651
Serratia liquefaciens	1,600	1,500	2,000	218	667	2,984	6,651
Serratia marcescens	1,600	1,500	2,000	3,489	21,352	11,935	13,302
Staphylococcus aureus	1,600	1,500	2,000	3,489	42,704	23,870	53,210
Streptococcus pyogenes	1,600	3,000	2,000	>51,200	51,200	12,800	25,600

Table 2. Minimum inhibitory concentration (MIC) of propionic acid, calcium propionate, sodium propionate, benzoic acid, sodium benzoate, sorbic acid, and potassium sorbate in broth media at pH 7.0 (continued)

Microorganism	MIC (ppm)[1)]						
	Propionic acid	Benzoic acid	Sorbic acid	Calcium propionate	Sodium propionate	Sodium benzoate	Potassium sorbate
Streptococcus salivarius subsp. *thermophilus*	6,400	1,500	>2,000	25,600	>51,200	25,600	6,400
Vibrio parahaemolyticus	1,600	1,500	2,000	3,489	51,200	11,935	13,302
Yersinia enterocolitica	1,600	1,500	2,000	>51,200	10,676	5,968	6,651
Brettanomyces bruxellensis	6,400	1,500	1,000	>51,200	25,600	3,200	6,400
Candida zeylanoides	1,600	1,500	2,000	>51,200	>51,200	51,200	25,600
Debaryomyces hansenii	1,600	1,500	2,000	>51,200	>51,200	51,200	51,200
Meyerozyma guilliermondii	1,600	1,500	2,000	51,200	>51,200	51,200	25,600
Ogataea polymorpha	1,600	1,500	1,000	>51,200	6,400	12,800	12,800
Saccharomyces cerevisiae	3,200	1,500	1,000	>51,200	25,600	25,600	12,800
Yarrowia lipolytica (*Candida lipolytica*)	3,200	3,000	2,000	>51,200	>51,200	>51,200	25,600
Zygosaccharomyces bailii	800	1,500	1,000	>51,200	25,600	12,800	12,800
Zygosaccharomyces rouxii	1,600	1,500	2,000	>51,200	12,800	6,400	25,600
Alternaria alternata	3,200	1,500	2,000	>51,200	51,200	25,600	25,600
Aspergillus flavus	1,600	1,500	2,000	>51,200	51,200	25,600	51,200
Aspergillus versicolor	1,600	1,500	1,000	>51,200	51,200	51,200	12,800
Aspergillus niger	800	1,500	2,000	51,200	>51,200	25,600	51,200
Aspergillus oryzae	800	1,500	1,000	51,200	51,200	25,600	25,600
Cladosporium cladosporioides	1,600	1,500	1,000	>51,200	51,200	25,600	12,800
Cladosporium sphaerospermum	1,600	1,500	1,000	51,200	51,200	25,600	12,800
Geotrichum capitatum	1,600	1,500	2,000	51,200	51,200	51,200	51,200
Mucor plumbeus	1,600	1,500	2,000	>51,200	>51,200	51,200	51,200
Penicillium roquefortii	800	1,500	2,000	51,200	25,600	25,600	51,200
Rhizopus oryzae	1,600	1,500	2,000	51,200	51,200	25,600	12,800

[1)] Value was obtained from three independent experiments which showed identical results.

medium to 4.5, 5.5, and 6.0. The five bacterial strains showed lower MIC of the preservative at lower pH (Table 3). The MIC of the preservative for *E. carotovora* were 50 ppm for propionic acid, 25 ppm for sorbic acid, and 50 ppm for benzoic acid at pH 5.5, which were lower MIC than those at pH 6.0. These results confirmed that the microbial growth prevention efficacy of the weak-acid type preservatives increased at low pH as presented in other research.

Minimum inhibitory concentrations of preservatives to food spoilage bacteria in animal products

Unprocessed animal products were inoculated with a mixture of the most sensitive foodborne bacteria selected by MIC to the preservatives, and the samples were stored at 10℃ until the bacterial cell counts of the control increased to >10^6 CFU/g, which is considered to be the level that the spoilage started. At this time the total bacteria in other samples were counted.

Table 3. Minimum inhibitory concentration (MIC) of propionic acid, benzoic acid and sorbic acid at pH conditions

Microorganism	MIC (ppm)[1)]								
	Propionic acid			Benzoic acid			Sorbic acid		
	pH 4.5	pH 5.5	pH 6.0	pH 4.5	pH 5.5	pH 6.0	pH 4.5	pH 5.5	pH 6.0
Campylobacter coli	ND	ND	50	ND	ND	200	ND	ND	100
Campylobacter jejuni	ND	ND	50	ND	ND	100	ND	ND	100
Erwinia carotovora subsp. *carotovora*	ND	50	50	ND	25	500	ND	50	500
Micrococcus luteus	ND	ND	50	ND	ND	500	ND	ND	500
Moraxella catarrhalis	ND	ND	75	ND	ND	200	ND	ND	100

[1)] Value was obtained from three independent experiments which showed identical results.
ND, not detected.

The MIC of preservatives in animal products are presented in Table 4. The MIC of propionic acid were 100 ppm in chicken legs, pork ribs, pork sirloin and beef ribs, 500 ppm in chicken breast, beef chunk and milk, and 1,500 ppm in eggs. The MIC of benzoic acid were 100 ppm in chicken legs, pork ribs, and pork sirloin, 500 ppm in chicken breast, beef ribs, beef chunk, and milk, and 1,500 ppm in eggs. The MIC of sorbic acid were 100 ppm in chicken breast, chicken legs, pork ribs, pork sirloin, beef ribs, and beef chunk, and 500 ppm in milk, and 1,200 ppm in eggs. The MIC of propionic acid, benzoic acid, and sorbic acid in processed butter and natural cheese were 100 ppm. In smoked eggs, MIC of propionic acid were 1,000 ppm, and MIC of benzoic acid and sorbic acid were 500 ppm. In our study, the MIC investigated in food were higher than pH in broth media. Specifically, the pH of ground meat was close to 6.0 and the MIC of propionic acid, benzoic acid, and sorbic acid were 1,500, >1,500, and >1,500 ppm, respectively. However, the MIC in the broth of the five strains of microorganisms used as inoculum were below 500 ppm at pH 6.0.

Table 4. Minimum inhibitory concentration (MIC) of preservatives to a mixture of *Campylobacter coli*, *Campylobacter jejuni*, *Erwinia carotovora*, *Micrococcus luteus*, and *Moraxella catarrhalis* in animal products

Food		pH	Inoculum concentration (Log CFU/g)	MIC (ppm)[1)]		
				Propionic acid	Benzoic acid	Sorbic acid
Unprocessed animal products	Eggs	7.53±0.02	3.5±0.3	1,500	1,500	>1,200
	Chicken breast	5.77±0.06	4.9±0.7	500	500	100
	Chicken legs	6.39±0.11	5.8±0.7	100	100	100
	Pork ribs	5.96±0.46	4.5±1.0	100	100	100
	Pork sirloin	6.25±0.30	5.2±0.2	100	100	100
	Beef ribs	6.48±0.08	4.2±0.3	100	500	100
	Beef chuck	5.97±0.11	4.6±0.8	500	500	100
	Milk	6.82±0.12	3.8±0.1	500	500	500
Processed animal products	Processed butter	6.77±0.02	3.5±0.3	100	100	100
	Ground meat product	5.90±0.25	5.6±0.5	1,500	>1,500	>1,200
	Natural cheese	5.42±0.14	4.1±0.8	100	100	100
	Smoked eggs	7.60±0.05	3.6±0.2	1,000	500	500

[1)] Value was obtained from three independent experiments which showed identical results.

Preservatives are food additives that inhibit microbial growth in food, but most studies have identified MIC in microbiological media rather than food. Although few studies have evaluated the MIC of preservatives in food, it is known that the MIC of preservatives in food were higher than those in microbiological media (Brocklehurst et al., 1995; Weiss et al., 2015). While the media have homogeneous structure and consist of simple composition, the food consists of various components (fat, protein, fiber, and antibacterial substances) and structures (Weiss et al., 2015). Lipid content and preservative activity are correlated (Glass and Johnson, 2004; Weiss et al., 2015). Organic acids such as propionic acid bind to phospholipids in the bacterial cell membrane. However, the fat component in food also competitively binds to lipophilic molecules, making it difficult for preservatives to bind to bacteria. Electrostatic and hydrophobic interactions also significantly affect the activity of acid-type preservatives that are dissociated (Weiss et al., 2015). These reasons may also have caused the differences in MIC between the broth media and animal products in our study.

Conclusion

Many studies evaluated MIC in broth media rather than in food matrix. In our study showed that MIC were higher in animal products than in the broth media. Thus, the case of the MIC determined in the animal products might be appropriate to be determine if the detected preservatives in food are added intentionally or not, because preservatives are added to inhibit microbial growth, and thus, the concentrations should higher than the MIC.

Conflicts of Interest

The authors declare no potential conflicts of interest.

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Author Contributions

Conceptualization: Seo Y, Yoon Y. Data curation: Seo Y, Sung M, Hwang J. Formal analysis: Seo Y, Sung M. Methodology: Seo Y, Sung M. Software: Sung M, Hwang J. Validation: Seo Y. Investigation: Seo Y, Sung M, Hwang J. Writing - original draft: Seo Y, Sung M. Writing - review & editing: Seo Y, Sung M, Hwang J, Yoon Y.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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