HPLC quantification of Ascorbic Acid, Thiamine and Nicotinamide in some local edible fruits

David Adeyemi, Kafayat Temitope, Oluwatosin Johnson and Gloria Ayoola
Department of Pharmaceutical Chemistry, Faculty of Pharmacy.
University of Lagos, Nigeria.
Idi-Araba Campus, P.M.B. 12003,
Lagos, Nigeria

Corresponding author: David Adeyemi
E-mail: dadeyemi@unilag.edu.ng Phone: +2348033871465

ABSTRACT

Background: Health and nutritional problem continues to be of increasing concern worldwide. Fruits are good source of vitamins and mineral, it plays a vital role in our health and also helps the human to maintain a healthy diet.

Objective: The current study was conducted for the determination of water-soluble vitamins like thiamine (vitamin B_1), niacin (vitamin B_3) and ascorbic acid (vitamin C) in twelve highly consumed local edible fruits named as Green and Red apple (*Malus domestica),* Soursop *(Annona muricata),* Cucumber *(Cucumis sativus)* Pear (*Pyrus communis*), Carrot (*Daucus carota*), Golden melon (*Cucumis melo*), Mango *(Magnifera indica*), Grape (*Citrus paradise*), Pinneaple (*Ananas comosus*), Orange (*Citrus sinensis*) and Lime (*Citrus aurantifolia*).

Methods: The analyses were performed by High Performance Liquid Chromatography (HPLC) using an analytical reversed phase C-8 (Zorbax Eclipse XDB RP C8 150x4.6mm, 5µm particle size) column and coupled to a UV detector.

Results: The ascorbic acid in fruits analysed varied from 0.249 to 4.439 mg/100g, nicotinamide varied from 4.190 to 44.031 mg/100g, while thiamine varied from 0.171 to 1.250mg/100g. Red apple had the highest content of ascorbic acid, golden melon had the highest for nicotinamide and lime fruit had the highest for thiamine.

Conclusion: It is hoped that these data will be helpful in the preparation of a complete food composition table which will be used for the preparation of diet therapy, for food based dietary guidelines, for nutrition education, for food security, safety and regulation, for the labelling of food in food industry, for nutritional survey and also for other research purposes.

Keywords: HPLC Analysis, fruits, vitamin B_1 vitamin B_3, vitamin C

Quantification par HPLC de l'acide ascorbique, de la thiamine et du nicotinamide dans certains fruits comestibles locaux

David Adeyemi, Kafayat Temitope, Oluwatosin Johnson et Gloria Ayoola
Département de chimie pharmaceutique, Faculté de pharmacie.
Université de Lagos, Nigéria.
Campus Idi-Araba, P.M.B. 12003, Lagos, Nigeria

Correspondance : David Adeyemi
E-mails : dadeyemi@unilag.edu.ng, Téléphone : +2348033871465

RESUME

Contexte : Le problème de santé et de nutrition continue d'être une préoccupation croissante dans le monde entier. Les fruits sont une bonne source de vitamines et de minéraux, ils jouent un rôle vital dans notre santé et aident également l'être humain à maintenir une alimentation saine.

Objectif: La présente étude a été menée pour la détermination de vitamines solubles dans l'eau comme la thiamine (vitamine B1), la niacine (vitamine B3) et l'acide ascorbique (vitamine C) dans douze fruits comestibles locaux très consommés appelés pommes vertes et rouges (*Malus domestica*), corossol (*Annona muricata*), concombre (*Cucumis sativus*) poire (*Pyrus communis*), carotte (*Daucus carota*), melon doré (*Cucumis melo*), mangue *(Magnifera indica*), raisin (*Citrus paradise*), ananas (*Ananas comosus*), orange (*Citrus sinensis*) et citron vert (*Citrus aurantifolia*).

Méthodes : Les analyses ont été réalisées par chromatographie en phase liquide à haute performance (HPLC) en utilisant une colonne analytique en phase inversée C-8 (Zorbax Eclipse XDB RP C8 150x4,6 mm, taille de particule 5µm) et couplée à un détecteur UV.

Résultats : L'acide ascorbique dans les fruits analysés variait de 0,249 à 4,439 mg/100g, le nicotinamide variait de 4,190 à 44,031 mg/100g, tandis que la thiamine variait de 0.171 à 1.250mg / 100g. La pomme rouge avait la teneur la plus élevée en acide ascorbique, le melon doré avait le taux le plus élevé de nicotinamide et le fruit de citron vert avait le taux le plus élevé de thiamine.

Conclusion : Nous espérons que ces données seront utiles pour la préparation d'un tableau complet de composition alimentaire qui sera utilisée pour la préparation d'une diète, pour les recommandations diététiques, pour l'éducation nutritionnelle, pour la sécurité alimentaire, la sécurité et la réglementation, pour l'étiquetage des aliments dans l'industrie alimentaire, pour les enquêtes nutritionnelles et à d'autres fins de recherche.

Mots-clés : Analyse HPLC, fruits, vitamine B1, vitamine B3, vitamine C

INTRODUCTION

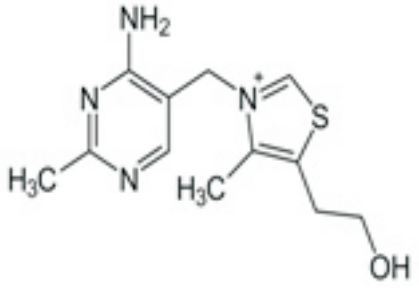
Fruit is a part of a flowering plant that is derived from specific tissues of the flower, one or more ovaries, and in some cases accessory tissues. Fruits are the means by which these plants disseminate seeds. Many of them that bear edible fruits, in particular, have propagated with the movements of humans and animals in a symbiotic relationship as a means for seed dispersal and nutrition, respectively; in fact, humans and many animals have become dependent on fruits as a source of food. [1] Fruits account for a substantial fraction of the world's agricultural output, and some (such as the apple and the pomegranate) have acquired extensive cultural and symbolic meanings. Fruits are good source of vitamins and minerals. Although, vitamins are required in small amount per day, they play a vital role in our health and also help human to maintain a healthy diet. [2] Vitamins are reported to reduce the damage by free radicals and check degenerative disease.[3] Vitamin B is water soluble vitamins that play important roles in cell metabolism. The term B-group vitamins usually refer to thiamine, riboflavin, pyridoxine, niacin, pantothenic acid, biotin, cyanocobalamine and folic acid. The deficiency syndromes of some B-vitamins are beri-beri (cardiac and dry), peripheral neuropathies, pellagra, and oral and genital lesions (related to riboflavin deficiency) which were once major public health problems in parts of the world. [4] Several vitamins of the B-group act mainly as coenzymes in the metabolism of foodstuffs to produce energy.[5]

Vitamin C is an essential nutrient for humans and certain other animal species. It is required for the prevention of scurvy and maintenance of healthy skin, gums and blood vessels. It functions in collagen formation, absorption of inorganic iron, reduction of plasma cholesterol level, inhibition of nitrosoamine formation, enhancement of the immune system, and reaction with singlet oxygen and other free radicals. As an antioxidant, it reportedly reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer.[6-9]

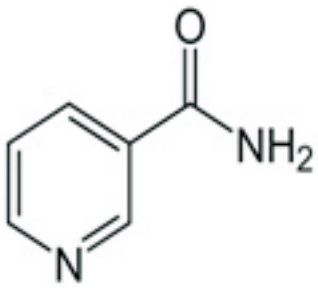
The human body can store only a certain amount of water soluble vitamins and the body stores are depleted, if fresh supplies are not consumed. In addition, vitamins are relatively unstable and can be lost during processing and storage, hence, qualitative and quantitative analyses are very crucial.

Some of the analytical techniques for determination of vitamins in literature include, electrochemical, microbiological and specific chemical titrimetric methods, which are typically designed for single vitamin analysis.[10] However, these methods are very laborious and time consuming. In recent times, chromatographic methods, including capillary electrophoresis and liquid chromatography (LC) coupled to different detectors are being employed for determination of vitamins in samples.[11] LC is a well-suited technique for simultaneous determination of multiple vitamins and for establishing vitamin profiles in a variety of matrices with various modes of detection.[12,13] Due to difference in solubility and reverse phase retention properties, water soluble vitamins are usually determined using an aqueous mobile phase with low organic solvent content, while fat soluble vitamins are determined using organic solvents.[14]

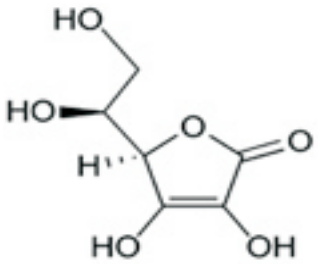
The purpose of the current study was to quantify some selected water soluble vitamins (Figure 1) including Thiamine (B1), Nicotinamide (B3) and ascorbic acids (Vitamin C) in some commonly available fruits in Lagos, Nigeria market employAscorbic aciding HPLC coupled to a UV detector.



Thiamine



Nicotinamide



Ascorbic acid

Figure 1: Structure of water soluble Vitamins

MATERIALS AND METHODS

All glassware were thoroughly washed and dried prior to use to avoid contamination of samples. Solvents including glacial acetic acid and acetonitrile 99.9% (Sigma-Aldrich) were HPLC grade. Reference standards of Vitamin B1 (Thiamine), B3 (Niacin) and Vitamin C (Ascorbic acid) were obtained from Merck chemical, Germany. Standard stock solution of each vitamin was freshly prepared by dissolving in acetonitrile, while working concentrations of vitamins B1, B3 and C were then prepared daily from the stock. Calibration curve were obtained for six different concentrations.

Collection of samples

Samples of twelve locally available fruits were randomly purchased from different stores in Mushin, Lagos, Nigeria. These include Green and Red apple (*Malus domestica),* Soursop *(Annona muricata),* Cucumber *(Cucumis sativus)* Pear (*Pyrus communis*), Carrot (*Daucus carota*), Golden melon (*Cucumis melo*), Mango *(Magnifera indica*), Grape (*Citrus paradise*), Pinneaple (*Ananas comosus*), Orange (*Citrus sinensis*) and Lime (*Citrus aurantifolia*). Each fruit samples were packed in a closed container in order to avoid risk of inadvertent damage and deterioration of nutrients during transport from point of collection. In the laboratory, samples were washed with distilled water and the edible portions of the sample was chopped into small pieces and homogenized.

Extraction procedure

Extraction was according to the modified method of Aslam *et al*.[15] Extraction solvent was prepared by mixing 50 mL of acetonitrile with 10 mL of glacial acetic acid and the volume was made up to 1000 mL in a volumetric flask with double distilled water. Each sample (10 g) was weighed, homogenized and transferred into conical flask, while 25 mL of extraction solvents was added, and sonicated in a water bath at 70 °C for 40 min. Thereafter, each sample was cooled, filtered using 0.45 µm glass fibre filter and made up to final of 50 mL with extraction solution.

Chromatographic conditions

HPLC analysis was carried out by using an Agilent Technologies, U.SA 1200 series HPLC system with a C-8 (Zorbax Eclipse XDB RP C8 150x4.6mm, 5µm particle size) column and coupled to a UV detector. A mobile phase: mixture of Acetonitrile and 10 mM Na Acetate in distilled water (40:60), Flow rate of 0.5ml/minute, Variable wavelength detector of 282nm at ambient temperature of 24^0C was used for the determination of Vitamin content in the fruits. A 20 µL was injected in a splitless mode while identification of Vitamins was by the retention time match with those of the standards.

Quality assurance

All glassware was thoroughly cleaned to avoid contamination and ensure method reliability. The calibrated equipment and validated standard operating procedures were employed. Blank of extraction solvents were run on HPLC to ensure there was no cross contamination. All the samples were carefully preserved prior to analysis and solutions were filtered through a 0.45 µm syringe filter to remove particles which may block the column. The calibration plot and regression equation from the HPLC analysis were obtained using the Microsoft excel 2007, while the mean concentrations were determined using the regression equation of the calibration plot.

RESULTS

HPLC is one of the most convenient and accurate analytical techniques. The method was found to possess linear calibration plot for the concentration range described in Figure 2, when average peak areas were plotted against concentration and good correlation coefficients (r^2) were obtained as 0.9995, 0.9997 and 0.9992 for ascorbic acid, thiamine and nicotinamide respectively.

The amount of thiamine (vitamin B_1) in the ten edible fruit samples ranged from 0.171 to 1.250 mg/100 g (Table 1). The content of vitamin B_1 was found highest in the lime fruit (1.250 mg/100 g). Orange fruit contained second highest amount of vitamin B_1 (1.228 mg/100 g). The amount of nicotinamide (vitamin B_3) in the ten edible fruit samples ranged from 4.190 to 44.031 mg/100g.The highest amount of vitamin B_3(44.031 mg/100 g) was estimated in golden melon fruit, while pear fruit contained 43.875mg/100g; the second highest content vitamin B_3. Among the ten edible fruits vitamin B_3 was lowest in mango (4.190 mg/100 g). The ascorbic acid (vitamin C) in fruits analyzed varied from 0.249 to 4.439 mg/100g. The highest content of vitamin C was present in red apple which was 4.439 mg/100g, while it was lowest in orange fruit (0.249mg/100g).

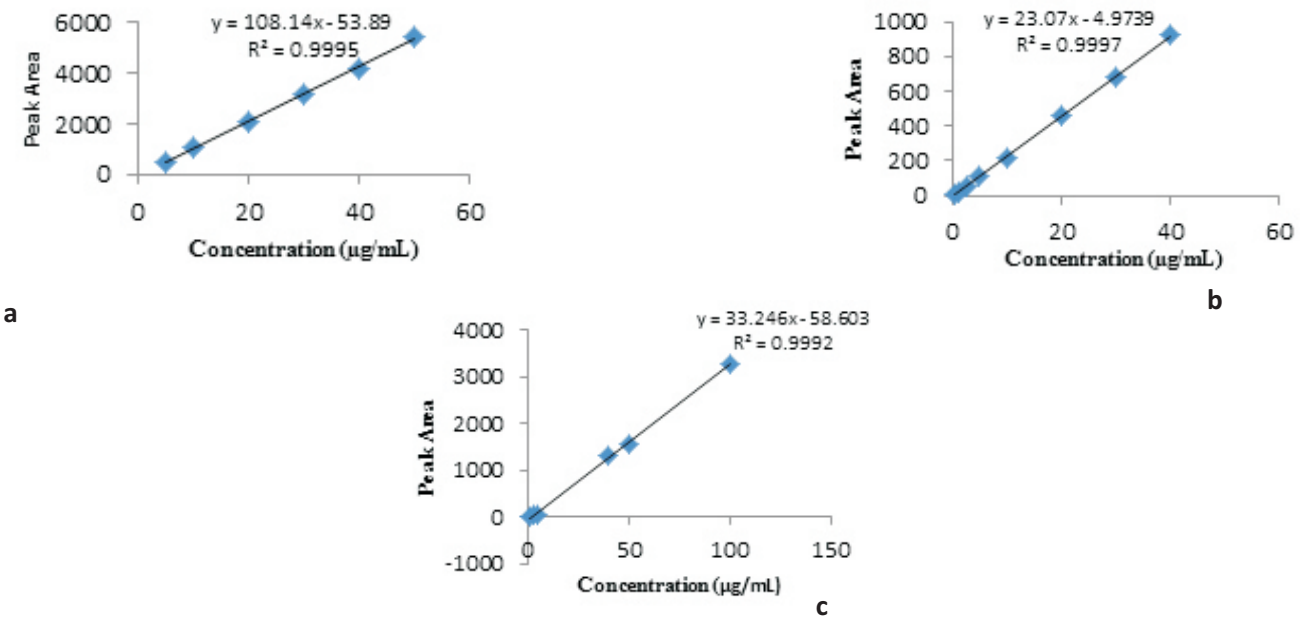


Figure 2: Calibration curve for a. ascorbic acid b. Thiamine and c. Nicotinamide

Table 1: Mean Concentrations (mg/100g) of Vitamins in fresh fruit samples

Common name of fruits	Botanical name	Family name	Ascorbic Acid (Vitamin C)	Nicotinamide (Vitamin B3)	Thiamine (Vitamin B1)
Soursop	*Annona muricata L.*	Annonaceae	2.352	4.846	0.171
Cucumber	*Cucumis sativum L.*	Cucurbitaceae	0.429	13.826	0.171
Green apple	*Malus pumila* Miller.	Rosaceae	1.654	9.101	0.171
Pear	*Pyrus communis L.*	Rosaceae	1.053	43.875	0.171
Carrot	*Daucus carota* Hoffm.	Apiaceae	1.173	12.594	0.171
Golden melon	*Cucumis melo L.*	Cucurbitaceae	1.405	44.031	0.171
Red apple	*Malus domestica* Borch.	Rosaceae	4.439	15.827	0.171
Mango	*Magnifera indica L.*	Anacardiaceae	2.609	4.190	0.171
Grape	*Citrus paradisi* Macfad.	Rutaceae	0.668	5.073	0.211
Pineapple	*Ananas comosus L.*	Bromeliaceae	0.478	7.016	0.171
Orange	*Citrus sinensis L.*	Rutaceae	0.249	31.066	1.228
Lime	*Citrus aurantifolia L.*	Rutaceae	0.295	10.696	1.250

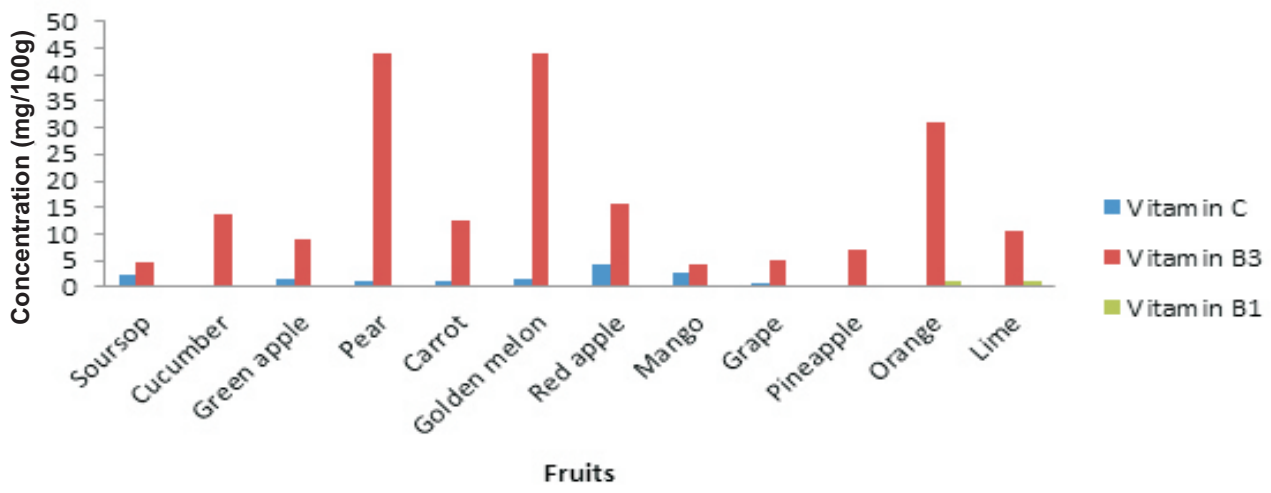


Figure 3: The total ascorbic acid, nicotinamide and thiamine content in fresh Fruits using HPLC technique

DISCUSSION

The HPLC method was successfully performed for the estimation of Thiamin (vitamin B_1), nicotinamide (vitamin B_3) and ascorbic acid (vitamin C) in ten local edible fruits. Vitamins are accessory factors, which must be present in the food in minute amounts to enable growth, health and life to be maintained. The common and easy sources of vitamins are fresh fruits. Comparison with published data by Ranajit and Dipak.[16] of similar fruits collected in Bangladesh, orange fruit had 0.12 mg/100g and 62 mg/100g content for thiamine and ascorbic acid, while the current study gave 1.228mg/100g and 0.249 mg/100g respectively. Pineapple fruit contained 0.20 mg/100g and 35 mg/100g for thiamine and ascorbic acid, while in the present study it gave 0.171mg/100g and 0.478 mg/100g respectively. According to current study, grape fruit contain 0.211mg/100g of thiamine and 0.668 mg/100g of ascorbic acid while the Ranajit and Dipak study reported that it contain 0.12 mg/100g and 31 mg/100g respectively. Mango fruit also contain 0.171mg/100g and 2.609 mg/100g (table 1) of thiamine and ascorbic acid, while previous report gave 0.08 mg/100g and 13 mg/100g respectively. However, the study of Ranajit and Dipak was focused at determination of thiamine and vitamin C content in grapefruits by biological standardization and 2,4-dinitrophenylhydrazine method respectively. To the best of our knowledge, the literature data for vitamin B_1 and B_3 in other fruits selected in this study are currently unavailable. Some values obtained from the present study vary from those of previous study and this variation may occur due to season, rainfall, climate, geographical and geological, in addition, the extent of maturity of the fruits can also be partly responsible for the variation noticed.

It is recommended that future studies be focused at determination of other vitamins and essential mineral ions composition to obtain a wider nutrient evaluation of the locally consumed fruits

CONCLUSION

The study provides the vitamins B_1, B_3 and C composition of twelve local edible fruits. Encouraging regular intake of these fruits can help to alleviate vitamins B_1, B_3 and C deficiency in Lagos, Nigeria.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Mr. Ojobor of Central Research Laboratory for the technical assistance received.

REFERENCES

1. Lewis RA (2002). *CRC Dictionary of Agricultural Sciences*. CRC Press. ISBN 0-8493-2327-4
2. Olaniyi AA (2000). Essential Medical Chemistry, 2nd Edition, Shaneson.
3. Jacab RA, Sotoudeh G. (2002). Vitamin C Function and Status in Chronic Disease. *Nutrition in Clinical Care* 5(2): 66-74.
4. FAO/WHO (2003). Thiamine, Riboflavin, Niacin, Vitamin B6, Pantothenic Acid and Biotin. Chapter 3.
5. Ball GMF (2006). Vitamins in Foods. Analysis, Bioavailability, and Stability. CRC Press, Boca Raton.

6. Rekha C, Poornima G, Manasa M, Abhipsa V, Pavithra DJ, Vijay KHT, Prashith TR. (2012). Ascorbic Acid, Total Phenol Content and Antioxidant Activity of Fresh Juices of Four Ripe and Unripe Citrus Fruits. *Chemical Science Transactions*. 1(2):303-310**.**
7. Choi Y, Jeong HS, Lee J. (2007). Antioxidant activity of methanolic extracts from some grains consumed In Korea. *Food Chemistry* 103:130-138.
8. Kaviarasan S, Naik GH, Gangabagirathi R, Anuradha CV, Priyadarshini KI. *(*2007).In vitro studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum* graecum) seeds. *Food chemistry* 103**:**31-37.
9. Yen GC, Duh PD, Su HJ. (2005). Antioxidant properties of lotus seed and its effect on DNA damage in human lymphocytes *Food Chemistry* 89: 379–385.
10. Salah MS, Yousif AMH. (2009). Sequential injection titrimetric analysis of Vitamin C in drug formulation using potassium permanganate. *Journal of Flow Injection Analysis* 26 (1): 53–57.
11. Fotsing L, Boulanger B, Chiap P, Fillet M, Hubert P, Crommen J. (2000). Multivariate optimization approach for the separation of water-soluble vitamins and related compounds by capillary electrophoresis. *Biomedical Chromatography* 14(1): 10–11.
12. Heudi O, Kilinç T, Fontannaz P. (2005). Separation of water-soluble vitamins by reversed-phase high performance liquid chromatography with ultra-violet detection: application to polyvitaminated premixes. *Journal of Chromatography A* 1070 (1–2): 49–56.
13. Goldschmidt R, Wolf W. (2010). Simultaneous determination of water-soluble vitamins SRM 1849 Infant/Adult Nutritional formula powder by liquid chromatography-isotope dilution mass spectrometry. *Analytical and Bioanalytical Chemistry* 397 (2): 471–481.
14. Moreno P, Salvado V (2000). Determination of Eight Water- and Fat-Soluble Vitamins in multivitamin Pharmaceutical Formulations by High-Performance Liquid Chromatography *Journal of Chromatography A* 870 : 207–215.
15. Aslam J, Khan SH, Khan SA. (2013). Quantification of water soluble vitamins in six date palm (*Phoenix dactylifera* L.) cultivar's fruits growing in Dubai, United Arab Emirates, through high performance liquid chromatography. *Journal of Saudi Chemical society*, 17: 9-16.
16. Ranajit KS, Dipak KP. (2004). Nutrients, vitamins and mineral content in common citrus fruits in the Northern region of Bangladesh. *Pakistan Journal of Biological Sciences*. 7 (2): 238 – 242.