Original Article

Common food antimicrobials: effects on cellular inflammation and oxidative damage and their estimated occurrence in Singapore

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Background and Objectives: The prevalence and potential health effects of common food antimicrobials in processed foods and beverages are relatively unknown in Singapore. The occurrence of chemical antimicrobials in processed foods and beverages and their effects on inflammation and oxidative stress in vitro were examined. **Methods and Study Design:** The occurrence of antimicrobials in 1605 processed food and 359 beverage items were examined by surveying the ingredients on the product labels. Human neutrophils were exposed to physiologically relevant concentrations of common antimicrobials. Established markers of inflammation, l Leukotriene B_4 and oxidative stress, F_2-isoprostanes were measured using stable-isotope dilution gas chromatography-mass spectrometry. **Results:** Antimicrobials were added to 23.2% of the processed foods and beverages. Sorbic, benzoic, lactic, propionic and acetic acids accounted for 84.8% of the added antimicrobials in the processed foods and beverages. 92.5% of the bread contained propionic acid. Lactic acid was the most common antimicrobial (44.4%) in cheeses. Sorbic acid was added to 63.2% of the margarines selected. Sauces (31.5%), energy drinks (50.0%), soft drinks (70.7%) and fruit cordials (66.6%) contained added benzoic acid. Benzoic and propionic acids at physiologically relevant concentrations augmented leukotriene B_4formation (benzoicacid, EC_{50} = ~100 µg $L^{-1}$and propionic acid, >200 µg L^{-1}). Lactic and sorbic acids dose-dependently inhibited the F_2-isoprostanes production (IC_{50} values ~100 µg L^{-1}) and myeloperoxidase activity (IC50values ~100 µg L^{-1}). **Conclusions:** Our results demonstrate that Singapore consumers are significantly exposed to food antimicrobials, and these molecules, in physiologically relevant concentrations, exert significant and differential effects in vitro.

Key Words: antimicrobial, prevalence, inflammation, myeloperoxidase, oxidative stress

INTRODUCTION

Food antimicrobials are added to food primarily to avoid and control contamination by microorganisms.[1] Some of the common food antimicrobials used in food are sorbic acids and sorbates (SA), benzoic acid and benzoates (BA), lactic acid (LA), and propionic acid and propionates (PA). Antimicrobial additives with restricted uses are SA (ADI 25 mg/kg BW/ day), BA (ADI 5 mg/kg BW/ day), and PA (quantum satis).[2] In Singapore, the uses of BA, SA, and PA in several food groups are authorised in the Singapore Food Regulations, provided that the maximum use rules are adhered to.[3] At present, there is limited data on the occurrence of these common food antimicrobials in the processed foods and beverages available to Singaporean consumers.

The roles of food antimicrobials in the pathogenesis of atherosclerosis are relatively unknown. Inflammation and oxidative stress are considered to play key roles in the development and progression of atherosclerosis.[4,5] Pro-inflammatory eicosanoid, leukotriene B_4 (LTB_4) mediates inflammatory processes in the vascular wall by promoting leukocyte–endothelial interactions and their subsequent inflammatory processes in the vascular wall by promoting leukocyte–endothelial interactions and their subsequent migration to the sub-endothelium.[6] LTB_4 was found to associate with symptoms of atherosclerotic plaque instability.[7] LTB_4 is also involved in other inflammatory diseases such as rheumatoid arthritis[8] and Dengue serotype II infection.[9] Oxidative modification of low-density lipoprotein (LDL) has been shown to associate with preclinical atherosclerosis, acute coronary syndrome, and coronary arterial atherosclerosis.[10] The generation of reactive oxygen species (ROS) by free radical oxidation and myeloperoxidase (MPO)-catalysed peroxidation are the identified pathways for LDL modification in vivo.[10] At

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Manuscript received 02 April 2016. Initial review completed 4June 2016. Revision accepted 01September 2016.
doi:10.6133/apjcn.032017.24

present, limited data is available to evaluate the effects of common food antimicrobials on the LTB_4 and ROS formation (via free radical or MPO pathways).

In this study, the prevalences of added antimicrobials in the processed food and beverage supply were examined. In a separate *in vitro* study, the abilities of the five most prevalent food antimicrobials to modulate pro-inflammatory LTB_4 production from human neutrophils were compared. Their capacity to influence arachidonic acid peroxidation and MPO reactivity was also investigated.

MATERIALS AND METHODS

Chemicals and materials

Arachidonic acid (AA), F_2-isoprostanes-d_4 and leukotriene B_4-d_4 were purchased from Cayman Chemical (MO, USA). Acetic acid, propionic acid, lactic acid, benzoic acid, sorbic acid, sodium hydrogen carbonate, guiacol, hydrogen peroxide (30% by volume), glucose, dextran 500, phorbol12-myristate 13-acetate (PMA), calcium ionophore A23187, MK-886, trypan blue, isooctane, Hank's balanced salt solution (HBSS), phosphate-buffered saline (PBS) and bis (trimethylsilyl) trifluoroacetamide (BSTFA) were purchased from Sigma-Aldrich (St. Louis, MO); acetonitrile, ethyl acetate, methanol, ethanol and sulfuric acid were from Tedia (OH, USA); and ficoll-paque was from GE Healthcare (Uppsala, Sweden).

Occurrence data collection at a local supermarket

All the processed food and beverage items on the shelves of one local supermarket were included in this study. The items were categorised according to the supermarket shelf labels. For foods: baking needs, biscuits, breads, butter, cakes, canned fruits, canned meat, canned seafood, canned vegetables, cereals, cheese, chips, coconut products, crackers, chocolates, dairy products, dried food, fish and seafood, frozen meat, frozen snacks, ice cream, instant noodles, jellies, margarine, nuts, prepacked noodles, puddings, sauces, soups, spreads, sweeteners, sweets and tofu; and for beverages: Asian drinks (beverages of flavours from Asian origin), coffee drinks, dairy-based products, energy drinks, fruit juices, health beneficial beverages (beverages with supposed health benefits, though there is no scientific evidence to support this classification), juice drinks, soft drinks, soy-based beverages, sports beverages, syrups or fruit cordials and tea. The name of each food or beverage item and the added chemical antimicrobials used in its production, as shown in the ingredient list on the food labels, were recorded by trained research personnel. The presence of added chemical antimicrobials should be listed on the ingredient list printed on the item's packaging as regulated by Singapore Food Regulations. The presence of antimicrobials in each processed food and beverage product category was computed as the percentage of the number of products which contain at least one antimicrobial to the total number of the products in the same category. The occurrence of the specific antimicrobial in each processed food and beverage category was computed as the percentage of the number of items which contain the antimicrobials to the total number of the items within the same category. The presence of specific antimicrobials in all surveyed processed food and beverage products was computed as the percentage of the number of products which contain the antimicrobials to the total number of the products. The occurrence of specific antimicrobial in the antimicrobial-containing food and beverage products was computed as the percentage of the number of products which contained the antimicrobials to the total number of the antimicrobial-containing products.

In vitro experiments

Neutrophils were isolated from the neutrophil/erythrocyte pellet of fresh human whole blood after Ficoll Paque gradient centrifugation and dextran sedimentation of red cells as previously described.[11] Cell viability was assessed using trypan blue exclusion and was typical >98%. The freshly isolated neutrophils were resuspended in HBSS at a concentration of 5×10^6 cells/mL.

The effect of AC, BA, LA, PA, and SA on the production of LTB_4 from peripheral neutrophils was examined. Briefly, the neutrophil suspension (5×10^6 cells/mL in HBSS, 1 mL) was incubated with either AC, BA, LA, PA or SA (final concentrations, 0, 10, 20, 50, 100, and 200 $\mu g\ L^{-1}$) and AA (final concentration, 10 $\mu mol\ L^{-1}$) at 37°C for 5 min prior to 5-lipoxygenase stimulation. AA, AC, BA, LA, PA, and SA were added using ethanol as a vehicle. The cells were stimulated with calcium ionophore A23187 (final concentration, 2.5 $mg\ mL^{-1}$) at 37°C for 15 min. Untreated cells with AA in ethanol were used as positive controls, while untreated cells incubated with the leukotriene biosynthesis inhibitor MK886 (300 $nmol\ L^{-1}$) in ethanol served as negative controls.[12] The supernatant from the cell suspension was collected and stored at -80°C before LTB_4 extraction and analysis. The release of LTB_4 from stimulated neutrophils was measured by stable isotope labelled gas chromatography-mass spectrometry (GC-MS).[11]

The ability of AC, BA, LA, PA, and SA to scavenge free radicals was examined by measuring the inhibition of F_2-isoprostanes (a stable marker of AA oxidation) formation in peripheral neutrophils. Briefly, the freshly isolated neutrophils (5×10^6 cells mL^{-1} in HBSS, 1 mL) were incubated with either AC, BA, LA, PA or SA (final concentrations, 0, 10, 20, 50, 100, and 200 $\mu g\ L^{-1}$) and AA (final concentration, 10 $\mu mol\ L^{-1}$) at 37°C for 5 min prior to stimulation. AC, AA, BA, LA, PA, and SA were added using ethanol as the vehicle. The cells were stimulated with PMA (final concentration, 200 $nmol\ L^{-1}$) at 37°C for 15 min. Arachidonic acid incubated with PMA-activated cells were used as positive controls, while AA incubated with untreated cells without PMA activation served as negative controls. The supernatant from the cell suspension was collected and stored at -80°C before F_2-isoprostanes extraction and analysis. F_2-isoprostanes was quantitated using stable isotope labelled GC-MS.[13]

To test the effect of AC, BA, LA, PA, and SA on MPO activity in peripheral neutrophils, freshly isolated cells (5×10^6 cells/mL in HBSS, 1 mL) were incubated with these compounds (final concentrations, 0, 10, 20, 50, 100 and 200 $\mu g\ L^{-1}$) for 5 min at 37°C before the incubate was removed. The neutrophils were resuspended on fresh HBSS and lysed by sonication. Functional MPO activity was determined by measuring its catalytic action on the oxidation of guaiacol in the presence of hydrogen peroxide as previously published.[14]

Statistical Analysis
The occurrence of antimicrobials was ranked in each of the food and beverage categories. Statistical analysis of the in vitro results (n=5 independent experiments using different batches of freshly isolated human neutrophils) was performed using SPSS version 22.0. ANOVA was performed on specific concentration points and the area under the curve in the concentration-response results. The results analysed were considered significantly different if *p*-value <0.05 based on 95% confidence interval. Error bars in all of the figures were presented as standard deviations (SD).

RESULTS

Prevalence of chemical antimicrobials
A total of 1605 processed food and 359 beverage items were grouped into 32 foods and 12 beverage categories according to typical supermarket shelf order (Table 1). The number of products in each category is listed in Table 1. At least one chemical antimicrobial was added to 21.4%, 31.5% and 23.2% of the processed foods, beverages, and both processed foods and beverages, respectively, sold in a typical local supermarket,. Of the thirty-three food and twelve beverage categories, more than 50% of the bread, cheese, margarine, canned meat, butter, sauce, fruit cordial, soft drink and energy drink contained added chemical antimicrobials (Table 1).

SA, LA, PA, AC, and BA (listed in descending order) were the five most common added chemical antimicrobial in the processed foods, and together they accounted for 23.0% and 79.9% of the added antimicrobials in processed foods and antimicrobial-containing foods, respectively (Figure 1). BA, SA, sulfur-containing compounds and LA (listed in descending order) were commonly found in processed beverages, and they accounted for 39.3% and 57.0% of the added antimicrobials in the beverages and antimicrobial-containing beverages, respectively (Figure 1). The five most commonly added chemical antimicrobials (SA, BA, LA, PA, and AC) accounted for 24.8% and 84.8% of the added antimicrobials in all processed foods and beverages, and those containing added antimicrobials, respectively.

92.5% of the bread contained PA (Table 1). LA was the most common antimicrobial (44.4%) added to cheeses. SA was added to 63.2% of the margarine (Table 1). Nitrite and nitrate were the predominant antimicrobials used in canned meat. 31.5% of the sauces, 50.0% of the energy drinks, 70.7% of the soft drinks and 66.6% of the fruit cordials contained added BA (Table 1).

In vitro experiments
AC, BA, LA, PA and SA did not significantly affect cell viability at all tested concentrations. The effects of AC, BA, LA, PA, and SA on LTB_4 production in human neutrophils are presented in Figure 2a. Excitatory and/or inhibitory activity was expressed as the percentage increase and/or reduction in LTB_4 production compared with the untreated positive control (producing 7.0 ng 10^{-6} cells). None of the negative controls (MK 886 treated) showed measurable LTB_4. BA and PA augmented the formations of LTB_4 (EC_{50} = ~100 µg L^{-1} for BA and >200 µg L^{-1} for PA) by the human neutrophils in a dose-dependent manner (Figure 2a). The increase in LTB_4 production was significantly greater by treatment with BA than with PA ($p<0.05$ using the comparison of the area under the curve (ANOVA), Figure 2a). AC, LA, and SA did not influence LTB_4 formation by the freshly isolated human neutrophils at all tested concentrations (Figure 2a).

The ability of AC, BA, LA, PA, and SA to influence the production of F_2-isoprostanes in the human neutrophils is presented in Figure 2b. Excitatory and/or inhibitory activity was expressed as the percentage increase and/or reduction in F_2-isoprostanes production compared with the untreated positive control. LA and SA dose-dependently inhibited the F_2-isoprostanes production from the freshly isolated human neutrophils with IC_{50} values of ~100 µg L^{-1}(Figure 2b). Significant effects on F_2-isoprostanes production in the human neutrophils were absent for AC, BA, and PA at all tested concentrations (Figure 2b). LA and SA showed significantly greater F_2-isoprostanes inhibitory activity than AC, BA and PA ($p<0.05$ using the comparison of the area under the curve (ANOVA), Figure 2b).

The ability of AC, BA, LA, PA, and SA to inhibit the MPO enzyme activity in the human neutrophils is presented in Figure 2c. Inhibitory activity was expressed as the percentage reduction in MPO activity compared with the untreated positive control. MPO activity was not affected by AC, BA, and PA at all tested concentrations (Figure 2c). LA and SA (IC_{50} = ~100 µg L^{-1}) inhibited MPO dose-dependently when compared with the positive control (Figure 2c). LA and SA inhibited MPO activity to significantly greater extents than AC, BA, and PA ($p<0.05$ using the comparison of the area under the curve (ANOVA), Figure 2c).

DISCUSSION

Our study is one of few which has provided data regarding the relative occurrence of chemical antimicrobials in Singaporean food supplies. The occurrence of chemical antimicrobials in the processed foods and beverages sold in Singapore was found to be 23.2%. One would expect a higher occurrence of chemical antimicrobials in the processed foods and beverages, especially in a city state, like Singapore, where processed food and beverage products are readily available and constitute significant proportions of the daily diet of its residents.

Our data showed that SA, LA, PA, AC, and BA were the common chemical antimicrobials found in the processed foods and beverages. While most other *in vitro* experiments have been conducted using testing molecules at high physiologically-unrealistic concentrations, our *in vitro* results were obtained by loading the cells with physiologically relevant concentrations of the test molecules. This was necessary when we needed to translate our in vitro results to become relevant in vivo. BA and PA, at physiologically relevant concentrations, exhibited pro-inflammatory responses by augmenting the production of pro-inflammatory LTB_4 by human neutrophils. PA has been previously shown to mobilise calcium ions in human neutrophils[15] and this may explain the increase in LTB_4

Table 1.(a) Food and (b) beverage categories sold in a local Singapore supermarket that contain added chemical antimicrobial, and the most prevalent antimicrobials in each category

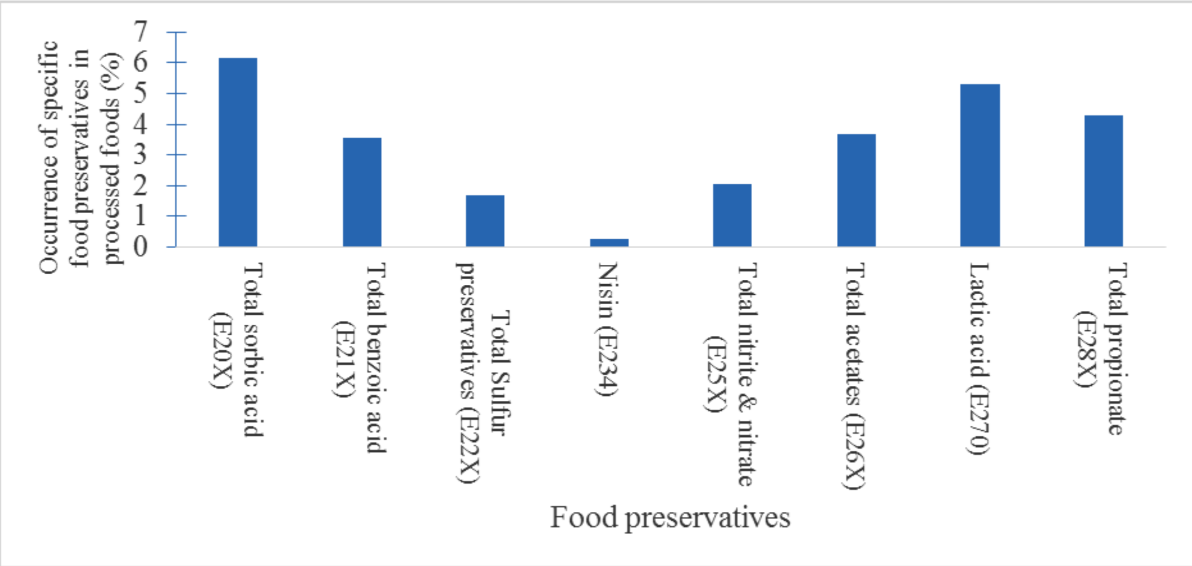
Category	Total number of items	Items with added chemical antimicrobial (%)†	Most prevalent chemical antimicrobials (%)‡
(a) Foods			
Baking needs	55	5.5	Sorbic acid, sulfur dioxide (3.6)
Biscuits	182	11.0	Sodium metasulfite (6.0)
Breads	68	97.0	Propionic acid (92.5)
Butter	12	58.3	Sorbic acid (41.5)
Cake	35	34.3	Sorbic acid (34.3)
Canned fruits	44	0	-
Canned meat	40	60.0	Sodium nitrite (40.0)
Canned seafood	58	1.7	Acetic acid (3.4)
Canned vegetables	82	6.1	Acetic acid (7.3)
Cereals	37	16.2	Sulfur dioxide (10.8)
Cheese	45	73.3	Lactic acid (44.4)
Chips	73	23.3	Acetic acid (15.1)
Coconut products	5	0	-
Cracker	32	0	-
Chocolate	82	0	-
Dairy product	46	34.8	Sorbic acid (21.7)
Dried food	2	0	-
Fish and seafood	29	0	-
Frozen meat	46	19.6	Sodium nitrite (13.0)
Frozen ice cream	39	0	-
Frozen snacks	62	17.7	Sorbic acid (9.7)
Instant noodle	79	21.5	Acetic acid (16.5)
Jelly	26	3.9	Benzoic acid (3.9)
Margarine	19	68.4	Sorbic acid (63.2)
Nuts	32	3.1	Sulfur dioxide (3.1)
Prepacked noodle	12	8.3	Acetic acid (16.7)
Pudding	12	0	-
Sauce	124	54.0	Benzoic acid (31.5)
Soup	76	1.3	Lactic acid (1.3)
Spread	75	5.3	Sorbic acid, sulfur dioxide (2.7)
Sweetener	13	0	-
Sweet	43	20.9	Lactic acid (16.3)
Tofu	21	0	-
Overall food items	1605	21.4	Sorbic acid (6.2)
(b)Beverages			
Asian drink	37	5.7	Benzoic acid (5.7)
Coffee drink	9	0	-
Dairy-based product	28	25.0	Lactic acid (14.3)
Energy drink	6	66.7	Benzoic acid (50.0)
Fruit juice	62	27.4	Sorbic acid (21.0)
Health beneficial drink	7	25.0	Benzoic acid, lactic acid (10.0)
Juice drink	59	28.3	Sorbic acid (15.2)
Soft drink	41	73.2	Benzoic acid (70.7)
Soy-based drink	22	4.6	Sorbic acid (4.6)
Sports drink	28	32.1	Benzoic acid (25.0)
Syrup or fruit cordial	30	78.1	Benzoic acid (66.7)
Tea	30	0	-
Overall beverage items	359	31.5	Benzoic acid (21.2)
Overall food and beverage items	1964	23.2	Sorbic acid (6.9)

†The presence of antimicrobials in each processed food and beverage product category was computed as the percentage of the number of items which contain at least one antimicrobials to the total number of the items in the same category.
‡The occurrence of the specific antimicrobial in each processed food and beverage product category was computed as the percentage of the number of items which contain the antimicrobials to the total number of the items within the same category.

release from neutrophils upon PA challenge. The human plasma concentration of PA has been reported to be ~76µgL^{-1},[16] though the proportions of endogenous and exogenous origins remain unknown. It was proposed that the exogenous food-sources of PA do not lead to significant amounts of PA in human circulation, since the quantity of PA derived from the anaerobic fermentation of undigested polysaccharides, oligosaccharides, long-chain fatty acids, protein, peptides and glycoprotein by the colonic micro biota is comparably greater.[17] The possible endogenous contribution of these short chain fatty acids should not be discounted, and further studies may be needed to work out the exogenous contribution to the in vivo pool.

LA and SA were demonstrated in our study to decrease the production of F_2-isoprostanes, which is an established

(a)



(b)

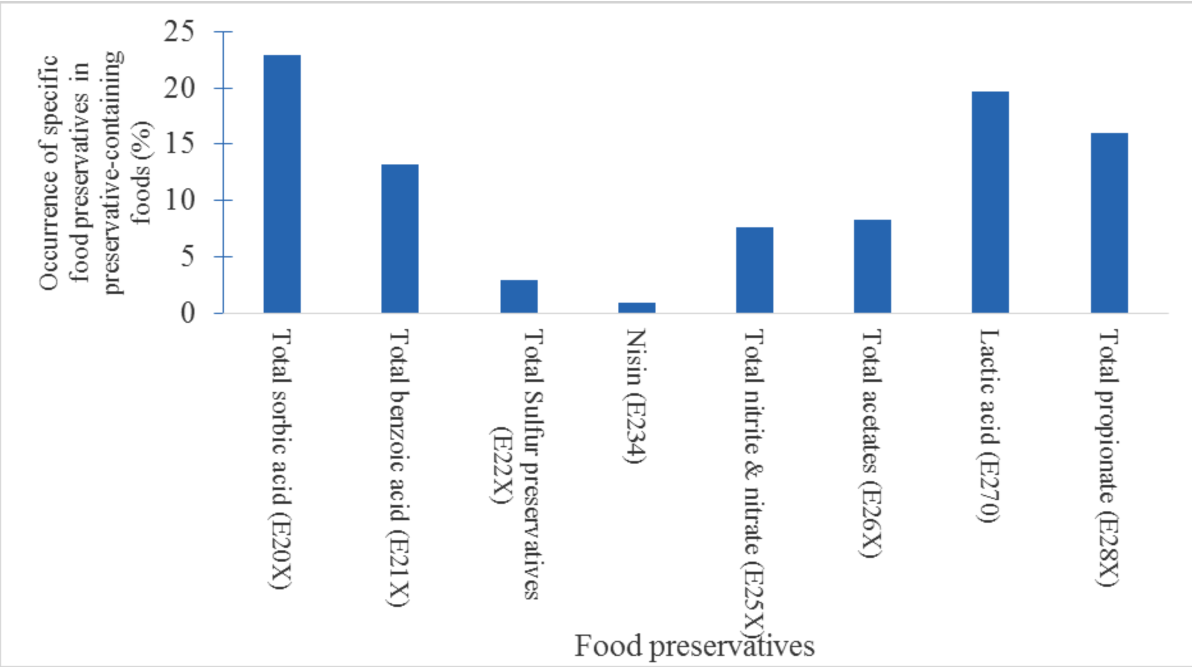


Figure 1. The occurrence of specific food antimicrobials in (a) all and (b) antimicrobial-containing processed foods sold in a local Singapore supermarket. The presence of specific antimicrobials in all surveyed products was computed as the percentage of the number of items which contain the antimicrobials to the total number of the items. The occurrence of specific antimicrobial in the antimicrobial-containing products was computed as the percentage of the number of items which contain the antimicrobials to the total number of the antimicrobial-containing items.

stable biomarker of cellular and systemic oxidative damage, in human neutrophils. Their antioxidant actions may be mediated through the inhibition of the MPO. Numerous studies have associated the presence of conjugated double bonds and α-hydroxy acid group to antioxidant activity.[18,19] Therefore, upon comparing with the absence of antioxidant activity of AC, BA, and PA, it is not difficult to attribute the observed antioxidant abilities to LA's α-hydroxy acid group and SA's conjugated double bonds. Previous studies have suggested that the conjugated double bonds present in SA's structure may be prone to nucleophilic attack, turning the molecule into a mutagenic compound.[20,21] The same conjugated double bonds may also donate electrons and become oxidised. EPR experiments exhibited direct radical scavenging activities of LA toward both superoxide and hydroxyl radical anions.[22] LA was also able to inhibit lipid peroxidation in hepatocyte culture.[22] Hertz at al 1997 proposed an indirect antioxidant effect of LA that the consumption of hydroxyl radical by LA produces pyruvate, which is a potent antioxidant capable of scavenging hydrogen peroxide and hydroxyl ion via decomposition into acetate and carbon dioxide.[23] It should be noted that the lactic dehydrogenase enzyme present in the human neutrophils catalyses the transformation of lactate into pyruvate, adding to the antioxidant capacity of LA.[23] Coincidentally, LA has been employed as a natural food antioxidant.[24]

It was not surprising to observe that over 90% of the

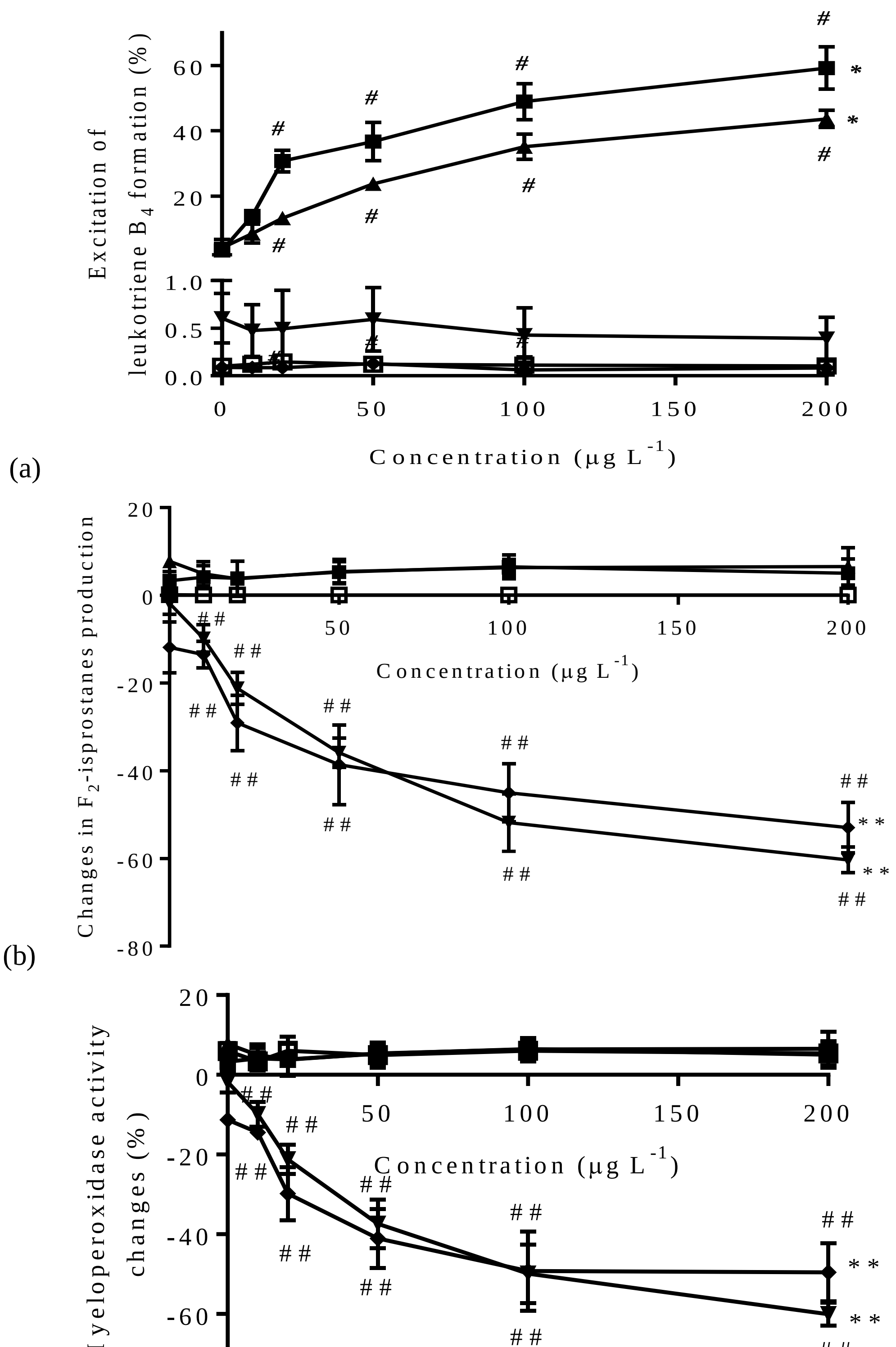


Figure 2. Change (%) inthe (a) leukotriene B_4, (b) F_2-isoprostanes production and (c) myeloperoxidase activity in freshly isolated human neutrophils by acetic (□), benzoic (■), lactic (♦), propionic (▲) and sorbic (▼) acids at concentrations up to 200 μg L^{-1} (N=5). $^{*}p< 0.05$ vs all other acids using the comparison of the area under the curve (ANOVA). $^{**}p< 0.05$ vs acetic, benzoic and propionic acids using the comparison of the area under the curve (ANOVA). $^{\#}p< 0.05$ vs all other acids (ANOVA). $^{\#\#}p< 0.05$ vs acetic, benzoic and propionic acids (ANOVA).

bread contained at least one chemical antimicrobial with PA as the predominant antimicrobial (92.5%). PA has been routinely used to delay bread mould and prolong shelf life in the bread baking industry. However it did raise elevated exposure concerns when 94.9% of the Singapore residents were reported to consume an average of 130.4 gram of bread daily in the National Nutrition Survey 2010.[25] The same survey also reported that the daily bread intake increased with the age groups of consumers. Older adults (aged 50 years and above) were found to consume more bread (227.7-243.3 gram daily) than their younger counterparts (118.3-157.0 gram daily),[25] and this trend may be due to the convenience of bread intake compared with alternative dietary carbohydrate sources.

The continuous intake of PA in the bread may pose significant health concerns, especially for older adults, although there is currently an absence of scientific evidence to limit the addition of these molecules. Irritability, restlessness, inattention and sleep disturbance were increased in a double-blind placebo-controlled study which fed twenty seven children with four slices of PA-added (2%) bread daily.[26] Similar symptoms may surface with adults. More studies should be carried out to discern the possible health risk to PA overexposure.

The prevalence of BA added into energy drinks, soft drinks and fruit cordials poses another concern when these beverages are popular among younger consumers. 73.5% of the surveyed Singaporean residents consumed sweetened drinks (which includes the BA-containing energy drinks, soft drinks, and fruit cordials) one or more times, with 15.6% consuming more than seven times per week.[25] After stratifying to the different age groups, 75.7% of young adults (age 18-29 years) and 68.8% of adults (age 29-39 years) ingested sweetened drinks twice or more weekly.[25] While the nutrition community focuses its attention on the sugar intake from these beverages, the exposure to BA and other food additives, such as artificial colourings, should also be taken into consideration. There are, until now, limited data on the effects of benzoic acid on inflammation, as more studies are required to ascertain its *in vivo* effects.

Our study is limited to the estimation of the occurrence of chemical antimicrobials added into the processed foods and beverages available to Singaporean consumers, which serves as an indication of the potential exposure of these molecules to consumers. We did not assess the actual exposure of the Singaporean consumers to these molecules, though our results can be indirectly translated as such, since the foods and beverages on retailing shelves might reflect the consumers' buying preferences. The study also did not measure the actual amount of the antimicrobials in the food and beverage products, as their measurement was not the focus of the study. It becomes important to study the occurrence of these antimicrobial additives in our food supply because of the following elements: (1) high intakes of processed foods and beverages were estimated in developed city-states, like Singapore; (2) there is a long list of food groups in which these additives are authorised; (3) feasibility to analyse; (4) they are important in bread and soft drinks for which consumption data are high and increasing; (5) these carboxylic acids are much less reactive than sulphites or nitrites resulting in residual levels closer to usage level. The human plasma concentrations of benzoic and propionic acids had been reported to be 14[27] and 76[28] $\mu g\ L^{-1}$, respectively. There is limited data on the physiological concentrations of acetic, benzoic, lactic, propionic and sorbic acids in humans. For the purpose of mimicking the *in vivo* conditions, the *in vitro* experiments were carried out using a range of treatment concentrations between 0 and 200 $\mu g\ L^{-1}$, within which the stated physiological concentrations have been previously reported.

Chemical antimicrobials were added to 23.2% of the processed foods and beverages sold in Singapore, of which SA, LA, PA, AC, and BA being the most common present. These molecules, at physiologically relevant concentrations, exert differential effects on the MPO activity, LTB_4 and F_2-isoprostanes production in human neutrophils. More relevant *in vitro* and *in vivo* studies should be carried out to elucidate the *in vivo* biological effects of these molecules to better infer or vindicate their potential health detrimental effects.

ACKNOWLEDGEMENTS
We thank Prime Supermarket (Singapore) for their assistance in the collection of the food ingredients data.

AUTHOR DISCLOSURES
The authors have no conflict of interest to declare. This work was supported by the Nanyang Polytechnic Student Final Year Project fund.

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