Taurine in Fresh Seafishes and Processed Seafishes

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ABSTRACT

The aim of this work was to study the effects of processed on taurine content in fishes samples from Gulf of Thailand. Taurine in samples were qualitative tested by thin layer chromatography (TLC) and quantitatively analysed by Ultraviolet –Visible Spectrophotometry technique. The method in the analysis was also validated. The results revealed that the rapid qualitative test by TLC showed all fresh fish samples contain taurine in their.The quantitative analysis of taurine was performed with high sensitivity and selectivity with LOD and LOQ as 6.27 µM amd 189.99 µM,respectively. Taurine in Russel's snapper contained the highest taurine level approximately as 5548.68 ± 7.78 mg/100g. Processed method such as frying , grilling and steaming effect on taurine content in fish samples.The experiment showed the effect on taurine content depend on processing method.

Keywords: 2-aminoethanesulfonic acid , Seafishes, Processed Seafishes, Taurine

INTRODUCTION

Taurine is a derivative of cysteine amino acid , its structural formula containing sulfhydryl group or sulfonate group[1], a basic amino group and two carbon in between. Its IUPAC name is known as 2–amino- ethanesulfonic acid.[2]Taurine exits naturally in animals including mammals ,birds, fish,oysters and mussels [3] However, taurine still also found in general plants but in lower level. An algae , fungi and other terrestrial plants contains the rich amount of taurine.[4]In mammals taurine involve in a particularly wide variety functions as biological roles in human body such as antioxidant[5,6], osmoregulation[7], membrane stabilization[8], cardiovascular function, retinal function, antiatherogenic for reduce triglyceride levels, antihypertensive in hypothalamus, function on central nervous system[9]and especially essential for support skeleton muscle.[10,11] Taurine has been shown to be an essential dietary requirement for cats.[12]There have several biosynthesis pathways study[13],an interesting pathway concern the cystein changing to hypotaurine and taurine in mammal system.[14,15] Nowaday, taurine from natural source and synthetic method were produced for sold in the world[16] and also serving in food industry, beverage industry such as energy drink and pharmaceutical applications as electrolyte products.[17,18] An important of taurine for human and animal function on complete biological activity as above details,so the aim of this work was to determine taurine content in Thai fresh seafishes and processed from seafishes for estimate the effect of processing method of seafish food.The qualitative test of taurine also studied. Since there have many methods to detect Taurine in sample such as popular method as high performance chromatography[19], Ion chromatography and Spectro- photometry[20]. In this work a simple method as UV-VIS Spectrophotometry was used in this analysis, the color complex from reaction between Taurine, phenol and sodium hypochlorite were performed and recorded an absorbance for calculation. The method was applied to analysis taurine content in all seafishes samples.

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MATERIALS AND METHODS

Chemicals and Apparatus

Taurine standard (2-aminoethanesulfonic acid)(AR grade) was purchased from Fluka. *0*-Phthaldialdehyde (OPA) (AR grade). Phenol, sodium hypochlorite solution (10–15 %), sodium bromide, sodium phosphate tribasic dodecahydrate, sodium hydroxide, hydrochloric acid was purchased from Sigma Aldrich Chemical Corp. (St. Louis, MO, USA). Propanol, glacial acetic acid, ethanol, methanol were purchased from Carlo Erbra. Trichloroacetic acid, Ninhydrin solution was purchased from BDH. Ready coated silica plate was from Na cherey–Nagel, Sil-G/UV254, microcap (Vertical).

Fish samples

Fresh fish samples: Fish samples such as Silver tiger fish (*Datniodes polota*), Target fish (*Terapon jarbua*) ,Whitespotted spinefoot (*Siganus canaliculstus*), Russell's snapper (*Lutinus russelli*) , Yellow stripe scad (*Selaroides leptolepsis*) ,Silver sillago (*Sillago sihama*) , Giant seaperch (*Lates calcarifer*), Silver pomfret (*Pampus argenteus*),Sharptooth snapper(*Pristipomoides typus*)

And Longtail tuna(*Thunnus tonggol*). All fresh fish samples were purchased from Ban Pae fresh market, Rayong Province.The fishermen from this area got all fresh fish from east seashore, area Gulf of Thailand.

Processed Fish samples: All fresh fishes were cooked as normally for food by frying in vegetable oil, grilling and steaming.

Preparation of sample for taurine analysis

Fresh samples and processed samples were chopped and weighed approximately as 5.xxxx g and mixed with 10 ml of 5% trichloroacetic acid solution. Then the mixtures were heated at 60^{o}C for 5 mins. and mixed again with 10 ml of 5 % sodium carbonate solution. After centrifugation at 2000 rpm, the samples solutions were filtered through filter paper (whatman no.1) and collected filtrates and stored in the freezer cabin of refrigerator until analysis.

Qualitative Analysis

The standard taurine solution was prepared as 10 ppm and spot on ready coated silica plate using standard microcapillary (microcap).The standard solution of cystein and methionine were also spot in the same plate for compare the R_f Value. TheTLC plate was placed into chromatographic tank that contain the mobile phase solvents.In this work the 3 solvent systems as following:

(modified from : Moua etal.,2002[21]) were studied.

system 1: butanol : glacial acetic acid: ethanol : water (4:2:3:3)

system 2 :propanol: water : glacial acetic acid: ethanol (5.2: 2 :2 : 0.8)

system 3: butanol : glacial acetic acid: water (4: 1 : 1)

The spot on each chromatogram were detected by spraying with ninhydrin reagent. The extracted solution from samples were also spot on the same Plate, compare with taurine standard for qualitative check before analysis.

Quantitative analysis

Part 1. Preparation of standard calibration curve.

The method was modified from Nejdi.,et al.,(2013). The standard taurine solution was prepared as series concentration in the range of 10 mM – 100 mM. Then each standard solution was pipetted 1 ml into micro test tube, mixed with 1 ml of 20 mM phosphate buffer (pH 10) , 1 ml of 200 mM phenol solution and 1 ml of 1000 mM sodium hypochlorite solution. Each tube was ready mixed again by Vortex mixture and left at room temperature for 10 mins. before heated at 60^{o}C for 30 mins.The blue complexed solutions were measured an absorbance at 630 nm. The standard calibration curve of taurine was plotted between standard taurine concentration and absorbance value.

Part2.Method of validation

The method for analysis taurine by spectrophotometry was validated by analysis of limit of detection (LOD) and limit of quantitation (LOQ) The calculation LOD , LOQ using the following equation as

$$LOD = 3SD/mean \quad \text{…..…….1)}$$

$$LOQ = 10SD/mean \quad \text{……2)}$$

Part 3. Analysis of taurine in samples.

The filtrate of fresh fish and processed fish samples were treated as taurine standard solution (as part 1). The blue complex of samples were recorded an absorbance and compared with the standard calibration curve in part 1 to calculated taurine content in samples.

Statistical analysis

All determinations were carried out at least five replicates, except in validation method the ten replications were applied and values were averaged. For all statistics, ANOVA and Microsoft Excel were used for calculate and graph presentation in this work.

RESULTS AND DISCUSSION

Qualitative analysis

After study the mobile solvent systems for three systems as system 1 – system 3, the result showed as in Fig 1.

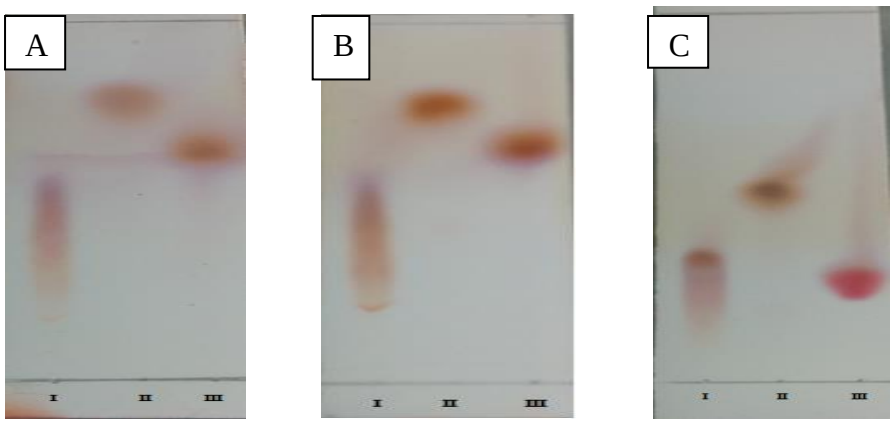

Figure 1. Chromatogram of standard taurine, cystein and methionine

Note: A = chromatogram of taurine from solvent 1 B = chromatogram of taurine from solvent 2 C = chromatogram of taurine from solvent 3

I = cystein, II = methionine and III = taurine

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The result from Thin layer chromatography for study the optimum mobile phase solvent systems for separation of taurine from an importance amino acid such as cysteine and methionine showed that the mobile phase system of propanol: water : glacial acetic acid: ethanol (5.2: 2 :2 : 0.8) should be the optimum mobile solvent systems for separate the amino acid as cysteine which was an importance precursor of taurine and methionine as the structural containing sulfur containing amino acid. The extracted sample from samples were tested by TLC and using the mobile phase system as propanol: water : glacial acetic acid: ethanol (5.2: 2 :2 : 0.8) for develop chromatogram and the example of TLC chromatogram showed in Fig2 .

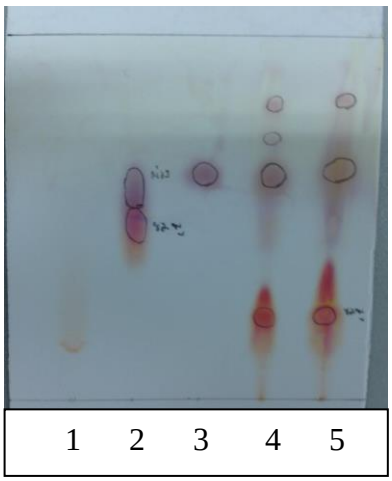

Figure 2. Chromatogram of standard taurine and fish sample develop in propanol: water : glacial acetic acid: ethanol = 5.2: 2 :2 : 0.8

Note : 1 = cystein , 2 = methionine , 3 = taurine 4 , 5 = sample

The TLC chromatogram of sample from fish samples showed taurine spot , this presented that sample contained taurine in it. However, all samples were qualitative tested as the same method to check the existence of taurine before further quantitative analysis of its content in samples.

Quantitative analysis

The preparation of standard calibration curve showed the good linearity (Figure 3) with the regression equation as y = 0.0933x , correlation coefficient as 0.9999.

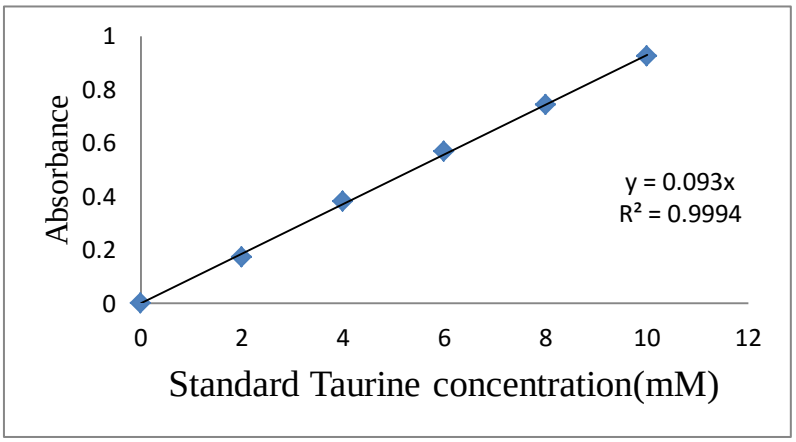

Figure 3. Standard calibration curve of standard Taurine

The method for analysis taurine by UV-VIS spectrophotometry was validated and revealed the limit of detection (LOD) and limit of quantitation(LOQ) as 6.27 µM and 189.9 µM respectively. This method showed high sensitivity with good selectivity for determination of taurine content in sample.

After the fish samples were prepared to sample solutions and followed the qualitative checked as part of qualitative analysis. The all samples showed that all samples contained taurine, so the samples were analysed taurine content. The quantitative analysis followed as in quantitative analysis step and the absorbance of colored complex reactions were recorded. Taurine content in those samples were calculated by regression equation from the standard calibration curve(Figure 3). The results of fresh seafishes showed in graph (Figure 4). All fresh seafishes contained taurine in the range of 304.14 ± 5.66 – 5548.68 ± 4.23 mg/100g,the fresh fish sample F4 name as Russell's snapper ,showed the highest taurine content.

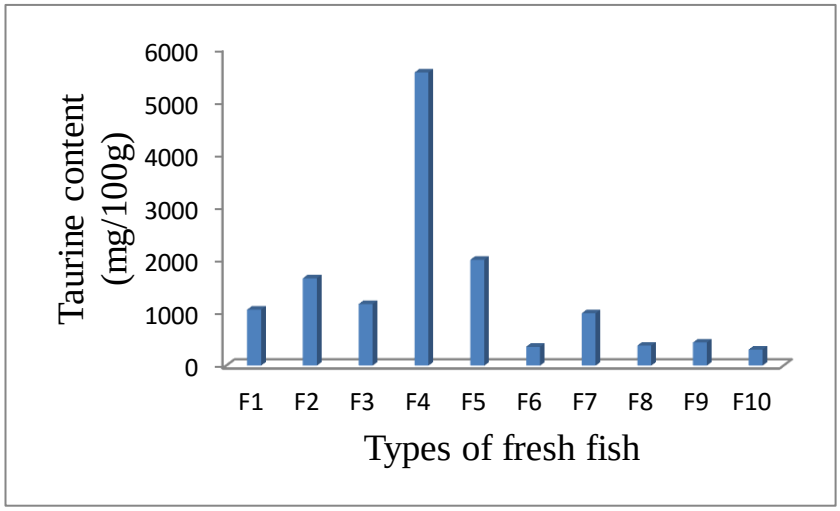

Figure 4. Taurine content in fresh fish samples

Note:

F1 = Silver tiger fish (*Datniodes polota*)

F2 = Target fish (*Terapon jarbua*)

F3 = Whitespotted spinefoot (*Siganus canaliculstus*)

F4 = Russell's snapper (*Lutinus russelli*)

F5 = Yellow stripe scad (*Selaroides leptolepsis*)

F6 = Silver sillago (*Sillago sihama*)

F7 = Giant seaperch (*Lates calcarifer*)

F8 = Silver pomfret (*Pampus argenteus*)

F9 = Sharptooth snapper(*Pristipomoides typus*)

F10= Longtail tuna(*Thunnus tonggol*)

All processed seafish samples showed taurine content as in fig 5.

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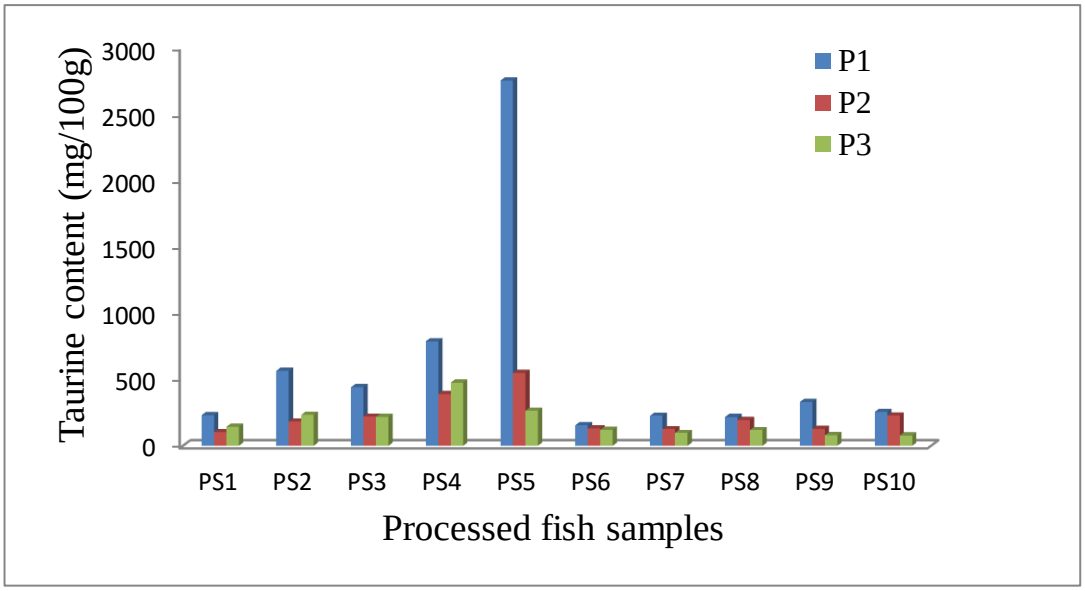

Figure 5 Taurine content in processed fish samples

Note :

PS1 = Processed fish of Silver tiger fish

PS2 = Processed fish of Target fish

PS3 = Processed fish of Whitespotted spinefoot

PS4 = Processed fish of Russell's snapper

PS5 = Processed fish of Yellow stripe scad

PS6 = Processed fish of Silver sillago

PS7 = Processed fish of Giant seaperch

PS8 = Processed fish of Silver pomfret

PS9 = Processed fish of Sharptooth snapper

PS10= Processed fish of Longtail tuna

P1 = method of cooking as frying

P2 = method of cooking as grilling

P3 = method of cooking as steaming

The result of taurine content in each sample showed the same trend and depend on method of cooking process.

This work showed the method to analysis taurine by qualitative test as screening test.The result from the referred part could rapidly checked the presentation of taurine in sample by thin layer chromatography, which using 3 mobile phase systems. The developing mobile phase system as propanol: water : glacial acetic acid: ethanol in the ratio of 5.2: 2 :2 : 0.8 was the optimum mobile solvent systems for separation ,because that system could separated the amino acid in sample as cysteine which was an importance precursor of taurine. However, the methionine amino acid was also completely separated from taurine too. After, the quantitative analysis of taurine by the ultraviolet visible spectrometry , taurine could reacted with phenol solution and sodium hypochlorite as the scheme in figure 6.

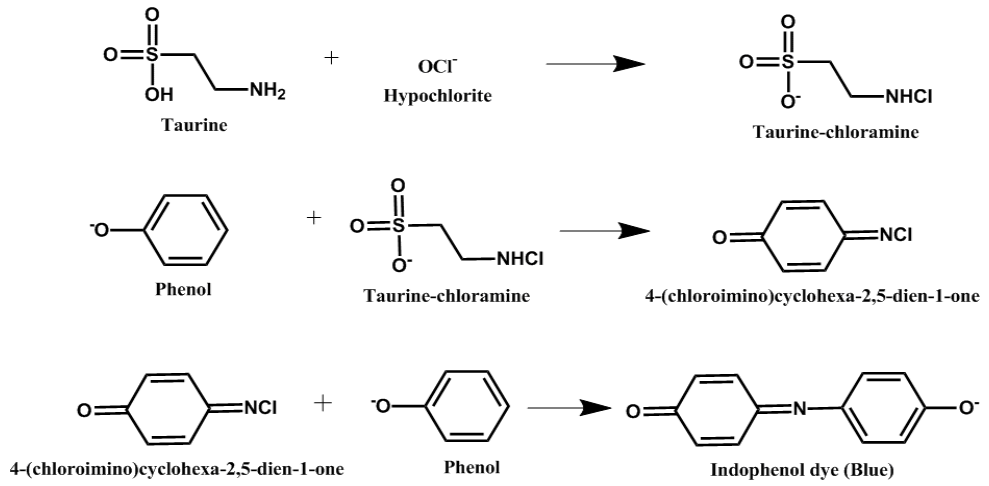

Figure6. Reaction between taurine , hypochlorite and phenol (modified from [20])

The indo- phenol blue complex was the products from the above reaction.It showed the specific reaction, this support the experimental of Nejdl. et al.,(2013). However, this work also studied the validation of the method such as LOD and LOQ.The LOD and LOQ from this modification method of taurine analysis gave the better value as 6.27 and 189.99µM. After this method was used to analyse the quantity of taurine in all seafish samples as in figure 4 ,the taurine in F4 sample showed the highest value. The F4 is Russell's snapper (*Lutinus russelli*) , it is a local seafish found in gulf of Thailand and popular as food for local people since its cheaper prices than many types of seafish such as F7 Giant seaperch (*Lates calcarifer*) ,F8 Silver pomfret (*Pampus argenteus*) ,F9 Sharptooth snapper(*Pristipomoides typus*) and F10 Longtail tuna(*Thunnus tonggol*).However, there has no report about taurine in these seafish samples ,but this result gave a good data for select type of fish for food and also showed the new knowledge that some fish which was not popular in the sold market as Mackerel also contains high taurine level[3].Taurine was claimed in Mackerel about 9.295 g/ kg or approx. 929.5 mg/100g[22].

The taurine in processed seafishes by normally cooking as grilling , frying and steaming also showed the different taurine value. The method to cook seafish samples effect on taurine content in sample , by the way cooking by frying method could save taurine in sample better than the others methods.The result from figure 5, all seafish samples that cook by frying method showed the same trend to maintain taurine content in their samples more than grilling and steaming methods.This result support the work from Spitze et al.,(2003)[23] and Hickman.M.A.et al.,(1990)[24],that referred the influence of cooking method on taurine content.This process relate to the nature of taurine that high soluble in water[25,26],so it can easily leak through water when food was placed into water medium.The cooking method that minimize taurine loss should not boiling or steaming in water.However,the result in taurine analysis in this work strongly support the usefulness of eating seafood which human body will also got an importance compound as taurine not only got the benefit from fatty acid[3].

CONCLUSIONS

This work presented the simple method as spectrophotometry technique in taurine analysis by reaction between taurine and hypochlorite reagent to form taurine chloramines followed by phenol reagent that change to indigo complex solution. This high sensitivity and selectivity method was applied to quantify taurine content in all fresh fishes and processed fishes. The samples were screening tested by thin layer chromatography for possible check the present of taurine in sample before analysis theirs content in sample.After the screening

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test , all fresh fishes samples approximately 10 types showed the taurine in them, and taurine content contained in Russell's snapper fish in the highest value.This fish was cheap but contains high taurine content,that is interesting for people to eat it. The processed of cooking as frying, grilling and steaming effect on taurine content. The way of cooking also showed the more loss of taurine content when the fish sample more contact to water such as steaming. From the all results proved taurine could be found in sea fish samples especially in fresh fish , the cooking method used to processed sea fish to food effect on taurine content.

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REFERENCES

[1]. Bittner, S., et al. (2005). Γ-L-glutamyltaurine. *Amino Acids, 28* (4): 343–356.

[2]. Carey, F.A. (2006). *Organic Chemistry* (6th ed.). New York: McGraw Hill.

[3]. Elizabeth K.L. (2013). Health benefits of seafood; Is it just the fatty acids? *Food Chemistry,140*(3): 413-420.

[4]. Brosnan, J., & Brosnan, M. (2006). The sulfur-containing amino acids: an overview *Journal of Nutrition.136* (6Suppl) :1636S–40S.

[5]. Huxtable, R.J. (1992). Physiological actions of taurine. *Physiological Review, 72* (1): 101–163.

[6]. Gürer, H., et al. (2001). Antioxidant effect of taurine against lead-induced oxidative stress. *Archives of Environmental Contamination and Toxicology,41* (4): 397–402.

[7]. Morales. H.P., & Schousboe.A,(1997). Role of taurine in osmoregulation in brain cells: Mechanisms and functional implications, *Amino Acids, 12*(3-4): 281-292.

[8]. Birdsall, T.C.,(1998). Therapeutic applications of taurine, *Alternative Medicine Review , 3* (2): 128-136.

[9]. Yang, H.J., et al. (2013). Dietary taurine can improve the hypoxia-tolerance but not the growth performance in juvenile grass carp Ctenopharyngodon idellus. *Fish Physiology Biochemistry*. *39* (5): 1071–1078.

[10]. Bouckenooghe, T., et al. (2006). Is taurine a functional nutrient?.*Current Opinion in Clinical Nutrition and Metabolic Care . 9* (6): 728–33.

[11]. Huxtable, R.J. (2000). Expanding the circle 1975–1999: Sulfur biochemistry and insights on the biological functions of taurine. Taurine 4: Taurine and excitable tissues. *Advances in Experimental Medicine and Biology*, *72* (1): 101–163.

[12]. Knopf, K., et al.(1978).Taurine.An essential nutrient for the cat. *Journal of Nutrition*. *108* (5): 773–778.

[13]. Stipanuk, M.H., et al.(2006).Mammalian cysteine metabolism:New insights into regulation of cysteine metabolism . *Journal of Nutrition. 136* (6):1652S–1659S.

[14]. Cavallini, D.(1956). *Metabolisme de la cystamine*. Colloque sur la Biochimie du Soufre, Roscoff, France.

[15]. Stipanuk, M. H., & Ueki, I. (2011). Dealing with methionine/homocysteine sulfur: cysteine metabolism to taurine and inorganic sulfur. *Journal of Inherited Metabolic Disease*. 34 (1): 17–32.

[16]. Tully, P.S. (2000). *Sulfonic acids. Kirk-Othmer encyclopedia of chemical technology.* John Wiley & Sons, Inc.

[17]. Chen,Y. (2014).*Process for Producing Taurine.United States Patent No 20140121405.* Qianjiang Yongan Pharmaceutical Co., Ltd.,

[18]. Higgins, J.P., et al. (2010). Energy beverages: Content and safety. *Mayo Clinic Proceeding*, *85*(11): 1033–1041.

[19]. Wang, Xi.F., et al.(2011).Determination of Taurine in Biological Samples by High-Performance Liquid Chromatography Using 4-Fluoro-7-Nitrobenzofurazan as a Derivatizing Agent,*Biomedical and Environmental Sciences, 24*(5):537–542

[20]. Nejdl, L., et al.(2013). Spectrometric and Chromatographic Study of Reactive Oxidants Hypochlorous and Hypobromous Acids and Their Interactions with Taurine. *Chromatographia.76*:363–373.

[21]. Moua, S., et al.(2002).Separation methods for taurine analysis in biological samples. *Journal of Chromatography B, 781*:251–267.

[22]. Whitton, P.S., et al.,(1995). Biosynthesis of taurine in tissues of the locust (Schistocerca americana gregaria) and the effect of physiological and toxicological stresses on biosynthetic rate of this amino acid. *Insect Biochemistry and Molecular Biology. 25* (1): 83–87.

[23]. Spitze, A.R., et al. (2003). Taurine concentrations in animal feed ingredients; cooking influences taurine content. *Journal of Animal Physiology and Animal Nutrition,87*: 251–262.

[24]. Hickman, M.A., Roger, O.R., & Morris, J.G., (1990). Effect of processing on fate of dietary[14C] taurine in cats.Journal of Nutrition.120(9):995-1000.

[25]. Jacobsan, I.G., & Smith, L.H.(1968).Biochemistry and physiology of taurine and taurine derivative,*Physiological Review,48*(2):424-511.

[26]. Kendler, B.S. (1989).Taurine and overview of its role in preventive medicine. *Preventive Medicine,18*(1):79-100.

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