ผลของดีคาร์บอกซีเลชั่นของกรดอะมิโนร่วมกันในการปรับปรุงอาหารไฮโดรเจนซัลไฟด์สำหรับการตรวจวิเคราะห์ซัลโมเนลลากลุ่มที่สามารถรีดิวซ์ไทโอซัลเฟตเบื้องต้น

COMBINED EFFECT OF AMINO ACID DECARBOXYLATION ON THE IMPROVEMENT OF HYDROGEN SULFIDE ENRICHMENT MEDIA FOR PRESUMPTIVE SCREENING OF THIOSULFATE – REDUCING SALMONELLA

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บทคัดย่อ

โรงงานอุตสาหกรรมอาหารต้องการวิธีการวิเคราะห์ที่รวดเร็วและเชื่อถือได้ สามารถทำได้สะดวกและค่าใช้จ่ายไม่สูงเพื่อใช้ในการตรวจสอบการปนเปื้อนของ *Salmonella* spp. เบื้องต้น งานวิจัยนี้จึงได้พัฒนาอาหารบ่งชี้การเกิดไฮโดรเจนซัลไฟด์ (H_2S) พร้อมวิธีทดสอบในเพลทระดับไมโครเวล สำหรับการตรวจหาซัลโมเนลลาเบื้องต้นอย่างรวดเร็ว

งานวิจัยนี้ได้ศึกษาผลของการใช้ดีคาร์บอกซีเลชั่นของกรดอะมิโนร่วมกันต่อการเพิ่มขึ้นของการเกิดตะกอนสีดำในรูปแบบอาหารเหลวบ่งชี้ก๊าซ H_2S (หรือ TFX) ร่วมกับไลซีน-ออร์นิทีน (TFXLO) ออร์นิทีน-อาร์จินิน (TFXOA) หรือไลซีน-อาร์จินีน (TFXLA) เพื่อปรับปรุงพัฒนาการเกิดไฮโดรเจนซัลไฟด์และการเกิดตะกอนสีดำที่เด่นชัด โดยทดสอบกับซัลโมเนลลาที่สามารถเกิดปฏิกิริยา H_2S^+ จำนวน 7 สายพันธุ์ และซัลโมเนลลาที่ไม่สามารถเกิดปฏิกิริยา H_2S^+ ได้ รวมถึงเชื้อที่ไม่ใช่ซัลโมเนลลาจำนวนรวม 13 สายพันธุ์ อาหารเหลว TFX

ที่ใส่ไลซีน (TFXL) เป็นอาหารควบคุม ปฏิกิริยาการเกิดตะกอนสีดำเป็นอินดิเคเตอร์สามารถตรวจติดตามจากการวัดค่าการดูดกลืนแสง (OD_{650}) ที่เปลี่ยนแปลงไปและด้วยตาเปล่า

ผลการวิจัยพบว่า ส่วนเสริมที่ใส่ลงไปร่วมกับกรดอะมิโนให้ผลการตกตะกอนของเฟอรัสซัลไฟด์ที่แตกต่างกัน และให้ค่าการดูดกลืนแสง OD_{650} ขึ้นอยู่กับซีโรวาร์ของซัลโมเนลลา การเกิดตะกอนสีดำของซัลโมเนลลามีความเข้มและคมชัดมากขึ้นเมื่อมีการปรับปรุงสูตรกรดอะมิโน โดยเฉพาะอาหารเหลวที่มีการใช้ออร์นีทีน-อาร์จินีนร่วมกัน ซึ่งจะให้ค่าการดูดกลืนแสงที่ 650 นาโนเมตร (OD_{650} = 1.7 – 2.5) เปรียบเทียบกับการใช้ไลซีนเพียงตัวเดียว (OD_{650} = 1.4 – 2.1) ปกติแล้วระบบไทโอซัลเฟต-เฟอร์ริกแอมโมเนียมซิเตรทนี้มักพบการเกิดตะกอนสีดำในซัลโมเนลลากลุ่มพิเศษ (*Salmonella* Anatum และ *Salmonella* Typhi) ค่อนข้างน้อย แต่เมื่อใช้ไลซีน-อาร์จินีนร่วมกัน มีผลช่วยเพิ่มความเข้มของอาหารเหลวไฮโดรเจนซัลไฟด์ จากที่ให้ค่า OD_{650} ที่ต่ำที่ 0.9 เพิ่มเป็น 1.6 ใน *S.* Anatum ได้ อย่างไรก็ตาม สำหรับ *S.* Typhi ยังไม่มีการใช้กรดอะมิโนร่วมกันสูตรใดที่สามารถปรับปรุงการเกิดไฮโดรเจนซัลไฟด์ได้ อาหารเหลวบ่งชี้ไฮโดรเจนซัลไฟด์ทุกสูตรสามารถแยกซัลโมเนลลาที่ไม่สามารถเกิดไทโอซัลเฟตรีดิวซ์ซึ่งออกจากซัลโมเนลลาสายพันธุ์ปกติได้ แต่ยังไม่สามารถแยกเชื้อแข่งขันของซัลโมเนลลาได้ ยกเว้น *Citrobacter freundii* สามารถแยกได้ในอาหารเหลวสูตร TFXL

โดยสรุปการใช้ออร์นิทีนและอาร์จินินเป็นสารอาหารพื้นฐานนั้น สามารถปรับปรุงระบบการเกิดปฏิกริยาไฮโดรเจนซัลไฟด์ให้ชัดเจนขึ้น อาหารเหลวบ่งชี้การเกิดปฏิกิริยาการเกิดไฮโดรเจนซัลไฟด์นั้นนับเป็นอาหารเหลวทางเลือกโดยเวลาที่ใช้ในการตรวจวิเคราะห์ทั้งหมดสำหรับผลการตรวจวิเคราะห์เบื้องต้นสั้นลงและรวดเร็วกว่าวิธีวิเคราะห์แบบทั่วไปที่ใช้เวลาถึง 48 ชั่วโมง

คำสำคัญ: ดีคาร์บอกซีเลชั่นของกรดอะมิโน การสร้างไฮโดรเจนซัลไฟด์ วิธีการไมโครเพลท การตรวจหาซัลโมเนลลาเบื้องต้น

Abstract

The food industry needs rapid and reliable methods that are convenient and cost-effective as primary screens for routine inspections of *Salmonella* spp. contamination. To fulfil *Salmonella* screening step, hydrogen sulfide (H_2S) indicator medium was proposed and tested in a microwell plate.

Method: The combined effect of amino acid decarboxylation on the increase of black precipitation, H_2S indicator broth (TFX) plus with lysine-ornithine (TFXLO), ornithine-arginine (TFXOA), or lysine-arginine (TFXLA) were studied in seven H_2S^+ *Salmonella* serovars and thirteen non-salmonellae *Salmonella* and non-salmonellae to improve hydrogen sulfide production and black precipitation contrast. The TFX broth containing lysine (TFXL) was compared as control. The black precipitates, as indicator of the reaction were followed by optical density (OD_{650}) changes and visualization.

Result: Different supplement with combined amino acids provided different ferrous sulfide precipitates and OD_{650} signals depending on *Salmonella* serovars. The black precipitates of all typical *Salmonella* had improved with more contrast in all combined amino acids, especially ornithine-arginine based broth (OD_{650}=1.7–2.5), compared to single lysine usage (OD_{650}=1.4–2.1). Thiosulfate-ferric ammonium citrate system commonly provided ineffective results for black precipitates in atypical *Salmonella* (*Salmonella* Anatum and *Salmonella* Typhi). The lysine-arginine combined effect could fix the low H_2S contrast OD_{650} from 0.9 to 1.6 in *S.* Anatum. However, none of combined amino acids was able to improve hydrogen sulfide production in atypical *S.* Typhi. All H_2S enrichment media could differentiate all non-thiosulfate reducing salmonellae competitors tested out of *Salmonella*. There was no differentiation of three H_2S^+ *Salmonella* competitors from *Salmonella* in any media, except *Citrobacter freundii* in TFXL broth.

Conclusion: The selected ornithine and arginine as the nutrient base was noted for further improvement in hydrogen sulphide reaction system. With applying H_2S indicator media alternative to conventional selective broths, the total detection time for presumptive results was shortened; more rapid than conventional protocol for up to 48 h.

Keywords: Amino acid decarboxylation, Hydrogen sulfide production, Microplate assay, *Salmonella* presumptive screening

Introduction

Salmonella, an Enterobacteriaceae, has been the leading foodborne illnesses for a century [1]. Consumption of *Salmonella*-contaminated food can cause the gastrointestinal illness, salmonellosis, producing sporadic food-borne outbreaks worldwide [2-5]. Although the most common sources are food products from animals that normally harbor *Salmonella*, many other types of food and beverage can carry *Salmonella* linked to foodborne outbreaks. Ready-to-eat foods are also considered to be serious sources of *Salmonella* contamination [1, 6]. Of several major foodborne pathogens, *Salmonella* has the most critical impact on public health and economic cost [4, 7]. Accordingly, there have been several attempts to improve and develop more efficient, rapid, convenient and reliable *Salmonella* detection methods during past decades [3, 8]. The interest points to the development of biochemical indicator medium, implementation of the miniaturization concept, as well as optical sensor [9-12] to presumptively screen for *Salmonella* contamination in food samples. Among *Salmonella* metabolic activities, reduction of thiosulfate to hydrogen sulfide has been widely used for identification and differentiation of *Salmonella* spp. from other species of Enterobacteriaceae in various conventional selective agars (e.g., xylose lysine decarboxylase agar) [13-15]. This reaction has been coupled with lysine decarboxylation to reduce the strong acid conditions. Strong acids produced by carbohydrate fermentation inhibit hydrogen sulfide production and ferrous sulfide precipitation resulting in low contrast of H_2S^+ black precipitates [15-16]. Based on this concept, the developed H_2S indicator enrichment media have been successfully developed using lysine, ornithine, or arginine as acid masking preventive system from excessive acidity coupled with optical detection approach [9 - 10]. However, none has been studied the combined effect of double and triple amino acid decarboxylation on hydrogen sulfide production in *Salmonella* and their competitors.

Objectives

This research aimed to study the combined effect of double and triple amino acid decarboxylation on hydrogen sulfide production and ferrous sulfide precipitation of *Salmonella* and non-salmonella in 96-microwell cultivation.

Methods

Bacterial pure cultures and culture preparation

All bacteria were obtained from the Department of Medical Sciences Thailand (DMST, Bangkok, Thailand) and Thailand Institute of Scientific and Technological Research) TISTR, Bangkok, Thailand). The target organisms, *Salmonella* were 5 non-typhoid serovars (*Salmonella* Anatum DMST 19600; *Salmonella* Enteritidis DMST 15673; *Salmonella* Rissen DMST 17365; *Salmonella* Typhimurium TISTR 292; and *Salmonella* Weltevreden DMST 10637) and 2 typhoid and paratyphoid serovars (*Salmonella* Typhi DMST 22842; and *Salmonella* Paratyphi B DMST 28118). The Gram-negative competitive bacteria included *Citrobacter freundii* DMST 16368; *Enterobacter aerogenes* DMST 8216; *Proteus mirabilis,* TISTR 100; *Proteus vulgaris* DMST 557; *Pseudomonas aeruginosa* DMST 4739; *Shigella flexneri* DMST 4423; *Shigella sonnei* DMST 561; *Serratia marcescens* DMST 8845; and *Yersinia enterocolitica* DMST 8012. Few Gram-positive competitive bacteria, *Enterococcus faecalis* DMST4736; *Listeria innocua* DMST 9011; and *Staphylococcus aureus* TISTR 808; were also tested.

All pure cultures were sub-cultured on tryptic soy agar (TSA, Lab M, UK) and one loopful of each strain was transferred into 10 ml of tryptic soy broth (TSB, Lab M, UK) in a 10-ml glass tube and incubated under a static condition at 37±1 °C for 24 h .The 10-fold serial dilutions were then done in 0.1 %w/v peptone water (PW, Difco Laboratories, Sparks, MD) to obtain the desired concentration.

Media

The behaviour of the organisms was studied in the following liquid media (pH 7.0±0.1) : (1) TFX, adapted from Xylose Lysine Decarboxylase (XLD agar), containing (g/L :soytone (USbiological, Salem, MA), 4.5; xylose (Acros organics, Fair Lawn, N), 1; ferric ammonium citrate (Fisher Scientific, Fair Lawn, NJ), 0.5; sodium thiosulfate, 0.1. The TFX broth was further added with lysine, lysine-ornithine, ornithine-arginine, or lysine-arginine, and lysine-arginine-ornithine (5 g of each amino acid type/L), to derive TFXLO, TFXOA, TFXLA, and TFXLOA broth. L-lysine, L-ornithine, and L-arginine used were from USbiological, Salem, MA. All prepared media was mixed and dissolved with mild heating and cooled to 25 ºC before pH adjustment to initial pH 7.0±0.1_by HCl (QRëC®, Malaysia) 1 N and NaOH (Carlo Erba, France) 1 N and then sterilized by filtration through a sterile nylon syringe filter membrane (13 mm diameter, 0.45 µm pore size, Filtrex, Thailand) before using.

Effect of double and triple amino acid decarboxylation on hydrogen sulfide production in TFX media

To study the effect of amino acid on the increase of black precipitation, each well of the 96-microwell plates was filled with 180 µL of TFXL, TFXLO, TFXOA, TFXLA, and TFXLOA media. Then each pure culture of salmonellae and non-salmonellae (20 µL) was individually inoculated into test media. The microplates were incubated under stationary condition at 37±1 ºC for 24 h. The optical density (OD) at 650 nm, the optimum wavelength derived from previous study [11], was measured during incubation time using media without inoculum as blank .The control medium was base broth without adding amino acid, TFX.

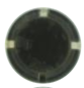
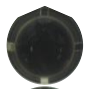
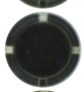
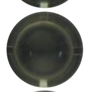
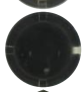
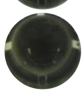
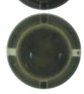
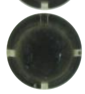
Results

The combined effect of lysine, ornithine, or arginine decarboxylation on H_2S production in thiosulfate-reducing *Salmonella* and non-salmonellae

As a consequence of single amino- acid decarboxylation, ornithine produced heavy black precipitates of FeS in most usual *Salmonella,* but modest in *S.* Paratyphi B, and very little in *S.* Anatum and *S.* Typhi. Arginine was great for *S.* Anatum, but worse in the others. Therefore, we assumed that mixing amino acids might achieve the highest H_2S production and black precipitation of iron sulfide. From results of single amino acids, the combination of ornithine and arginine was suggested. However, we further studied double and triple combinations of all three amino acids in the following set of experiments.

The basal medium broth, TFX, was prepared and then added with (1) lysine (L) and ornithine (O), (2) ornithine (O) and arginine (A), (3) lysine (L) and arginine (A), (4) lysine (L), ornithine (O) and arginine (A), to give TFXLO, TFXOA, TFXLA, and TFXLOA broths, respectively. All thiosulfate-reducing *Salmonella* and non-salmonellae (7 log CFU/mL) were tested in the prepared media to study the effect of amino-acid decarboxylation on black precipitation while selecting *Salmonella* competitors out. The control medium for amino acids was TFX with lysine, TFXL broth.

Table 1 Hydrogen sulfide production of salmonellae (7 log CFU/mL) in thiosulfate-ferric ammonium-citrate based broths (TFX) supplemented with different amino acids; (1) lysine (L) and ornithine(O), (2) ornithine (O) and arginine (A), (3) lysine (L) and arginine (A), (4) lysine (L), ornithine (O) and arginine (A), to give TFXLO, TFXOA, TFXLA, and TFXLOA broths under aerobic cultivation at 37 ºC after 24 h. The control medium was TFX with lysine, TFXL broth.

Test strains	H_2S indicator broth				
	TFXL	TFXLO	TFXOA	TFXLA	TFXLOA
Salmonellae					
Typical strains					
S. Enteritidis					
S. Rissen					
S. Typhimurium					
S. Weltevreden					
S. Paratyphi B					

Test strains	H_2S indicator broth				
	TFXL	TFXLO	TFXOA	TFXLA	TFXLOA
Atypical strains					
S. Anatum					
S. Typhi					

Table 1 and 2 show the black precipitation resulting from *Salmonella* and non-salmonellae, respectively, in TFX broths with double and triple amino acids after 24 h incubation as evaluated by eye. The black precipitates appeared more in LO, OA, LA, and LOA than in L broths with all typical *Salmonella* and *S.* Anatum. The most optimal amino acid combination for *S.* Anatum tended to be LA. No improvement was observed in *S.* Typhi in all media. All broths with two or three amino acids support H_2S production and black precipitation of iron sulfide. Based on eye observation, it was hard to decide which amino acid combinations were most effective; therefore, we used OD_{650} measurement to quantify the black precipitation. We plotted the OD_{650} time course of each organism in each medium to determine the optimum amino acids, which provide the most black precipitation in broth media.

Table 2 Hydrogen sulfide production of non-salmonellae (7 log CFU/mL) in thiosulfate-ferric ammonium-citrate based broths (TFX) supplemented with different amino acids; (1) lysine (L) and ornithine (O), (2) ornithine (O) and arginine (A), (3) lysine (L) and arginine (A), (4) lysine (L), ornithine (O) and arginine (A), to give TFXLO, TFXOA, TFXLA, and TFXLOA broths under aerobic cultivation at 37 ºC after 24 h. The control medium was TFX with lysine, TFXL broth.

Test strains	H_2S indicator broth				
	TFXL	TFXLO	TFXOA	TFXLA	TFXLOA
Non-salmonellae, Gram-negative					
Citrobacter freundii					
Proteus mirabilis					
Proteus vulgaris					
Enterobacter aerogenes					
Escherichia coli					

Test strains	H_2S indicator broth				
	TFXL	TFXLO	TFXOA	TFXLA	TFXLOA
Non-salmonellae, Gram-negative					
Klebsiella pneumoniae					
Serratia marcescens					
Pseudomonas aeruginosa					
Shigella flexneri					
Shigella sonnei					
Yersinia enterocolitica					
Non-salmonellae, Gram-positive					
Enterobacter faecalis					
Staphylococcus aureus					
Listeria innocua					

In Figure 1, we compared the OD_{650} values at 24 h of media with single and double/triple amino acids among different *Salmonella* strains to select the most effective amino acid mixtures for H_2S production. The OD_{650} signals of typical *Salmonella* were significantly improved when changing lysine to other amino acids. The different amino acids promoting the highest OD_{650} values varied depending on the *Salmonella* serovars, for example ornithine (*S.* Enteritidis) and ornithine plus with arginine (*S.* Rissen). None provided the highest OD_{650} in all typical *Salmonella*, the most optimum formula; OA was selected since it gave OD_{650} values above 1.7.

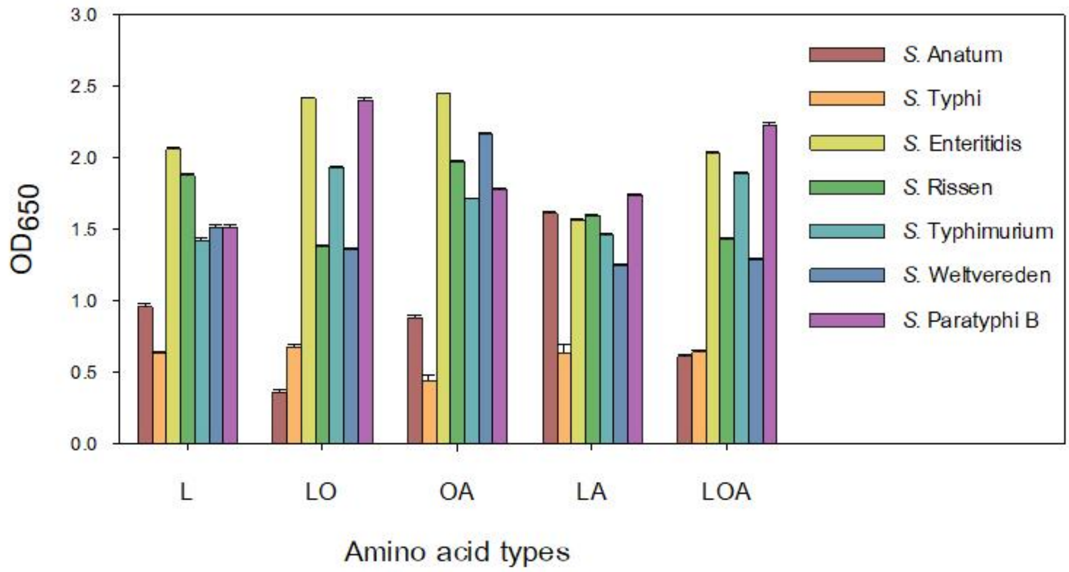


Figure 1. Optical density profiles of hydrogen sulfide broths with different amino acids measured at 650 nm after incubation at 37°C for 24 h.

The major goal for double or triple amino acid optimization was to improve the OD signal in atypical *S.* Anatum and *S.* Typhi. However, no double or triple amino acid combination increased the OD_{650} of *S.* Anatum relative to arginine alone (OD_{650} = 2.147). In addition, all amino acid mixtures resulted in less precipitates and low OD_{650} in *S.* Typhi (OD_{650} = 0.425 – 0.796). The highest OD_{650} of *S.* Typhi was from the formula supplemented with ornithine (OD_{650} = 0.796).

However, combinations of amino acids was not able to improve hydrogen sulfide production in atypical *Salmonella* serovars (*S.* Anatum and *S.* Typhi), but fortunately ornithine and arginine in combination improved the signals in typical strains compared to other amino acids tested. Consequently, the selected ornithine and arginine as the nutrient base was noted for further improvement. Since sugar (type fermentation significantly affects thiosulfate reduction and hydrogen sulfide production in bacteria [17], the effect of various fermentable sugars supplemented in TFOA broth is interesting for future research.

Conclusions and Discussion

To achieve the rapid *Salmonella* screening for practical use in food industry, the H_2S indicator enrichment media based on the *Salmonella* selective agar formula and coupled with microplate assay was proposed .The TFXL broth was improved by substituting with double and triple amino acid decarboxylation (lysine, ornithine, or arginine). Different supplement with combined amino acids provided different ferrous sulfide precipitates and OD_{650} signals depending on *Salmonella* serovars. It seemed the combination of ornithine and arginine in TFX broth (OD_{650}=1.7–2.5) was able to enhance optical density change and thiosulfate-reducing metabolism of most *Salmonella* comparing to single lysine usage (OD_{650}=1.4–2.1) and other amino acid mixtures (OD_{650}=1.4–2.4). Atypical *Salmonella* (*S.* Typhi and *S.* Anatum) gave low OD_{650}

in all media except in TFXLA that *S.* Anatum showed high black precipitations. No differentiation was observed on three common thiosulfate-reducing non-salmonellae (*Citrobacter* and *Proteus* spp.). Only *C. freundii* could be deleted out or showed negative hydrogen sulphide in TFXL media. The further study on fermentable sugar types could possibly increase the sensitivity and selectivity of this H_2S ornithine-arginine based media. The developed H_2S indicator media can be applied for rapid *Salmonella* presumptive screening, alternative to conventional selective enrichment broth with given presumptive result with 48 h from pre-enrichment step (conventional method; 24 h for pre-enrichment and 24 h for selective enrichment followed by agar plating with additional 24 – 48 h for those result).

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References

[1] Alakomi, H.L., and Saarela, M. (2009). *Salmonella* Importance and Current Status of Detection and Surveillance Methods. *Quality Assurance and Safety of Crops and Foods*. *1*(3), 142-152.

[2] Torlak, E.; Akan, I.M., and Inal, M. (2012). Evaluation of RapidChek Select for the Screening of *Salmonella* in Meat and Meat Products. *Journal of Microbiological Methods*. *90*(3), 217-219.

[3] Lee, K.M.; Runyon, M.; Herrman, T.J.; Phillips, R., and Hsieh, J. (2015). Review of *Salmonella* Detection and Identification Methods: Aspects of Rapid Emergency Response and Food Safety. *Food Control*. *47*, 264-276.

[4] CDC. (2014). Bad bug book – Aflatoxins. Retrieved September 20, 2019, from www.cdc.gov/foodborneburden/2011-foodborne-estimates.html#annual

[5] Borowsky, L.M.; Schmidt, V., and Cardoso, M. (2007). Estimation of Most Probable Number of *Salmonella* in Minced Pork Samples. *Brazilian Journal of Microbiology*. *38*, 544-546.

[6] Forshell, L.P., and Wierup, M. (2006). *Salmonella* Contamination: a Significant Challenge to the Global Marketing of Animal Food Products. *Revue Scientifique Et Technique*. *25*(2), 541-554.

[7] Hoffmann, S., and Anekwe, T.D. (2013). Making Sense of Recent Cost-of-Foodborne-Illness Estimates. *Economic Information Bulletin*. *2013*(118), 1-13. Retrieved September 20, 2019, from https://www.ers.usda.gov/webdocs/publications/43796/40344_eib118.pdf?v=0

[8] Tietjen, M., and Fung, D.Y.C. (1995). Salmonellae and Food Safety. *Critical Reviews in Microbiology*. *21*(1), 53-83.

[9] Khueankhancharoen, J.; Saranak, J., and Thipayarat, A. (2017). Optimization of Amino Acid Decarboxylation and Sugar Fermentation to Enhance Hydrogen Sulfide Production for Rapid Screening of *Salmonella* during Selective Enrichment. In Proceedings of The 13rd Asian Congress on Biotechnology 2017 (ACB 2017), pp 120-1 - 120-12. July 23-27, 2017, Khon Kaen, Thailand.

[10] Shelef, L.A., and Tan, W. (1998). Automated Detection of Hydrogen Sulfide Release from Thiosulfate by *Salmonella* spp. *Journal of Food Protection*. *61*(5), 620-622.

[11] Khueankhancharoen, J.; Thipayarat, A., and Saranak, J. (2016). Optimized Microscale Detection of Amino Acid Decarboxylase for Rapid Screening of *Salmonella* in the Selective Enrichment Step. *Food Control*. *69*, 352-367.

[12] Shelef, L.A.; Surtani, A.; Kanagapandian, K., and Tan, W. (1998). Automated Detection of Amino Acid Decarboxylation in Salmonellae and other Enterobacteriaceae. *Food Microbiology*. *15*, 199-205.

[13] ISO. (2002). Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Detection of *Salmonella* spp. *International Organization for Standardization*. Geneva.

[14] Barrow, G.I., and Feltham, R.K.A. (1993). Cowan and Steel's Manual for the Identification of Medical Bacteria (3rd ed.): Cambridge University Press.

[15] Barrett, E.L., and Clark, M.A. (1987). Tetrathionate Reduction and Production of Hydrogen Sulfide from Thiosulfate. *Microbiological Reviews. 51*(2), 192-205.

[16] Bulmash, J.M., and Fulton, M. (1964). Discrepant Tests for Hydrogen Sulfide. *Journal of Bacteriology*. *88*(6), 1813.

[17] Park, S.H.; Ryu, S., and Kang, D.H. (2012). Development of an Improved Selective and Differential Medium for Isolation of *Salmonella* spp. *Journal of Clinical Microbiology*. *50*(10), 3222-3226.