Journal of Applied Sciences

ISSN 1812-5654

science alert

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Journal of Applied Sciences 12 (6): 568-574, 2012
ISSN 1812-5654 / DOI: 10.3923/jas.2012.568.574

A New Simple and Rapid Colorimetric Screening Test for Semi-qualitative Analysis of Vitamin C in Fruit Juices Based on Prussian Blue

S. Teepoo, P. Chumsaeng, S. Jongjinakool, K. Chantu and W. Nolykad
Department of Chemistry, Faculty of Science and Technology,
Rajamangala University of Technology Thanyaburi, Pathumthani 12110, Thailand

Abstract: A simple and rapid screening test to determine the real amount of vitamin C contained in fruit juices daily products was established by Prussian blue. The ferric chloride was reduced by vitamin C and then reacted with potassium ferricyanide to form a blue solution of Prussian blue. The developed color was monitored at absorption wavelength of 709 nm. Optimum conditions of substrate concentrations were investigated. Interference effects of various substances including glucose, fructose and sucrose have been studied. The vitamin C kit was fabricated under the optimum conditions. The kit contains all necessary reagents and operates on a very simple protocol. The detection level of this semi-quantitative kit has been set at 0.01 mM of vitamin C with acceptable of precision and accuracy. It has been successfully applied to analyze the vitamin C in fruit juices and the results were compared well to a titration method. These results demonstrate that the screening test was suitable for the quality control of vitamin C with simple, rapid, precise and accurate and convenient approaches for analysis of fruit juices.

Key words: Vitamin C, Prussian blue, fruit juices, screening test

INTRODUCTION

Vitamin C or L-ascorbic acid is a water soluble vitamin which plays an important role in antioxidant activity for prevention and treatment of several diseases, i.e., cancer and AIDS (Antonelli *et al*., 2002; Iqbal *et al*., 2004a). Vitamin C is also an essential organic substance for the metabolism. However, it cannot be produced in sufficient quantities by the human organism. Therefore, the consumption of vitamin C on a daily basis in adequate quantities is needed. Fruit juice is a rich source of vitamin C and is a vital and important contributor of vitamins and minerals in the diet (Khalid *et al*., 2011; Iqbal *et al*., 2004b; Falade *et al*., 2003). With this reason, the consumption of fruit juices has increased at very quick rates. Since vitamin C is sensitive to the light, some producers added vitamin C to juice beyond what is naturally found in the fruit juices (Ajibola *et al*., 2009; Hossain *et al*., 2011; Ayo and Sinkalu, 2007). Therefore, the determination of real content of vitamin C in the fruit juices is particularly important to control the quality of the product.

Many analytical techniques, i.e., spectrophotometry (Guclu *et al*., 2005; Grudpan *et al*., 1999), electrochemical (Ensafi-Ali *et al*., 2010; Castro-Suely *et al*., 2001), fluorescence (Wu *et al*., 2003; Miura *et al*., 2002) and chromatographic (Romeu-Nadal *et al*., 2006; Karlsen *et al*., 2005) methods have been developed for the determination of vitamin C in fruit juices. However, these methods have some shortcoming of time consuming, required several separation steps and special equipped laboratories and/or skillful personnel. Therefore, there is a need to develop a rapid and simple test for vitamin C. The developed method has to be able to apply for real sample analysis. Moreover, the method should be versatile and can be carried out using a low-cost instrumentation. One approach is a screening test which is very easy to handle and produce results faster than the previous conventional methods (Skopelitou and Labrou-Nikolaos, 2010; Khan-Ashfaq *et al*., 2005; Ballesteros *et al*., 2003; Phillips *et al*., 2001; Benamar *et al*., 2010; Addo *et al*., 2008; Jamal *et al*., 2005). In this work, we are interested to develop the screening test to determine the amount of vitamin C in fruit juices based on Prussian blue.

The root of this interest goes back as far as the 18th century when Diesbach accidentally produced a complex of deep blue color and now known as Prussian blue. Prussian blue can be synthesized chemically by mixing of ferric (ferrous) and hexacyanoferrate ions with different oxidation state of iron atoms: either Fe^{III} + $[Fe^{II}(CN)_6]^{4-}$ or Fe^{II}+$[Fe^{III}(CN)_6]^{3-}$. After mixing, an immediate formation of a dark blue colloid is observed. On the contrary, the mixed solutions of ferric (ferrous) and hexacyanoferrate

Corresponding Author: S. Teepoo, Department of Chemistry, Faculty of Science and Technology,
Rajamangala University of Technology Thanyaburi, Pathumthani 12110, Thailand

ions with the same oxidation state of iron atoms are apparently stable (Karyakin-Arkady, 2001). A significant development of analytical biosensors and chemical sensors based Prussian blue has been observed during the last 10 years. Especially, there was a report using Prussian blue as spectroscopic probe reagent to detect glucose (Lenarczuk *et al*., 2001a), cesium (Faustino *et al*., 2008) and urea (Koncki and Wolfbeis, 1999) which resulted in dramatic change of its optical. This indicates that Prussian blue play a role as the indicating reagent for chemical analysis fields. In this work, we presented a new chemical sensor for screening test of vitamin C. To the best of our knowledge, the developing semi-qualitative analysis of vitamin C based on Prussian blue has not been reported. The detection principle relies on the ability of vitamin C ($C_6H_8O_6$) to reduce Fe^{III} and form Prussian blue colloidal (Lenarczuk *et al*., 2001b; Nobrega and Lopes, 1996). The first step is the reduction of Fe^{III} by vitamin C ($C_6H_8O_6$) to produce Fe^{II}.

$$2Fe^{III}+C_6H_8O_6 \rightarrow 2Fe^{II}+C_6H_6O_6+2H^+ \quad (1)$$

The second step is the formation of Prussian blue ($KFe^{III}[Fe^{II}(CN)_6]$) when Fe^{II} reacted with potassium ferricyanide ($K_3[Fe^{III}(CN)_6]$).

$$Fe^{II}+K_3[Fe^{III}(CN)_6] \rightarrow KFe^{III}[Fe^{II}(CN)_6]+2K^+ \quad (2)$$

Prussian blue is a co-ordination compound which shows the intense absorption band near 700 nm (Koncki, 2002). Thus, it is powerful for application in optical chemical sensors. Beside the color property of Prussian blue, in our paper, we developed screening test for vitamin C detection based on the production of Prussian blue resulting from the reduction of Fe^{III} by vitamin C. The experimental conditions related to the performance of the fabricated test were investigated in detail. Moreover, in order to demonstrate the usefulness and feasibility of this assay, in this paper the assay was applied to analyze vitamin C in a wide range of commercial fruit juices and fresh fruits including orange, lemon, grape, apple, pineapple and cocktails. The results from the developed test kit were then compared with the classical titration method.

MATERIALS AND METHODS

Chemicals: Vitamin C (L-Ascorbic Acid) and potassium ferricyanide were obtained from Unilab. Ferric chloride was obtained from POCH. Nitric acid was obtained from J.T. Baker. All other chemicals were analytical reagent grade. All solutions used in this work were prepared with distilled water.

Standard solutions of vitamin C between 0.005 to 10 mM were fresh prepared in 0.15% (v/v) nitric acid to prevent the oxidation of vitamin C.

Instruments: The color change of the vitamin C detection was measured by a UV-vis spectrophotometer (Model UV 1601, Shimadzu) using a 1 cm path length conventional cuvette. The pH measurements were carried out using a model UB-10 digital ion analyzer (FormaScientific, Inc).

Screening detection of vitamin C: The detection of vitamin C is performed based on the visible color of Prussian blue. Briefly, 1.0 mL of different concentrations of standard vitamin C solution (or samples), 1.0 mL of 1.0 mM ferric chloride and 1.0 mL of 5.0 mM potassium ferricyanide were mixed together by vigorous stirring for 10 min. The reduction of Fe^{III} by vitamin C and the formation of Prussian blue as described in Eq. 1 and 2 were obtained. The blue colloidal solution of Prussian blue was obtained and can be observed by naked eyes. For the semi-quantitative analysis, the color change was monitored by spectrophotometer at a maximum wavelength of 709 nm. The optimizations of the screening test condition were carried out to meet the high performance. The effected parameters including ferric chloride concentration, potassium ferricyanide concentration and pH were studied.

Real sample analysis in juices

Commercial fruit juices: Commercial fruit juice samples were apple, orange, lemon, pineapple and cocktail. They were obtained from the local supermarkets in Pathumthani, Thailand. The samples were filtered through no. 42 Whatman paper and diluted with appropriate volume of distilled water to obtain the concentrations between 0.01 and 5.0 mM. The samples were then analyzed by a developed screening test.

Fresh fruit juices: One hundred grams of fresh fruits (orange, lemon and pineapple) were squeezed. The juices were subsequently centrifuged at 5,000 rpm for 30 min. The supernatant was filtered (filter paper; Whatman no. 42) and diluted with distilled water in working range concentrations from 0.01 to 5.0 mM.

RESULTS AND DISCUSSION

Screening detection of vitamin C: The design of the colorimetric sensor array was based on the detection of the color change. Prussian blue reaction was used as an

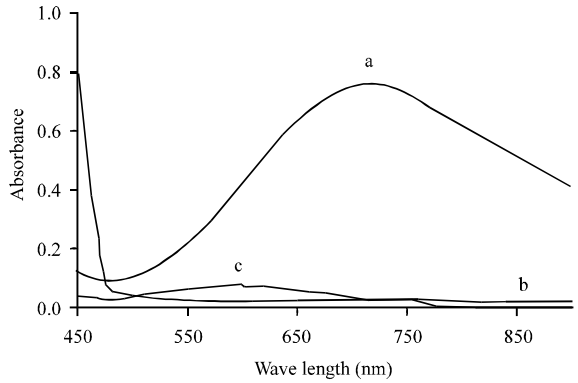

Fig. 1: UV-visible absorption spectra of (a) $FeCl_3$ mixed with vitamin C and $K_3[Fe(CN)_6]$ as Prussian blue (b) $FeCl_3$ mixed with $K_3[Fe(CN)_6]$ as reagent blank (c) vitamin C solution in nitric acid. The concentrations of each component were 1.0 mM of $FeCl_3$, 5.0 mM of $K_3[Fe(CN)_6]$ and 0.5 mM of vitamin C

indicating reagent to detect vitamin C. First, Fe^{III} was reduced by a strong reducing of vitamin C to Fe^{II} which further reacted with potassium ferricyanide. Then color of this mixed solution was dramatically changed to blue (called Prussian blue) within 2 sec. The maximum absorbance of Prussian blue showed as a single sharp peak at wavelength of 709 nm as Fig. 1a. This was due to a transition from the ground state to an excited state upon which an electron is transferred from a ground state $Fe^{III}Fe^{II}$ to an excited state $Fe^{II}Fe^{III}$ form (Koncki, 2002). Whereas, the absorbencies of the reagent blank ($FeCl_3$+$K_3[Fe(CN)_6]$) (b) and vitamin C (c) were almost weaker than Prussian blue in the range of 500-900 nm, respectively. Therefore, all the following measurements were carried out at 709 nm which resulted from Prussian blue.

Such dramatic color changes were useful for screening test purposes. The reaction time and the stability of developed Prussian blue were studied by monitoring the absorbance change every one minute for 60 min at the maximum wavelength (709 nm) using 0.5 mM of vitamin C. Figure 2 showed the influence of reaction time on the absorbance. It was found that absorbance increased rapidly when reaction time increased from 1 to 10 min and reach the plateau at 10 to 60 min. This clearly indicated that Fe^{III} was completely reduced by vitamin C and reacted with excess potassium ferricyanide to produce Prussian blue within 10 min. After 10 min the developed color of Prussian blue did not change and it was stable up to 60 min. Therefore, this method was feasible and powerful to develop as a rapid colorimetric screening sensor for vitamin C analysis on filed.

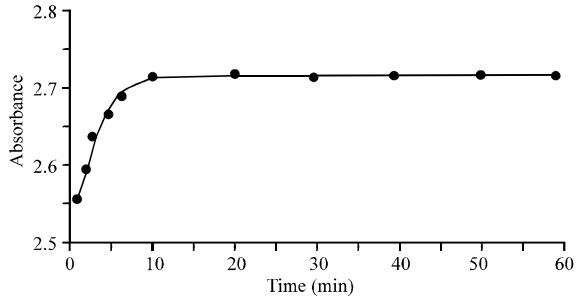

Fig. 2: Absorbance change of Prussian blue in a function of time at the maximum wavelength of 709 nm. Condition concentrations: 1.0 mM of $FeCl_3$, 5.0 mM of $K_3[Fe(CN)_6]$ and 0.5 mM of vitamin C

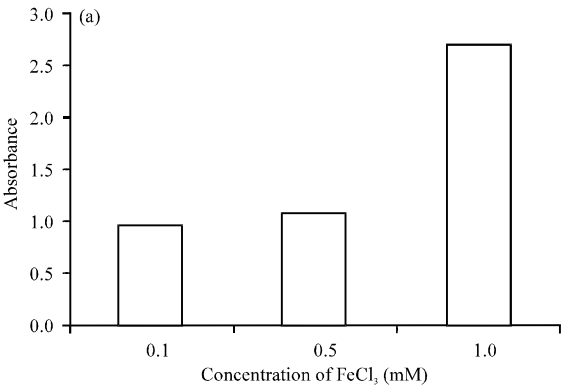

Fig. 3a: The influence of $FeCl_3$ concentration on the formation of Prussian blue using 0.5 mM of vitamin C and 10 min of reaction time

Influence of ferric chloride concentration: To ensure that vitamin C will be completely used as a reducing agent, the amount of Fe(III) should be excess to obtain high sensitivity of screening test. Thus, the effect of ferric chloride concentration was evaluated from 0.1 to 1.5 mM. The tested concentration of vitamin C was 0.5 mM. The results are shown in Fig. 3a. The increase in ferric chloride concentration provided more formation of Prussian blue compound led to the increase of the intensity of Prussian blue color. This caused the increase in the absorbance. The absorbance reached the maximum value when the amount of ferric chloride was 1.0 mM. At higher concentration (1.5 mM) the color developing from Prussian blue cannot be detectable because the precipitation solution was obtained. Therefore, 1.0 mM of ferric chloride was selected as an optimum concentration for the ongoing experiment.

Influence of potassium ferricyanide concentration: The excess potassium ferricyanide will prevent the occurrence of precipitation (Vogel, 1979). Therefore, various

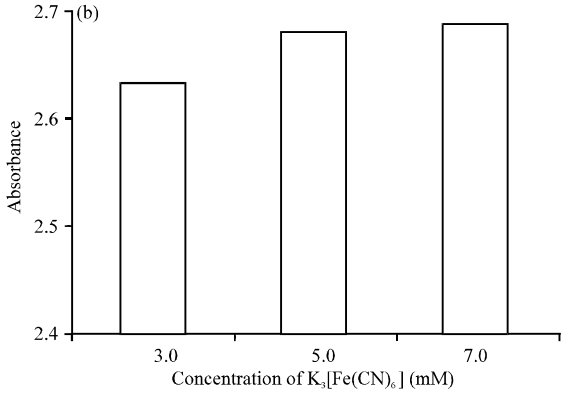

Fig. 3b: The influence of $K_3[Fe(CN)_6]$ concentration on the formation of Prussian blue using 0.5 mM of vitamin C and 10 min of reaction time

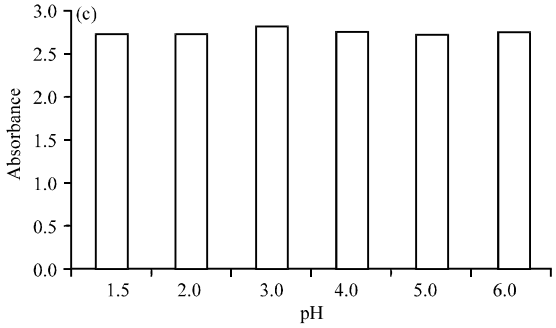

Fig. 3c: The influence of pH on the formation of Prussian blue using 0.5 mM of vitamin C and 10 min of reaction time

concentrations of potassium ferricyanide between 3.0 mM and 7.0 mM were tested and the highest absorbance value was obtained at 5.0 mM (Fig. 3b). With this concentration, the color of Prussian blue was clearly observed and the absorbance can be measured without any precipitation of the Prussian blue compound. Thus, the optimal concentration of potassium ferricyanide was selected at 5.0 mM.

Influence of pH: Normally, manufactured processed fruit juices are mostly acid-producing. However, each type of fruit juices is different in pH. Therefore, the influence of sample pH was examined due to the obtained verity of samples. The experiment was carried out by changing the pH of nitric acid between 1.5 and 6.0 during vitamin C preparation. As shown in Fig. 3c, the pH of sample did not influence the Prussian blue production. This is corresponded to the special property of Prussian blue that has a good stability in acidic solution (Bai *et al*., 2009). Therefore, there is no need to adjust the pH of sample

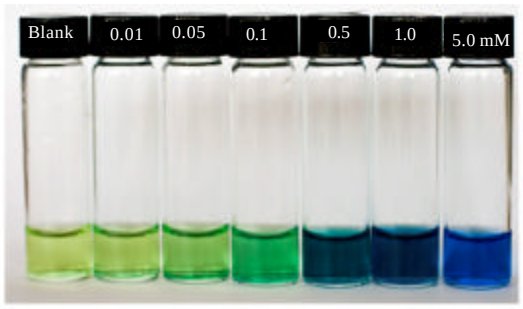

Fig. 4: Colorimetric responses of the screening test to different concentrations of vitamin C from 0.01-5.0 mM

before the analytical process resulting in short analysis time. Moreover, this rapid screening test is easy and makes it practical in real applications.

Validation of the method: Under the optimum conditions, working range concentration was studied by using different concentrations of vitamin C from 0.005 to 10 mM. The developed color of Prussian blue was observed by naked eye. The color change depended on the amount of vitamin C as shown in Fig. 4. The working range concentration was between 0.01 and 5.0 mM of vitamin C. The developed color of Prussian blue of this concentration range can be observed by the naked eyes from yellow to blue. At the concentration of 0.01 mM of vitamin C, the obtained color of Prussian blue can be distinguished from blank by naked eye. Therefore, the detection limit of this screening test was 0.01 mM of vitamin C.

The precision was determined as repeatability and reproducibility of the method. The repeatability (intra-day) of the method was evaluated by analyzing 0.5 mM of vitamin C. A relative standard deviation (RSD) value of 3.1% was obtained for seven successive determinations which indicated a good repeatability of method. For the reproducibility (inter-day) was studied by analyzing 0.5 mM of vitamin C for seven consecutive days. The RSD was 3.6% which demonstrated the reliability of the assay. The proposed method of screening test showed high precision.

The accuracy of vitamin C screening test for the semi-quantitative measurement of vitamin C concentration was evaluated with sample blank. Standard of vitamin C at different concentrations were spiked into sample blank. The concentrations of all samples were measured after spiking by comparing obtained blue color intensity of samples with the one produced by the vitamin C

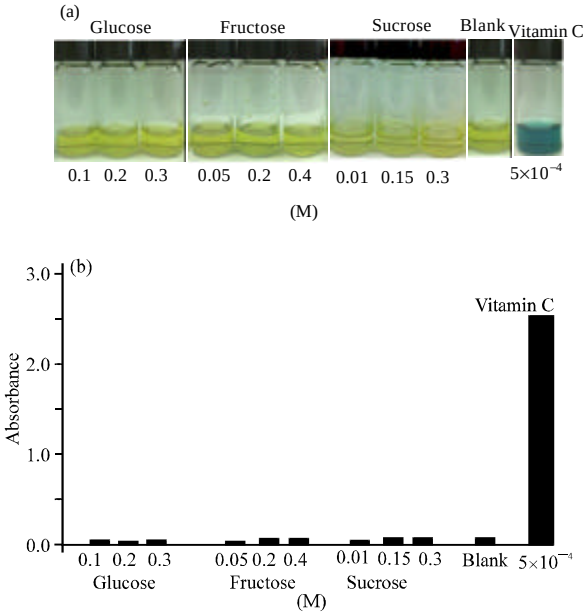

Fig. 5 (a-b): (a) The color change of the screening test when various concentrations of interferences were tested and compared with blank and vitamin C (0.5 mM). (b) The absorbance change obtained from the interference test at the same condition as Fig. 5(a).

Table 1: The analysis of sample blank spiked with different concentrations of standard vitamin C using the developed screening test.

Spiked concentration (mM)	Concentration detected by the developed screening test (mM)
0.01	0.01
0.05	0.05
0.1	0.10
0.3	0.10-0.5
0.5	0.50
1.0	1.00
3.0	1.00-5.0
5.0	5.00
7.0	>5.00

standards. As shown in the Table 1, the results of the visual estimation of the vitamin C concentrations matched very well with the real spiked concentration value. Therefore, developed screening test provided a good accuracy for vitamin C detection.

Selectivity of screening test: The evaluation of the selectivity of the proposed method was studied. The possible interferences of some ingredients in fruit juices, i.e. glucose, fructose, sucrose were investigated. Different concentrations of interferences (0.1-0.3 M of glucose, 0.05-0.4 M of fructose and 0.01-0.3 M of sucrose) which normally present in fruit juices were added to the instead of vitamin C. The results in Fig. 5 clearly indicated that

Fig. 6: The screening test for vitamin C consisted of plastic syringes, reagent 1, reagent 2 and color comparison paper

Fe^{III} could not be reduced by glucose, fructose and sucrose resulting in no Prussian blue formation. This was shown as clear yellow solution (Fig. 5a). The same result was observed in blank (using distilled water). So, the presence of glucose, fructose and sucrose could not interfere the screening test of vitamin C under the testing conditions which indicated that the method had a remarkable high selectivity for vitamin C (the right bar in Fig. 5b).

Screening test fabrication: The screening test was constructed as show as Fig. 6. The package consists of plastic syringes, reagent 1 (ferric chloride), reagent 2 (potassium ferricyanide), a color comparison paper and a protocol. Doing this screening test is very easy, just mix the 1.0 mL volume of reagent 1 and sample (1.0 mL). Then the reagent 2 was added into this mixture solution and incubated for 10 min for color development at room temperature and evaluate the results visually comparing the color of the samples to the one of the standards by using a color comparison paper. In case of the sample contains vitamin C concentration higher than 5.0 mM, the sample must be dilute before analysis.

Comparison between the screening test and titration method: Different concentrations of vitamin C standard were prepared and were analyzed with the screening test and the titration method. The titration method is based on the reaction of vitamin C with an iodine solution, using starch as an indicator for the end point of the titration. Results were validated by comparing between vitamin C concentrations obtained by screening test with those obtained by titration method. The results obtained by proposed method showed that there is no difference when

Table 2: Comparison of the vitamin C concentration obtained from the developed screening test and the titration method when the samples were fruit juices and fresh fruits.

	Concentration of vitamin C (mM)	
Sample	Screening test	Titration method
Fruit juices		
Sample-1	0.01-0.05	0.015
Sample-2	0.5-1.0	0.9
Sample-3	0.05	0.05
Sample-4	1.0-5.0	1.2
Sample-5	1.0-5.0	4.0
Sample-6	0.1	0.1
Sample-7	<0.01	Not detectable
Sample-8	1.0-5.0	1.4
Sample-9	0.1-0.5	0.4
Fresh fruits		
Sample-10	1.0-5.0	4.6
Sample-11	1.0-5.0	2.3
Sample-12	1.0-5.0	1.6

compared with titration method, indicating the utility of the proposed method for analysis of vitamin C in real samples.

Application of the method in real samples: To evaluate the performance of the proposed screening test, this assay was applied for the determination of vitamin C in various commercial fruit juices including orange juices, lemon, grape, apple and cocktail and also fresh fruits (orange, lemon and pineapple). In all instances, samples were analyzed with no previous pretreatments such as chromatographic assays or elimination of interferences. In every samples, volumetric titration method was used as a reference method according to the monograph of the USP (United States Pharmacopeia, 2007) for the analysis of vitamin C. pH of all samples were ranging from 2.0 to 6.0. The analytical results are given in Table 2. It was found that the results obtained from a new screening test were very well the results obtained by reference method.

CONCLUSIONS

In this work, we have demonstrated a simple, cost-effective and rapid visible colorimetric assay for semi-quantitative analysis of vitamin C. It is based Prussian blue as a spectroscopic probe for the first time to fabricate screening test kit. The assay involves the optimizations of effective parameters, validation method and application in real samples. The method reported here showed relatively good selectivity for vitamin C with the lowest detection concentration of 0.01 mM. The detection of vitamin C could be observed by the naked eye and completed within 10 min. So, the method is simple, rapid and very cheap (about 10 cents per analysis). In addition, the proposed screening could be successfully applied for monitoring vitamin C levels in fruit juices.

ACKNOWLEDGMENT

Financial support of this work was provided by the Office of the National Research Council of Thailand (97514).

REFERENCES

Addo, M.G., W.G. Akanwariwiak, P. Addo-Fordjour and K. Obiri-Danso, 2008. Microbiological and sensory analysis of imported fruit juices in Kumasi, Ghana. Res. J. Microbiol., 3: 552-558.

Ajibola, V.O., O.A. Babatunde and S. Suleiman, 2009. The effect of storage method on the vitamin C content in some tropical fruit juices. Trends Applied Sci. Res., 4: 79-84.

Antonelli, M.L., G.D. Ascenzo, A. Lagana and P. Pusceddu, 2002. Food analyses: A new calorimetric method for ascorbic acid (vitamin C) determination. Talanta, 58: 961-967.

Ayo, J.O. and V.O. Sinkalu, 2007. Effects of ascorbic acid on diurnal variations in rectal temperature of shaver brown pullets during the hot-dry season. Int. J. Poult. Sci., 6: 642-646.

Bai, J., B. Qi, C. Ndamanisha-Jean and L.P. Guo, 2009. Ordered mesoporous carbon-supported Prussian blue: Characterization and electrocatalytic properties. Micropor. Esopor. Mat., 119: 193-199.

Ballesteros, B., D. Barcelo, D.A. Dankwardt, P. Schneider and M.P. Marco, 2003. Evaluation of a field-test kit for triazine herbicides (SensioScreen TR500) as a fast assay to detect pesticide contamination in water samples. Anal. Chim. Acta., 475: 105-115.

Benamar, H., W. Rached, A. Derdour and A. Marouf, 2010. Screening of Algerian medicinal plants for acetylcholinesterase inhibitory activity. J. Biol. Sci., 10: 1-9.

Castro-Suely, S.L., R. Balbo-Valdir, J.S. Barbeira-Paulo and R. Stradiotto-Nelson, 2001. Flow injection amperometric detection of ascorbic acid using a Prussian Blue film-modified electrode. Talanta, 55: 249-254.

Ensafi-Ali, A., M. Taei, T. Khayamian and A. Arabzadeh, 2010. Highly selective determination of ascorbic acid, dopamine and uric acid by differential pulse voltammetry using poly(sulfonazo III) modified glassy carbon electrode. Sensor. Actuat. B-Chem., 147: 213-221.

Falade, O.S., O.R. Sowunmi, A. Oladipo, A. Tubosun and S.R.A. Adewusi, 2003. The level of organic acids in some Nigerian fruits and their effect on mineral availability in composite diets. Pak. J. Nutr., 2: 82-88.

Faustino, P.J., Y. Yang, J.J. Progar, C.R. Brownell and N. Sadrieh *et al*., 2008. Quantitative determination of cesium binding to ferric hexacyanoferrate: Prussian blue. J. Pharm. Biomed. Anal., 47: 114-125.

Grudpan, K., K. Kamfoo and J. Jakmunee, 1999. Flow injection spectrophotometric or conductometric determination of ascorbic acid in a vitamin C tablet using permanganate or ammonia. Talanta, 49: 1023-1026.

Guclu, K., K. Sozgen, E. Tutem, M. Ozyurek and R. Apak, 2005. Spectrophotometric determination of ascorbic acid using copper(II)-neocuproine reagent in beverages and pharmaceuticals. Talanta, 65: 1226-1232.

Hossain, M.N., M. Fakruddin and M.N. Islam, 2011. Effect of chemical additives on the shelf life of tomato juice. Am. J. Food Technol., 6: 914-923.

Iqbal, K., A. Khan and M.M.A.K. Khattak, 2004a. Biological significance of ascorbic acid (vitamin C) in human health-A review. Pak. J. Nutr., 3: 5-13.

Iqbal, N., F. Sen and N.A. Virk, 2004b. Effect of inflorescence types on fruits quality of Owari cultivar of *Satsuma mandarin* (*Citrus unshiu*, Marc.). Pak. J. Biol. Sci., 7: 1840-1846.

Jamal, P., M.Z. Alam, M. Ramlan, M. Salleh and M.M. Nadzir, 2005. Screening of aspergillus for citric acid production from palm oil mill effluent. Biotechnology, 4: 275-278.

Karlsen, A., R. Blomhoff and E. Gundersen-Thomas, 2005. High-throughput analysis of Vitamin C in human plasma with the use of HPLC with monolithic column and UV detection. J. Chromatogr. B., 824: 132-138.

Karyakin-Arkady, A., 2001. Review Prussian Blue and Its Analogues: Electrochemistry and Analytical Applications. Electroanal. 13: 813-819.

Khalid, U., F. Nosheen, M.A. Raza, M. Ishaque, M. Ahmad, S.R. Ahmad and F. Rubab, 2011. A comparative study about the daily intake of fruits and vegetables among female students of two universities of Faisalabad. Pak. J. Nutr., 10: 684-689.

Khan-Ashfaq, A., M. Khan-Hasan and H. Delincee, 2005. DNA Comet Assay- a rapid screening method for detection of irradiated cereals and tree nuts. Food Control., 16: 141-146.

Koncki, K., 2002. Chemical sensors and biosensors based on Prussian blues. Crit. Rev. Anal. Chem., 32: 79-96.

Koncki, R. and O.S. Wolfbeis, 1999. Composite films of Prussian blue and N-substituted polypyrroles: covalent immobilization of enzymes and application to near infrared optical biosensing. Biosens. Bioelectron., 14: 87-92.

Lenarczuk, T., D. Wencel, S. G^3 and R. Koncki, 2001a. Prussian blue-based optical glucose biosensor in flow-injection analysis. Anal. Chim. Acta., 447: 23-32.

Lenarczuk, T., S. G^{3}ab and R. Koncki, 2001b. Application of Prussian blue-based optical sensor in pharmaceutical analysis. J. Pharmaceut. Biomed. Anal., 26: 163-169.

Miura, Y., M. Hatakeyama, T. Hosino and R. Haddad-Paul, 2002. Rapid ion chromatography of L-ascorbic acid, nitrite, sulfite, oxalate, iodide and thiosulfate by isocratic elution utilizing a postcolumn reaction with cerium(IV) and fluorescence detection. J. Chromatogr. A., 956: 77-84.

Nobrega, J.A and G.S. Lopes, 1996. Flow-injection spectrophotometric determination of ascorbic acid in pharmaceutical products with the Prussian Blue reaction. Talanta, 43: 971-976.

Phillips, T.M., A.G. Seech, H. Lee and J.T. Trevors, 2001. Colorimetric assay for Lindane dechlorination by bacteria. J. Microbiol. Meth., 47: 181-188.

Romeu-Nadal, M., S. Morera-Pons, A.I. Castellote and M.C. Lopez-Sabater, 2006. Rapid high-performance liquid chromatographic method for Vitamin C determination in human milk versus an enzymatic method. J. Chromatogr. B., 830: 41-46.

Skopelitou, K. and E. Labrou-Nikolaos, 2010. A new colorimetric assay for glutathione transferase-catalyzed halogen ion release for high-throughput screening. Anal. Biochem., 405: 201-206.

United States Pharmacopeia, 2007. Validation of Compendial Methods. United States Pharmacopeia 30, National Formulary 25, The United States Pharmacopeial Convention Inc., Rockville, Md., USA.

Vogel, I., 1979. Reactions of the Anions. In: Vogel's Textbook of Macro and Semimicro Qualitative Inorganic Analysis, Vogel, A.I. and G. Svehla (Eds.). 5th Edn. Longman, London, UK., ISBN-13: 9780582443679, pp: 297-313.

Wu, X., Y. Diao, C. Sun, J. Yang, Y. Wang and S. Sun, 2003. Fluorimetric determination of ascorbic acid with ophenylenediamine. Talanta, 59: 95-99.