Available online at www.scholarsresearchlibrary.com



Scholars Research Library

Annals of Biological Research, 2012, 3 (11):5145-5150 (http://scholarsresearchlibrary.com/archive.html)



Evaluation of anti-nutrient contents of watermelon Citrullus lanatus

¹ Johnson J.T., ²Iwang E.U., ³Hemen J.T., ⁴Odey M.O., ²Efiong E.E. and ²Eteng, O.E.

¹Department of Chemical Sciences, College of Natural Sciences University of Mkar, Mkar, P.M.B. 017. Benue State Nigeria. ²Department of Biochemistry, College of Medical Sciences University of Calabar, P.M.B 1115 Calabar, Cross River State Nigeria. ³Department of Biological Sciences, College of Natural Sciences University of Mkar, Mkar, P.M.B. 017. Benue State Nigeria.

⁴*National Research Institute for Chemical Technology P.M.B.* 1052 Zaria, Kaduna State Nigeria.

ABSTRACT

The anti-nutrient contents of the pulp, seeds and rind of Citrulluslanatus were evaluated. The study was carried out on both fresh and dried samples. Results of the investigation revealed that the anti-nutritional components such as saponin, alkaloid, hydrocyanic acid, phenols, oxalate, tannins, phytates were detected in all the samples but at a varying tolerable concentration. However, other anti-nutrient constituents were in small insignificant amount in all the parts of the fruit investigated. Although there were significant difference (p < 0.05) in the anti-nutrient compounds in the different parts of the fruit, seeds and rind which are the parts always discarded on the basis of acclaim toxicity, these can contribute immensely to recommended daily allowance and maintenance of good nutritional status and hence good health for both man and animals.

Keywords: Evaluation, anti-nutrient contents, watermelon citrulluslanatus.

INTRODUCTION

Humans possess great capacity to adapt physiologically to different types of foods. In spite of this, nutrition science has demonstrated that there are certain foods that cannot be eliminated, such as fruits and fresh vegetables [1]. As reported by [2], fruits offer the most rapid methods of providing adequate supplies of vitamins, minerals and fibres to people living in the tropics. Most fruits and vegetables have low energy density and are recommended for weight management [3]. The optimal diet for everyone as recommended by the world health and food and agriculture organization is a low-fat, and fibre diet rich in complex carbohydrate characterized by a frequent consumption of fruits and vegetables are consumed in Nigeria on a daily basis, and they form an integral part of our diet. However, most times only the fleshy pulp of these fruits are consumed leaving the seed and the rind. Fruits contain a high percentage of water averaging 85%, fats and protein in very small varying amounts, a fair proportion of carbohydrate present as cellulose, starch in small quantity and sugar. Beside their low energy value, they are known for their high micronutrients concentrations including carotene or provitamin A, vitamin k, ascorbic acid, riboflavin, iron, iodine and other mineral elements [5]. The main contribution of fruits in nutrition is

Scholars Research Library

vitamins and the main source from which humans and animals derive their vitamins is from fruits and vegetables. Fruits such as pawpaw, oil palm, carrots and pumpkins provide large quantities of carotene. [2]reported a value of 200 IU each for avocado pear and passion fruit. Fruits and vegetables provide vitamin and minerals in quantities high enough to provide the body with its needs [6]. They have been linked to the management of anaemia because of their vitamin C content. When consumed with meals, they enhance iron status of the individual. Their high content of vitamin C improves absorption of iron [7]. Seeds and peels of grapes and pomegranates are also rich sources of natural antioxidant [8]. Research studies have recently shown that a diet rich in the vitamin antioxidants (Vitamin C and E) and the carotenoids is associated with improved health and a lower risk of coronary heart disease and cancer [1]. Food of vegetable origin rich in fibre, minerals and vitamins, also bring substances to the diet that although, not well understood nor classified as nutrients, display potent anti-carcinogenic and curative effects on a variety of diseases and illnesses, these substances are known as phytochemcials[1]. The fibre content of fruits and vegetables has been reported to have beneficial effects on blood cholesterol and they aid in the prevention of large bowel diseases [9, 10]. It has also been reported that populations that consume diet rich in fruits and vegetables have significantly lower rates of many types of cancers [11]. Fruits have high vitamin, mineral, fibre, phytochemcial and antioxidant in their pulps, seeds and rinds but they have not been given much importance in the diets of many Nigerians especially the seed and rind which most times are discarded. Due to ignorance of the nutritive value and their curative advantages, lack of proper storage facilities, poor distribution, rising cost of fruits, poor accessibility and affordability [12], most low income groups have not given fruit consumption much importance in their daily diet. Citrullius lanatus plant is a herbaceous creeping plant of the botanical family cucurbitaceous, which produces from 3 to 5 fruits weighing from 3 to 10 kilogram; the 'florida giant' may weigh up to 20 kilogram [1]. Citrulluslanatus fruit is round, oval or oblong, with a light green to very dark green skin, variously patterned or stripped and red, yellow or orange flesh. The seeds are flat and smooth, varying in size and may be white tan, brown, black red, green or motted[13]. The Citrulluslanatus fruit has a smooth exterior rind (green, yellow and sometimes white and a juicy, sweet interior flesh). The rind is used in preserves, jellies and conserves and to make pickles [14, 15]. Citrulluslanatus can be used for smoothes, sorbets or granite depending on the texture whether smooth or coarse. The rind is also edible and is sometimes used as vegetable [16]. In China, they are stir fried, stewed or more often pickled. The de-skinned and de-fruited rind is cooked with olive oil, garlic, chilipepper, scallions, sugar and rum. Pickled Citrulluslanatus rind is also commonly consumed in the Southern United States [17].The inner rind which is usually light green or white contains many hidden nutrients and is edible, but most times is avoided due to its unappealing flavor. It contains mainly citrulline which is a known stimulator of nitric oxide [18]. Citrulluslanatus juice can be made into wine the seeds are consumed as snacks in china, Israel and elsewhere. The pulp is cooked and seeds eaten in Sudan, Nigeria and Egypt [19]. Citrulluslanatus contains a significant amount of citrulline and after consumption of several kilograms an elevated concentration is measured in blood plasma [17]. In Africa, seed may be ground into coarse flour or oil may be extracted from them, mature fruit may be prepared and used as summer squash [20].Studies have shown that fruits and vegetables contain among other vital nutrients, an appreciable quantity of vitamins, fibre, antioxidants, phytochemicals and a daily consumption of at least 5 to 10 servings of a wide variety of fruits and vegetables is an appropriate strategy for significantly reducing the risk of chronic diseases and to meet nutrient requirement for optimum health [21]. These fruits are consumed, fresh, canned or processed and its consumption results in the production of vast amount of agricultural waste from their seeds and rind, disposal of these Agricultural wastes can have a serious environmental impact which is becoming harder to solve. Much effort will therefore be needed to develop the nutritional and industrial potential of by-products waste and these under- utilized agricultural products. Despite the numerous nutritional benefits from fruits only a small portion of plant material is utilized directly for human consumption [22], the remaining part may be converted into nutrient for either food or feed or into fertilizer. Although several research work have been done on the nutritional evaluation of some locally available fruits, [23, 24, 25, 26, 27, 28, 29, 30].Not much has been done on the nutritional and anti-nutrient contents of many locally available fruits: their pulp, seeds and rind which is most times discarded. The knowledge of the nutritive and the anti-nutrient content of various parts of these fruits will encourage their consumption in diverse ways and re-utilization of the vast amount of seeds and peels discarded as waste for human food, animal feed and fertilizer. Much has been reported on the nutritional value of fruits and how it can be used to effectively prevent nutritional deficiency in man as well as treat nutritional diseases. The nutritional value and anti-nutrient content of many fruits, seeds and their rind has not be given much attention such that most times these parts of fruit are discarded even with their hidden nutrients. The seeds and rind which are often the waste part of the fruits have not generally received much attention with a view to being used or recycled rather than discarded. Interestingly the seed and rind of some fruits have higher vitamins, fibres, minerals and other essential nutrients activity than the pulp fractions [8]. It is therefore necessary to evaluate the nutritional and anti-nutrients contents of these fruits and their waste materials so that the knowledge derived can be used to

encourage adequate consumption of fruits and re-utilization of the seeds and rind in possible value added applications.

MATERIALS AND METHODS

SOURCES OF MATERIALS

8kgs of *Citrulluslanatus* (water malon) were bought from the local markets in Calabar, Obudu and Obubra Local Government Area of Cross River State, Nigeria. The samples were bought when available in their fresh state and in sufficient quantity for the analysis.

COLLECTION AND TREATMENT OF SAMPLES

Four *Citrulluslanatus* weighing 2kgs each were used for both the nutritional analysis and phytochemical screening. The fruit were bought at different times for the nutritional and the anti-nutrient analysis.4kgs of *Citrulluslanatus*.The samples for drying were washed and cut openned with a knife into small pieces. The seeds were removed from the pulp before separating the red pulp from the rind. The seeds were washed, allowed to drain and placed on a foil. The pulp was chopped into shreds, allowed to drain and placed in another tray lined with foil, the rind was chopped into tiny cubes and placed in a separate tray line with foil. They were transferred into the oven. The dried samples were removed and grounded separately in a steel-blade grinding mill to pass through a 30-mesh sieve [31]. The samples were stored in airtight containers and labeled accordingly from which required quantities were scooped out for phytochemical screening.The fresh state. The samples were divided into two portions, one for chemical evaluation and the other for phytochemical screening.

PHYTOCHEMICAL ESTIMATION

Qualitative and quantitative analysis were carried out on each of the test samples using diverse methods viz:

Tanninswere estimated using the method of[32], Oxalate was done using the method of [33], Hydrocyanic acidwas estimated using the method of[34] while Alkaloid determination was done using the alkaloid precipitation gravimetric method described by [32].Flavonoids estimation was done using ethyl acetate precipitation graviemetric method [32].Phenols content was evaluated according to the folin-cioccitean colorimetric method [35]. However, Phytatewas estimated by spectrophotometer method as described by [36, 37].Saponin determination was done using forth and emulsion test by [32].

ANALYSIS OF DATA

The results of the proximate analysis and anti-nutrient screening were analysed for statistical significance by one way ANOVA (F- ratio)[38] and student 't' test were applicable values at (p<0.05) were regarded as significant in comparison with appropriate control. All data were expressed as MEAN \pm SEM.

RESULTS

The results of the Anti-nutrient contents of Citrulluslanatusis presented in (table 1) based on mg/100g fresh and dried matter.Statistical evaluation showed that there was no significant difference with the Saponin content of fresh *Citrulluslanatus* pulp, seed and rind at (P<0.05). The dry sample however revealed that the rind (2.93 ± 0.03) was significantly higher than the seed (2.33 ± 0.18) but lower than the pulp (3.08 ± 0.05) at (P<0.05) as shown in table 1.However, the alkaloid results showed that there was no significant difference between the pulp (0.12 ± 0.00) and seed (0.16 \pm 0.00) of fresh *Citurlluslanatus*, but the rind (1.01 \pm 0.01) was significantly higher than the pulp and seed at (P<0.05). The dry pulp (0.35 ± 0.00) and seed (0.37 ± 0.02) were not significantly different but the rind (1.42) ± 0.01) was significantly higher than the pulp and seed at (P<0.05) as shown in table 1. Beside, HCN content of fresh *Citrulluslanatus* seed (1.47 ± 0.01) was significantly higher than the pulp (0.50 ± 0.00) and rind (0.51 ± 0.01) at (P<0.05). The rind was however not significant with the pulp. HCN was only present in the dry seed (0.95 ± 0.03) of *Citrulluslanatus*, but was not detected in the pulp and rind. Tannin content of fresh *Citrulluslanatus* seed $(0.40 \pm$ 0.01) was significantly higher than the pulp (0.03 ± 0.00) but lower than the rind (1.47 ± 0.00) at (P<0.05). The dry seed (0.52 ± 0.01) was also significantly higher than the pulp (0.18 ± 0.01) but lower than the rind (1.15 ± 0.01) at (P<0.05) as shown in the table below. More so, Phytate content of fresh *Citrulluslanatus* rind (0.10 \pm 0.00) was significantly higher than the pulp (0.06 ± 0.00) but when compared with the seed (0.23 ± 0.01), was lower at (P<0.05). The dry rind (0.43 \pm 0.04) was significantly higher than the pulp (0.10 \pm 0.01) but lower than the seed (0.63 ± 0.04) at (P<0.05). Phenol content of fresh rind (0.18 ± 0.00) was significantly higher than the seed $(0.05 \pm$ DWMS

DWMR

±0.18*

2.93

±0.02*

1.42

0.00) and pulp (0.04 \pm 0.01) at (P<0.05). There was no significant difference with the pulp and seed. The dry seed (0.11 ± 0.03) was significantly higher than the pulp (0.06 ± 0.00) but lower than the rind (0.45 ± 0.04) at (P<0.05).Oxalate content of fresh *Citrulluslanatus* seed (0.11 ± 0.03) was significantly higher than the pulp ($0.09 \pm$ 0.01) and rind (0.07 \pm 0.01) at (P<0.05) but there was no significant difference with the rind and pulp at (P<0.05). The dry seed (0.08 \pm 0.03) was also significantly higher than the pulp (0.03 \pm 0.00) and rind (0.04 \pm 0.00) at (P<0.05) but there was no significant difference with the rind and pulp.Flavonoid content was also evaluated and results showed that the flavonoid content of fresh seed (40.16 \pm 0.01) was significantly higher than the rind (8.71 \pm 0.01) but lower than the pulp (58.10 \pm 0.33) at (P<0.05). The dry rind (2.63 \pm 0.02) was significantly higher than the seed (2.00 ± 0.00) but lower than the pulp (3.20 ± 0.10) at (P<0.05).

	Saponin	Alkaloid	HCN	Tannin	Phytate	Phenol	Oxalate	Flavonoids	
	1.01	0.12	0.50	0.03	0.06	0.04	0.09	58.10	
FWMP	±0.00	± 0.00	± 0.00	± 0.00	± 0.00	±0.01	±0.01	±0.33	
	1.15	0.16	1.47	0.40	0.23	0.05	0.11	40.16	
FWMS	±0.01	±0.00*	±0.01*	±0.01*	±0.01*	±0.00	±0.03*	±0.01*	
	1.24	1.01	0.51	1.05	0.10	0.18	0.07	8.71	
FWMR	±0.02	$\pm 0.01^{*,a}$	$\pm 0.01^{a}$	$\pm 0.00^{*,a}$	$\pm 0.00^{a}$	$\pm 0.00^{*,a}$	$\pm 0.01^{a}$	$\pm 0.01^{*,a}$	
	3.08	0.35	0.00	0.18	0.10	0.06	0.03	3.20	
DWMP	±0.05	±0.00	± 0.00	±0.01	±0.01	± 0.00	±0.00	±0.10	
	2.33	0.37	0.95	0.52	0.63	0.11	0.08	2.00	

TABLE 1 Anti-nutrient contents (mg/100g) of fresh and dried watermelon (Citrulluslanatus)

±0.03*,ª ±0.01*,a ±0.01*,a FWMP = fresh water melon pulp; DWMP = dry water melon pulp;

±0.01*

1.15

 ± 0.03

+0.00

0.00

FWMS = fresh water melon seed; DWMS = dry water melon seed;

FWMR = fresh water melon rind; DWMR = dry water melon rind.

±0.02*

0.43

+0.04

±0.03*

+0.03*

0.45

 ± 0.00

 $+0.01^{\circ}$

0.07

±0.00*

±0.02*^{,a}

2.63

Values are expressed as mean \pm SEM, n = 3.

p < 0.05 vs pulp; a = p < 0.05 vs seed.

DISCUSSION

Eight phytochemical components found in the fresh and dried pulp seed and rind of Citrulluslanatus were evaluated and there are saponins, alkaloids, tannins, phytates, oxalate, phenols, HCN and flavonoids, known phytochemicals with antioxidant properties. The highest content of all the anti-nutritional factors detected was in the alkaloid and saponin content of fresh and dry pulp, seed and rind. Pulp and seed of recorded a high value of flavonoidthan the other parts of the fruits although the values were low compared to earlier work. The high flavonoid noticed in the pulp and seed of Citrulluslanatus agrees with the findings of [39] who reported that natural colouring in plant based food with antioxidant, anti-inflammatory and diuretic effect is due to flavonoid as is observed in the pulp and seed of Citrullus. The toxic levels of these anti-nutrients however have not been established as reported by [40]. He also stressed that saponins have bitter taste and can reduce plant palatability, some of which are toxic to cold blooded animals at a particular concentration. There is therefore need for further research to define the role of these natural products in their host organism which have been described as poorly understood till date. Oxalate, phytate, tannin, HCN and phenols were the least in concentrations. In 1991 however, FAO/ WHO recommended that HCN levels in mammals is 10mg/kg dry weight (10ppm) which is higher than what was obtained in this study. [41]reported that phytic acid intake of 4-9mg/100g is said to decrease iron absorption by 4-5 folds in humans and the lethal level of oxalate in man is 3-5g as reported by [33]. Recently, [42] reported that a daily intake of 450mg of oxalic acid has been reported to interfere with various metabolic processes. The values obtained for phytate and oxalate are lower than the lethal dosage reported in other studies while the toxic effect of these anti-nutrients may not occur when these fruits are consumed because their levels are not enough to elicit toxicity.Tannins are known to affect the digestive tracts and their metabolites are toxic [43]. The precise toxic amount of tannin to cause depression in human is not known, but the values obtained for these phenolic substance is within the range of 0.03mg - 1.72mg/100g. From this study, it was observed that HCN and flavonoid content were more in the fresh than in the dry sample. The percentage reduction for HCN was 35.35% in the dry sample and flavonoid was 94.49 and 95.02% in the pulp and seeds of Citrulluslanatus. This result is similar to that of [44, 45, 46] who reported that numerous processing methods including some drying, roasting, heat treatment like frying, drying and boiling have been shown to reduce HCN content of protein containing foods such as legumes and a 45-50% reduction in HCN content in cassava at 50° C and 53° -60% at 70° C.[47] also reported that leaching in dry samples is due to heat treatments which results in

Scholars Research Library

a change in the solubility or the chemical reactivity of polyphenols causing an apparent decrease in assayable polyphenols.

CONCLUSION

The results obtained from this study have shown that the seeds, pulp and rind of the fruitcontain a significantly high amount of Saponin alkaloid and flavonoid in their pulp, seed and rind, the level at which the other phytochemicals occur is relatively small. However, there is a significant difference in the phytochemical content in the pulp,seed and rind of the fruit. Flavonoid was significantly high in the pulp and seed of *Citrulluslanatus*. Alkaloid, Tannin, Phenol were significantly high in the rind of the fruit compared with the pulp and seed while Phytate and Oxalate were significantly high in the seeds compared with the pulp and rind of each fruit. The anti-nutrient compounds in the different parts of the fruits, the seeds and rind which are the parts always discardedwhere below FAO/ WHO recommended save levels. Thus the pulp and rind can contribute immensely to recommended daily allowance and maintenance of good nutritional status and hence good health for both man and animals.

REFERENCES

[1]. Pamplona-roger, G. D, Healthy Foods. First Edition, San Fernando de Henares, Madrid, Spain: European Union, **2008**.

[2]. Ngoddy, P. O. & Ihekoronye, A. I, Integrated food science and technology. First Edition, Macmillan Publication London, **1985**, 293-311.

[3]. Rolls, B. J. & Ello-martin, J. A. & Johill, B. C, Nutrition Review, 2004, 62:1-17.

[4]. WHO & FAO, Diet, nutrition & the prevention of chronic diseases: In report of a joint WHO/FAO Expert consultation. Geneva: WHO.2003

[5]. Shiundu, K, M, AJFNS, 2004, 2(2)96-97.

[6]. Fraser, D. M. & Cooper, M. A, *Elsevier Sciences*, 2006, 4(7)333-336.

[7]. Wardlaw, G. M. & Hamplh, J. S, Perspective in nutrition Seventh Edition. New York: McGraw Hill**2007**, 244 - 54.

[8]. Jayaprakasha, G. K. Singh, R. P. & Sakariah, K. K, *Foodchemistry*, **2001**, (73)285-90.

[9]. IFT, Institution of food technology quality of foods and vegetables: A scritafic status summary by the last of food technology expert. Panel on food safety of nutrition, 1990, 44(6)15.

[10]. Jenkins, D. J. A, British Medical Journal, 1978, (1)1392-4.

[11]. Voorrips, L., E., Goldbohm, R. A., Van Poppel, G., Sturmans, F., Hermus, R. J. J. & van den Brandt, P. *epidemiology*,**2000**, 152(11)1081-1092.

[12]. Tindall, H. D, Rambutan cultivation: FAO plant products and protection Paper 121, Rome.2004, 163.

[13]. FAO, Legumes in Human Nutrition: Food and Agricultural Organization Report of the United Nations, 1994.

[14]. Dane, F. Lang & P., Bakhtiyarova, R, *Theoretical Appllied Genetics*, 2004, (108)958-966.

[15]. Dane, F. & Liu, J, Genet Resource Crop Evolution, 2007, (54)1255-1265.

[16]. Wada, M, Biochemistry, 1930, 2(4)221-229.

[17]. Mandel H, Levy, N., Izkovitchs&Korman, S. H, Berichte der deutschenchemischen Gesellschaft, 2005, 28(4):467-472.

[18]. Science Daily, **2008**-05-16.

[19]. Goda, M, Biodiversity centre, 2007, 3(5)17-23.

[20]. U S Department of Agriculture and U S Department of Health and Human service dietary guidelines for Americans, Washington, DC: U S government printing office**2003** and **2006** respectively.

- [21]. Liu, R. H, Journal of nutrition, 2004, (4)134:3475.
- [22]. El-adawy, T. A., Palma E. H., El-bedawy, A. A.&Gafar, A. M, Nahrang, 1999, (43)385-391.
- [23]. Obizoba, A. S., Vellon, L., Colomer, R; & Lupu, R, Journal BiologyApplied Chemistry, 2005, (12)14-18.
- [26]. Adepoju, O. T. & Adeniji, P. O, Journal Nutrition Science, 2008, 5(29)15-23.
- [27]. Ene-obong, H. N, Eating right: University of Calabar Press, Calabar.2001, Pp 39-49.
- [29]. Essien, E. U, Plant Food Human Nutrition,1994,(45)47-51.
- [30]. Dye, V. B,weed, 1956, (4)55-60.
- [31]. AOAC, Official Methods of Analysis: Association of Official Analytical Chemist, 1991.

[32]. Harbone, J.B, Phytochemical methods. A guide to modern technique of plant Analysis: Chapman and Hall, London, New York, **1973**, 182-191

[33]. Munro, A. & Bassir, O, Journal BiologyApplied Chemistry, 1973, (12)14-18.

- [34]. Balogoplan, C, Cassava in food, feed and industry: C R C press Incorperation. Florida, 1998, 187-89
- [35]. AOVC, Association of Vitamin chemist: Methods of vitamin Assay. Third Edition, Interscience publication John Wiley & Sons New York, **1990**, 97, 123.
- [36]. Onwuka, G. J, Food Analysis and Instrumentation, theory and practice. First Edition, Naphtali prints. Lagos. Nigeria, **2005**, Pp. 1-219.

[37]. Oberleas, D, The determination of phytate and inositol phosphates: In glick D(ed.) methods of Biochemical Analysis. Willey: New York, **1983**, pp325-329.

[38]. Welkowitz, R. S, Statistic for biomedical research. Washington: Howard press, 1978, pp18-21.

[39]. FAO/WHO, Joint FAO/WHO food standards programmes: In: CordexAlimentarius Commission, xii (Suppl. 4). Rome: FAO, 1991.

[40]. Forester, H, American JournalClinial Nutrition, 2006, 103(15)66-71.

[41]. Hurrel, R. F., Jullert, M. B., Reddy, S. R., Lynch, S. D., & Cook, J. D, American JournalClinial Nutrition, 1992, (56)573-578.

[42]. Amaowo, E. U., Ndon, B. A., & Etuk, E.U. Mineral and antinutrient in fluted pumpkin *Telferaoccidentalis:* Hook F. (ed) *Food Chemistry*, **2000**, 70:235-240.

[43]. Ene-obong, H. N. Nutritional evaluation, consumption pattern and processing of wider exploited traditional plant food with particular reference to the African yam bean (*Sphenostylussternocarpa*). Ph. D thesis, University of Nigeria, Nsukka, **1992**.

[44]. Coursey, D. G. Cassava as food: Toxicity and Technology. In: chronic cassava toxicity (edited by B. L. Nestle and R. MacIntyre). International Development Research Centre: Ohawa, Canada, **1973**, PP 27-36.

[45]. Wannick, T. M, Journal of plant foods for Human Nutrition, 1993, (46)117-120.

[46]. Nambisan, B. Acta Horticulture, 1994, (375)193-201.

[47]. Butler, L. G. Effects of condensed tannins on Animals nutrition in Chemistry and Significance of condensed Tannis: R. W. Heming way and J. J. Karchesy Edition: Plenum Press New York, **1989**, pp391-402.