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## 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

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**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  $1,912\times g$  and 15 min for  $4^{\circ}C$ , 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  $\mu L$  of diluted preservative solutions with different concentrations were placed in the wells. Each well was inoculated with 20  $\mu L$  of the inocula at 4 Log CFU/mL. The 96 well microtiter plates were incubated at  $35^{\circ}C$  for 24 h for the growth of the bacteria and yeast, and at  $35^{\circ}C$  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)
<b>Bacteria</b>			
<i>Acetobacter aceti</i>	KCTC12290	BHIB	25
<i>Acetobacter pasteurianus</i>	KCTC12289	BHIB	25
<i>Acinetobacter calcoaceticus</i>	NCCP16013	BHIB	25
<i>Aeromonas salmonicida</i>	KCCM40239	BHIB	25
<i>Alcaligenes faecalis</i>	KCTC2678	TSB	37
<i>Alcaligenes xylooxidans</i> ssp. <i>xylooxidans</i>	NCCP15702	TSB	30
<i>Bacillus cereus</i>	NCCP16296, 15910, 15909, 14796, 14043	TSB	30
<i>Campylobacter coli</i>	ATCC33559	CA	42
<i>Campylobacter jejuni</i>	ATCC33560	CA	42
<i>Carnobacterium maltaromaticum</i>	KCTC3602	TSBYE	30
<i>Clostridium perfringens</i>	NCCP15912, 15911	BHIB	37
<i>Enterobacter aerogenes</i>	NCCP16285	TSB	37
<i>Enterobacter amnigenus</i>	NCCP15837	TSB	30
<i>Enterobacter cloacae</i>	NCCP14672	TSB	37
<i>Enterococcus casseliflavus</i>	KCCM40712	BHIB	37
<i>Enterococcus faecium</i>	KCCM12118	BHIB	37
<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	KCCM11319	BHIB	30
<i>Escherichia coli</i>	NCCP16186, 16185, 15663, 15651, 13588	TSB	37
Enterohemorrhagic <i>E. coli</i>	NCCP15961, 15957, 15739, 15656, 14541	TSB	37
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	KCTC3636	MRSB	37
<i>Listeria monocytogenes</i>	ATCC BBA-839, 51774, 13932	TSBYE	30
<i>Micrococcus luteus</i>	KCCM11211	TSB	25
<i>Moraxella catarrhalis</i>	KCCM42707	BHIB	37
<i>Proteus mirabilis</i>	KCTC2566	TSB	37
<i>Proteus vulgaris</i>	KCTC2579	TSB	37
<i>Pseudomonas fluorescens</i>	KCTC42821	TSB	30
<i>Pseudomonas putida</i>	KCCM11348	TSB	25
<i>Salmonella</i> Enteritidis	NCCP14544, 13701, 12243, 12236	TSB	37
<i>Salmonella</i> Typhimurium	NCCP12441, 12219	TSB	37
<i>Serratia liquefaciens</i>	KCTC42170	TSB	30
<i>Serratia marcescens</i>	KCTC42171, 2516	TSB	30
<i>Staphylococcus aureus</i>	NCCP14400, 14401, 14402, 14403, 14404, 14405, 14406, 14407	TSB	37
<i>Streptococcus pyogenes</i>	KCCM40411	BHIB	37
<i>Streptococcus salivarius</i> subsp. <i>thermophilus</i>	KCTC3779	MRSB	37
<i>Vibrio parahaemolyticus</i>	ATCC43996, 33844, 27519, 17802	Marine broth	37

**Table 1. Microorganisms examined in this study (continued)**

Microorganism	Strain	Culture conditions	
		Media	Temp. (°C)
<i>Yersinia enterocolitica</i>	KVCC BA2100003, BA2100004, BA2100005, NCCP12713	BHIB	30
Yeast			
<i>Brettanomyces bruxellensis</i>	KCCM11490	YMB	25
<i>Candida lipolytica</i>	NCCP32688	PDB	30
<i>Candida zeylanoides</i>	KCTC27413	PDB	25
<i>Debaryomyces hansenii</i>	KCCM50192, 12084	PDB	25
<i>Meyerozyma guilliermondii</i>	KCTC27416	PDB	25
<i>Ogataea polymorpha</i>	KCTC17566	PDB	25
<i>Saccharomyces cerevisiae</i>	KCTC7296, 7107	PDB	25
<i>Yarrowia lipolytica</i>	KCTC17170, 7272	PDB	25
<i>Zygosaccharomyces bailii</i>	KCTC7539	PDB	25
<i>Zygosaccharomyces rouxii</i>	KCTC7880	PDB	25
Mold			
<i>Alternaria alternata</i>	NCCP32766	PDB	30
<i>Aspergillus flavus</i>	KCCM60330	PDB	25
<i>Aspergillus niger</i>	NCCP32627	PDB	37
<i>Aspergillus oryzae</i>	NCCP32629	PDB	30
<i>Aspergillus versicolor</i>	KCCM60336	PDB	25
<i>Cladosporium cladosporioides</i>	KCTC26745	PDB	25
<i>Cladosporium sphaerospermum</i>	KCTC26739	PDB	25
<i>Geotrichum capitatum</i>	NCCP32601	PDB	30
<i>Mucor plumbeus</i>	KCCM60265	PDB	25
<i>Penicillium roqueforti</i>	KCTC6080	PDB	25
<i>Rhizopus oryzae</i>	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°C. Poultry and processed meat products were stored at 5°C, and smoked eggs were stored at 25°C. 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<sup>®</sup> 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<sup>™</sup> Petrifilm aerobic count plate, 3M, St. Paul, MN, USA) to quantify the total bacteria. The AC Petrifilms were incubated at 35°C 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  $\alpha=0.05$  with the general linear model procedure (proc glm) of SAS<sup>®</sup> (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) <sup>1)</sup>						
	Propionic acid	Benzoic acid	Sorbic acid	Calcium propionate	Sodium propionate	Sodium benzoate	Potassium sorbate
<i>Acetobacter aceti</i>	1,600	3,000	2,000	>51,200	51,200	25,600	25,600
<i>Acetobacter pasteurianus</i>	1,600	1,500	2,000	>51,200	51,200	25,600	25,600
<i>Acinetobacter calcoaceticus</i>	800	1,500	1,000	1,744	5,338	5,968	6,651
<i>Aeromonas salmonicida</i>	800	1,500	1,000	6,400	6,400	3,200	1,600
<i>Alcaligenes faecalis</i>	800	1,500	2,000	6,978	42,704	2,984	6,651
<i>Alcaligenes xylosoxidans</i> ssp. <i>xylosoxidans</i>	1,600	1,500	2,000	6,978	51,200	11,935	13,302
<i>Bacillus cereus</i>	1,600	3,000	2,000	>51,200	85,407	23,870	26,605
<i>Campylobacter coli</i>	800	750	250	1,744	2,669	746	104
<i>Campylobacter jejuni</i>	800	375	250	1,744	3,200	800	104
<i>Carnobacterium maltaromaticum</i>	1,600	3,000	>2,000	6,400	>51,200	12,800	25,600
<i>Clostridium perfringens</i>	1,600	1,500	1,000	>55,822	42,704	5,968	13,302
<i>Enterobacter aerogenes</i>	1,600	1,500	2,000	6,978	21,352	11,935	13,302
<i>Enterobacter amnigenus</i>	1,600	1,500	2,000	1,744	21,352	5,968	6,651
<i>Enterobacter cloacae</i>	1,600	3,000	2,000	13,956	85,407	11,935	13,302
<i>Enterococcus casseliflavus</i>	1,600	3,000	2,000	>51,200	85,407	47,741	53,210
<i>Enterococcus faecium</i>	1,600	3,000	2,000	>51,200	>51,200	51,200	51,200
<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	400	750	1,000	1,600	400	3,200	1,600
<i>Escherichia coli</i>	1,600	1,500	2,000	13,956	85,407	11,935	13,302
Enterohemorrhagic <i>E. coli</i>	1,600	1,500	2,000	13,956	42,704	11,935	13,302
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	3,200	>3,000	2,000	6,400	51,200	3,200	6,400
<i>Listeria monocytogenes</i>	1,600	1,500	2,000	>55,822	21,352	5,968	6,651
<i>Micrococcus luteus</i>	800	750	1,000	12,800	>51,200	1,600	25,600
<i>Moraxella catarrhalis</i>	400	750	500	6,400	800	1,600	800
<i>Proteus mirabilis</i>	1,600	3,000	2,000	27,911	85,407	23,870	26,605
<i>Proteus vulgaris</i>	1,600	1,500	2,000	>55,822	42,704	23,870	26,605
<i>Pseudomonas fluorescens</i>	1,600	1,500	2,000	12,800	12,800	5,968	6,651
<i>Pseudomonas putida</i>	1,600	1,500	1,000	436	2,669	5,968	6,651
<i>Salmonella</i> Enteritidis	1,600	1,500	2,000	6,978	42,704	11,935	13,302
<i>Salmonella</i> Typhimurium	1,600	1,500	2,000	6,978	42,704	11,935	6,651
<i>Serratia liquefaciens</i>	1,600	1,500	2,000	218	667	2,984	6,651
<i>Serratia marcescens</i>	1,600	1,500	2,000	3,489	21,352	11,935	13,302
<i>Staphylococcus aureus</i>	1,600	1,500	2,000	3,489	42,704	23,870	53,210
<i>Streptococcus pyogenes</i>	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) <sup>1)</sup>						
	Propionic acid	Benzoic acid	Sorbic acid	Calcium propionate	Sodium propionate	Sodium benzoate	Potassium sorbate
<i>Streptococcus salivarius</i> subsp. <i>thermophilus</i>	6,400	1,500	>2,000	25,600	>51,200	25,600	6,400
<i>Vibrio parahaemolyticus</i>	1,600	1,500	2,000	3,489	51,200	11,935	13,302
<i>Yersinia enterocolitica</i>	1,600	1,500	2,000	>51,200	10,676	5,968	6,651
<i>Brettanomyces bruxellensis</i>	6,400	1,500	1,000	>51,200	25,600	3,200	6,400
<i>Candida zeylanoides</i>	1,600	1,500	2,000	>51,200	>51,200	51,200	25,600
<i>Debaryomyces hansenii</i>	1,600	1,500	2,000	>51,200	>51,200	51,200	51,200
<i>Meyerozyma guilliermondii</i>	1,600	1,500	2,000	51,200	>51,200	51,200	25,600
<i>Ogataea polymorpha</i>	1,600	1,500	1,000	>51,200	6,400	12,800	12,800
<i>Saccharomyces cerevisiae</i>	3,200	1,500	1,000	>51,200	25,600	25,600	12,800
<i>Yarrowia lipolytica</i> ( <i>Candida lipolytica</i> )	3,200	3,000	2,000	>51,200	>51,200	>51,200	25,600
<i>Zygosaccharomyces bailii</i>	800	1,500	1,000	>51,200	25,600	12,800	12,800
<i>Zygosaccharomyces rouxii</i>	1,600	1,500	2,000	>51,200	12,800	6,400	25,600
<i>Alternaria alternata</i>	3,200	1,500	2,000	>51,200	51,200	25,600	25,600
<i>Aspergillus flavus</i>	1,600	1,500	2,000	>51,200	51,200	25,600	51,200
<i>Aspergillus versicolor</i>	1,600	1,500	1,000	>51,200	51,200	51,200	12,800
<i>Aspergillus niger</i>	800	1,500	2,000	51,200	>51,200	25,600	51,200
<i>Aspergillus oryzae</i>	800	1,500	1,000	51,200	51,200	25,600	25,600
<i>Cladosporium cladosporioides</i>	1,600	1,500	1,000	>51,200	51,200	25,600	12,800
<i>Cladosporium sphaerospermum</i>	1,600	1,500	1,000	51,200	51,200	25,600	12,800
<i>Geotrichum capitatum</i>	1,600	1,500	2,000	51,200	51,200	51,200	51,200
<i>Mucor plumbeus</i>	1,600	1,500	2,000	>51,200	>51,200	51,200	51,200
<i>Penicillium roquefortii</i>	800	1,500	2,000	51,200	25,600	25,600	51,200
<i>Rhizopus oryzae</i>	1,600	1,500	2,000	51,200	51,200	25,600	12,800

<sup>1)</sup> 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°C until the bacterial cell counts of the control increased to >10<sup>6</sup> 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) <sup>1)</sup>								
	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
<i>Campylobacter coli</i>	ND	ND	50	ND	ND	200	ND	ND	100
<i>Campylobacter jejuni</i>	ND	ND	50	ND	ND	100	ND	ND	100
<i>Erwinia carotovora</i> subsp. <i>carotovora</i>	ND	50	50	ND	25	500	ND	50	500
<i>Micrococcus luteus</i>	ND	ND	50	ND	ND	500	ND	ND	500
<i>Moraxella catarrhalis</i>	ND	ND	75	ND	ND	200	ND	ND	100

<sup>1)</sup> 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) <sup>1)</sup>			
			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 <sup>1)</sup>	>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

<sup>1)</sup> 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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