



## Effect of Thermal Processing on Elimination of Antinutritional Factors in *Irvingia smithii* Almond

**V.G. Gindo Mbaya<sup>1\*</sup>, P.E. Sumbu Zola<sup>2</sup>, E. Kimbemuken Thasur<sup>3</sup>, D. Mayele Kipoy<sup>4</sup>**

<sup>1,2,3,4</sup>Department of Chemistry and agricultural Industries, Faculty of Agronomy, University of Kinshasa, Democratic Republic of Congo

Email: gindombaya@gmail.com

### **Abstract:**

*The objective of this work is to identify and eliminate antinutritional factors in the fruit kernel of *Irvingia smithii* (wild mango tree) from the KISANTU botanical garden (DRC) by thermal effect at different temperatures (T1: sun drying, T2: roasting in an oven at 130° C for 30 minutes, T3: boiling for 45 minutes) as a function of time. It appears from the results of our analyzes that the fruit kernels of *Irvingia smithii* contain antinutritional factors in low proportions: phytates (536.34mg/100g), oxalates (14.84mg/100g), nitrates (12.71mg/100g, 8), and nitrites (50.15mg/100g.). Saponins were not found in these almonds. Treatment of almonds by boiling for 45 minutes (T2 treatment) proved to be the best eliminator of antinutritional factors (60%) followed by treatment of almonds by roasting (T3) (43%), probably by the dissolution of antinutritional factors in medium aqueous (hydrolysis) and their evaporation by boiling which takes place during cooking. Wild mango (*Irvingia smithii*) levels of antinutrients are low enough to cause poisoning in their consumers; they have not yet been detected in human tissue or the urine of their consumers. We recommend that the roasting of the almond is done before cooking which is done by boiling, a process which will eliminate almost all the antinutritional factors.*

### **Keywords:**

*wild mango; *Irvingia smithii*; antinutritional factors; thermal effect*

## I. Introduction

Nowadays, non-timber forest products take an important place in cultural and economic development of local population depending on ecosystems (Awono and al., 2009; Vermeulen and al., 2009; Debroux and al., 2007).

Among them, four non-timber forest products are the most commercialized and consumed in central Africa are: *Dacryodes edulis* fruits, *Ricinedendron heudelotti* seeds, almonds of *Irvingia gabonensis* and *Irvingia wombulu* (Awono and al., 2009; Silou and al., 2004; Vermeulen, 2009).

*Irvingia smithii*, subject of the present study, is a wild mango tree in the family of Irvingiaceae (Tobe and Raven, 2001) widely distributed in dense humid forests and gallery forest of Africa. Its natural distribution area extends from Nigeria to Angola via the Gulf of Guinea, Cameroon, Gabon, the Central African Republic, the Democratic of Congo and the Congo Brazzaville (Anonyme 1, 2008; Tailfer, 1989).

Its pulp is less consumed such as the mango one, hence the name of the wild mango tree attributed to its tree (Gindo, 2017). But its almond is widely used to season dishes and thicken sauces in the same way as with peanuts and squash (Silou, 2004; Loumouamou et al., 2013; Gindo et al., 2015).

Studies on nutritional quality and chemical composition of *Irvingia smithii* show that its organic matter contains variable amounts of bioactive substances (proteins, especially lipids and minerals) and toxic and/or antinutritional chemical substances (alkaloids, oxalates, phenols, phytates, cyanides, nitrates, nitrites, etc.) (Drogba, 2010).

In addition, similar studies reveal the presence of low amounts of antinutritional factors in the kernels of wild mangoes of the genus *Irvingia*: *Irvingia gabonensis* (Ogungbenle, 2014; J.E Ayivor et al, 2011; Abena et al., 2013), *Irvingia wombolu* (Ekpo et al, 2007) and *Irvingia smithii* (Gindo, 2015). Some of these antinutritional factors could associate with food components and lead to harmful consequences for consumers.

Moreover, the techniques for eliminating antinutritional factors generally involve imbibition in an aqueous medium (hydrolysis) and/or heating (boiling) or simply drying (evaporation) (Simon-Gérard, 1980; Bokanga, 1996; FAO, 2008).

Present study aimed at identifying antinutritional factors in almonds of *Irvingia smithii* and disposing of them by thermal treatment at different temperature as a function of time.

## II. Material and Methods

### 2.1 Material

Biological material used to carry out the present study consisted of fruits of *Irvingia smithii* harvested at the Botanical Garden of Kisantu, province of Kongo-Central (DRC), on 24 June 2015.

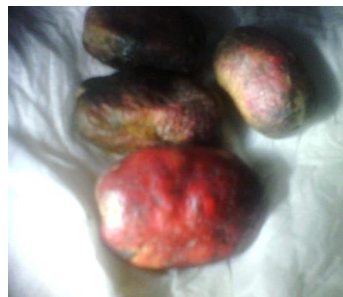
### 2.2 Methods

#### a. Sampling

The whole fruits were dried using solar energy for two weeks, then crushed in order to recover the almonds which were the subject of analyses.



**Figure 1.** Fresh fruits of *I. smithii*



**Figure 2.** Fruits being dried



**Figure 3.** Almonds of *I. smithii*

Almonds were separated into four groups at a rate of 20 grams per batch to undergo four different treatment modalities:

- T0: 20g almonds that have not undergone any heat treatment (control)
- T1: 20g almonds dried by sun exposure during one week
- T2: 20g almonds boiled for 45 minutes
- T3: 20g almonds roasted at 120°C for 30 minutes



*Figure 4. Batches of Irvingia smithii sample*

After drying, the different samples (T0, T1, T2, T3) subsequently underwent grinding using the Moulinex brand PHILIPPS HR2027. Powder obtained has been defatted by soxhlet using ether. Remaining cakes were dried in an oven at 50°C for 24 hours, and stored in hermetically sealed plastic jars before being analyzed.

#### **b. Chemical Analysis**

Moisture content determination and delipidation have been realized in the Laboratory of Food research and nutritional analysis, Faculty of Sciences, University of Kinshasa (DRC), whereas determination of antinutritional factors has carried out the Mining and geological Research Centre (CRGM) and in the Veterinary Laboratory of Kinshasa (Labovet).

Determination of the water content was carried out using the classic method of drying in an oven (brand MEMMERT model 100-800) at 105°C as described by Vervack (1982) with an accuracy balance (OHAUS brand).

Phytic acid was measured by colorimetry at 830 nm using a spectrophotometer (HACH DR 2000) by exploiting its ability to form colored solutions with an ammonium molybdate and mercury reagent in a sulfuric acid medium. Solution absorbance is proportional to the concentration of phytic acid according to the Lambert–Beer law.

Oxalic acid was measured by colorimetry at 420 nm using a spectrophotometer (HACH DR 2000). Its absorbance at this maximum obeys the Lambert–Beer law; thus, it can be dosed, first, by drawing a calibration line from solutions of known concentrations. Saponins were qualitatively determined by the formation of foam in the filtrate (Trease and Evans, 2002).

Nitrates and nitrites were measured by colorimetry respectively at 500 nm and 585 using a spectrophotometer (HACH DR 2000).

The metallic cadmium reduces the nitrate present in the sample to nitrite in an acid medium.

Nitrite forms with sulphanilic acid an intermediate which is a diazonium salt. The diazonium salt couples with gentisic acid to form an amber-colored solution whose optical density at 500 nm is proportional to the nitrite content and therefore nitrate content. The nitrite ions present in a sample are reduced in an acid medium by iron (II) sulphate to nitrogen monoxide (NO) which combines with the Fe<sup>++</sup> ions, forming a complex of greenish color whose absorbance at 585 nm is proportional to the concentration of nitrites present in the sample (Vervack, 1982; Mbemba et Remacle, 1992; Fouassin et Noirfalèse, 1995 et Lutete, 1994; 21. Samir and al., 2014).

### c. Statistical Analysis

Means and standard deviations were used to describe the data; while the statistical analysis referred to the analysis of variance using STATISTIX software version 8.0. The Student's T test, tests of multiple comparison, of LSD, at the 5% threshold allowed to examine the differences observed between the means of the groups (difference between the treatments).

## III. Results and Discussion

### 3.1 Results

Results of the determination of the levels and elimination of the antinutritional factors of different treatments of almond samples of *Irvingia smithii* are given in tables 1 and table 2.

**Table 1.** Residual Antinutritional Factors Content in *Irvingia smithii* Almonds after Thermal Treatment (mg/100g)

Treatments/ time	Parameters measured (mg/100g)				
	Phytates	Oxalates	Nitrates	Nitrites	Saponins
<b>T<sub>0</sub> (Control)</b>	536,34±1,43	14,84±1,5231	12,71±0,3601	51,15±1,6519	-
<b>T<sub>1</sub>: solar/ 7days</b>	391,53±1,313	12,15±1,5706	8,4±1,1177	40,64±1,1903	-
<b>T<sub>2</sub>: Ebullition/ 45 minutes</b>	240,6±1,745	6,34±0,5622	4,66±0,5396	18,03±0,8703	-
<b>T<sub>3</sub> : toasting at 120°C/ 30 minutes</b>	340,54±1,1829	8,33±0,5211	6,19±0,3231	31,53±1,0420	-

#### Legend:

T<sub>0</sub> : almonds that have not undergone any heat treatment (control)

T<sub>1</sub> : almonds dried by sun exposure during one week

T<sub>2</sub> : almonds boiled for 45 minutes

T<sub>3</sub> : almonds roasted at 120°C for 30 minutes

**Table 2.** Amount of Antinutritional Factors Eliminated due to Thermal Treatment According to the Time

Treatment/times	Eliminated antinutritional factors (%)				
	Phytates	Oxalates	Nitrates	Nitrites	Means
T <sub>1</sub> : solar/7j	30	18	34	21	25,75
T <sub>2</sub> : Ebullition/45 minutes	66	52	64	65	61,75
T <sub>3</sub> : Roasting at 120°C/30 minutes	37	44	51	39	42,75

**Legend :**

T<sub>0</sub> : almonds that have not undergone any heat treatment (control)

T<sub>1</sub> : almonds dried by sun exposure during one week

T<sub>2</sub> : almonds boiled for 45 minutes

T<sub>3</sub> : almonds roasted at 120°C for 30 minutes

**3.2 Discussion**

Tables 1 and 2 show an overall reduction in the levels of antinutritional factors in almonds having undergone heat treatments at different temperatures (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>) compared to control (T<sub>0</sub>) not subjected to any heat treatment.

Antinutritional factors content found in the Control (T<sub>0</sub>) corroborates those found by Gindo et al. (2015) in wild mango kernels of the species *Irvingia smithii* from the DRC.

The analysis of variance of the chemical composition of the wild mango almonds (*Irvingia smithii*) subjected to the thermal effect shows that there is a large significant difference in the elimination of the antinutritional factors between the treatments having been subjected to the thermal effect (T<sub>1</sub>, T<sub>3</sub>, and T<sub>2</sub>).

The LSD multiple comparison t-test at the probability threshold 5% shows these differences in elimination of antinutrients between treatments:

- *for the phytates*: almonds subjected to boiling treatment (T<sub>2</sub>) recorded a significant elimination of antinutritional factors (66%) (295,54 mg/100g) followed by that of roasted almonds (T<sub>3</sub>) which eliminated 37% (195,80 mg/100g); sun dried almonds recorded (T<sub>1</sub>) showed an élimination of 30% (144 ,81 mg/100g).
- *for the nitrites*: an significant elimination (65%) has been recorded for almonds submitted to boiling treatment (T<sub>2</sub>) (33,12 mg/100g), followed by the roasted almonds (T<sub>3</sub>) which eliminated 39% (19,62 mg/100g). Sun dried almonds (T<sub>1</sub>) eliminated 21% (10,51 mg/100g).
- *for the oxalates*: T<sub>2</sub> eliminated 52 % (8,51 mg/100g), followed by (T<sub>3</sub>) with 44% (6,51 mg/100g) and then (T<sub>1</sub>) that eliminated 18% (soit 2,69 mg/100g).
- *for the nitrates*: a low elimination level has been recorded (34%) by T<sub>1</sub> (4.31 mg/100g); roasted almonds (T<sub>3</sub>) eliminated 51% (6,55 mg/100g); the high elimination (64 %) was recorded by T<sub>2</sub> (soit 8,05 mg/100g).

These results show that boiling treatment (T<sub>2</sub>) is the best eliminator (62% on average) followed by the roasting treatment, T<sub>3</sub> (43%).

The dissolution of antinutritional factors in aqueous media (solubilization) which is accompanied by hydrolysis and their evaporation by boiling during cooking would probably be the basis of this significant elimination. The T2 treatment is a combined treatment of hydrolysis and evaporation (Bokanga, 1996; Simon-Gérard, 1980).

#### IV. Conclusion

Present study aimed to identifying and eliminating different antinutritional factors present into almond of *Irvingia smithii* fruits from botanical garden of Kisantu, using thermal processing.

Our analyzes show that the fruit kernels of *Irvingia smithii* do not contain saponin, but contain a low proportion of phytates (536.34 mg /100g), oxalates (14.84 mg/100g), nitrates (12.71 mg/100g,) and nitrites (50.15 mg/100g).

Thermal processing has played an important role in the elimination of antinutritional factors, mostly with T2 (ebullition) that eliminated 62 % of these compounds. This is the best eliminating treatment before T3 (43%) and T1 (26%).

Thanks to the present study, consumers are recommended to proceed first by roasting and pre-grinding of the almonds of wild mango (*Irvingia smithii*) before cooking, as for other seeds rich in protein lipid (peanuts, squash).

#### References

- Abena A. B. (2013). Assessment of some health beneficial constituents of edible portions of four underutilized fruits, A Thesis submitted to the Department of Food Science and Technology, Kwame Nkrumah University of Science and Technology in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE, Department of Food Science and Technology, College of Science.
- AFNOR (Association Française de Normalisation), 1981. Corps gras, graines oléagineuses et produits dérivés. Recueil des normes françaises. AFNOR, Paris (France) 2ème éd.438p.
- Anonyme, 2008. Mangue sauvage *Irvingia* spp. CIFOR Central Africa C/o IITA Humid Forest Ecoregional Center, B.P. 2008 Yaounde, Cameroon Tel: +237 222 74 49 / +237 222 74 51 Fax: +237 222 74 50 E-mail: cifor.cameroon@cgiar.org www.cifor.cgiar.org
- Awono A., Manirakiza D. Et Ingram V., 2009. « Mobilisation et renforcement des capacités des petites et moyennes entreprises impliquées dans les filières des produits forestiers non ligneux en Afrique centrale » Etude de Base Du Ndo'o (*Irvingia spp.*) dans les Provinces du Centre, Sud et Littoral Cameroun, CIFOR/GCP/RAF/408/EC Yaoundé, Janvier 2009, 99 pages.
- Ayivor J.E., Debrah S.K., Nuviadenu C. and Forson A., 2011. Evaluation of Elemental Contents of Wild Mango (*Irvingia gabonensis*) Fruit in Ghana, Advance Journal of Food Science and Technology 3(5): 381-384, 2011.
- Bokanga, M., 1996. Biotechnologie et transformation du manioc en Afrique. La Recherche à l'IITA; Institut International d'Agriculture Tropicale, Ibadan, pp38 – 50.
- Debroux L., Hart T., Kaimowitz D., Karsenty A., et Topa G., 2007. Les forêts en république démocratique du Congo post conflit : analyse d'un agenda prioritaire, Eds 2007.
- Drogba, Alexis Sahoré, 2010. Propriétés physico-chimiques et fonctionnelles des tubercules et des amidons d'Ignames (*Dioscorea*). Cas de quelques espèces d'Ignames spontanées.

- Drogba Alexis Sahoré, Jean Gnopo Nemlin, Achille Fabrice Tetchi, 2012. Study of Physicochemical Properties of Some Traditional Vegetables in Ivory Coast: Seeds of *Beilschmiedia mannii* (Lauraceae), Seeds of *Irvingia gabonensis* (Irvingiaceae) and *Volvariella volvacea* *Food and Nutrition Sciences*, 2012, 3, 14-17.
- Ekpo, I. W.; Amor, I. D. and Morah, F. N. I., 2007. Seed oils and nutritive studies on the seeds of *gabonensis* and *wombolu* varieties of *irvingia gabonensis*, The Nigerian Academic Forum, Volume 13 No. 1, 3 pages, November, 2007.
- FAO, 2008. Le sorgho et les mils dans la nutrition humaine – Inhibiteurs nutritionnels et facteur toxiques. Archives de documents de la FAO, 9p.
- Gindo Mbaya V.G., Sumbu Zola L.P.E., Silou Thomas, Tshiombe Mulamba V.E. et Akumbakinayo M. P., 2015. Contribution to the chemical characterization of wild mango kernels of *Irvingia smithii* species (*Irvingiaceae*) in DRC / Congo Basin. *International Journal of Agricultural and Food Science* 2015; 5(1): 27-32.
- Gindo Mbaya V.G., 2017. Composition chimique et étude nutritionnelle des amandes de mangues sauvages du genre *Irvingia* de la Rd Congo, Usages et perspectives de développement durable des oléagineux du Bassin du Congo, Thèse de Doctorat Unique, Faculté des Sciences et Techniques/Université Marien Ngouabi de Brazzaville, 178 pages.
- Loumouamou B.W., Gomoufatan J.P.M, Silou T., Nzikou J.M., Gindo Mbaya V.G., Figueredo G. And Chalard J.P., 2013. Extraction and Chemical Composition of Seed Kernel Oil from *Irvingia smithii* of Congo Basin. *Advance Journal of Food Science and Technology* 5(5): 506-513.
- Mbemba F. et Remacle J., 1992. Inventaire et composition chimique des aliments et denrées alimentaires traditionnelles du Kwango-Kwilu du Zaïre, Presse universitaire.
- Ogungbenle Henry Niy, 2014. Chemical and Amino Acid Composition of Raw and Defatted African Mango (*Irvingia gabonensis*) Kernel, *British Biotechnology Journal* 4(3): 244-253, 2014.
- Samir. B.Y., Oboh. M., 2014. Introduction à l'enseignement de toxicologie
- Silou T., Biyoko S., Heron S., Tchaplà A. et Maloumbi M.G., 2004. Caractéristiques physico-chimiques et potentialités technologiques des amandes de *Irvingia gabonensis* *Rivista Italiana delle sostanze Grasse*; 81, 49 - 57.
- Simon-Gérard E., 1980. Foods consumed and Endemic Goiter in the Ubangi. In Role of cassava in the ethiologie of endemic goiter and cretinism. Ermans A.M., Mbulamoko N., Delenge F. and Ahluwalia (Ed.) *International Development Research Center (IDRC)*, Ottawa, Canada, pp38 – 50.
- Tailfer Y., 1989. La forêt dense d'Afrique centrale; Identification pratique des principaux arbres: CTA postbus wageningen; 380 pages.
- Tobe H. Et Raven P.H.J., 2001. Embryology of the *Irvingiaceae*, a family with uncertain relationship among the Malpighiales; *Journal of Plant Resources* 124; 577-591 p.
- Vermeulen C., Schippers C., Julve C., Ntouné M.F.D., Bracke C., Doucet J.-L., 2009. Enjeux méthodologiques autour des produits forestiers non ligneux dans le cadre de la certification en Afrique centrale; *BOIS ET FORÊTS DES TROPIQUES*, 2009, N°300 (2); 69 -79 pp.
- Vervack, W., 1982. Méthode d'analyse des aliments, Faculté des sciences agronomiques. Laboratoire de biochimie de la nutrition, UCL, Louvain-la-Neuve.

## **Annex 1: Basic Datas**

*Table 3. Residual antinutritional factors content in Irvingia smithii almonds after thermal treatment (mg/100g)*

Treatments	Repetitions	Parameters measured in mg/100g				
		Phytates	Oxalates	Nitrates	Nitrites	Saponins
T <sub>0</sub>	1	536,71	16,51	12,55	52,02	absents
	2	534,77	13,52	13,13	49,11	absents
	3	537,56	14,51	12,47	51,92	absents
	Average	536,34	14,84	12,71	51,15	
T <sub>1</sub>	1	392,93	10,75	8,03	42,02	absents
	2	390,33	13,85	7,52	39,91	absents
	3	391,33	11,86	9,66	40,01	absents
	Average	391,53	12,15	8,4	40,64	
T <sub>2</sub>	1	242,33	6,07	4,5	19,04	absents
	2	238,84	5,97	5,27	18,03	absents
	3	240,63	6,99	4,23	17,04	absents
	Average	240,6	6,34	4,66	18,03	
T <sub>3</sub>	1	341,53	7,83	5,92	32,4	absents
	2	339,23	8,29	6,11	30,42	absents
	3	340,86	8,87	6,55	31,77	absents
	Average	340,54	8,33	6,19	31,53	
		532,74	15,51	13,51	53,01	absents

### **Legend :**

T<sub>0</sub> : almonds that have not undergone any heat treatment (control)

T<sub>1</sub> : almonds dried by sun exposure during one week

T<sub>2</sub> : almonds boiled for 45 minutes

T<sub>3</sub> : almonds roasted at 120°C for 30 minutes

## **Annex 2 : Statistical analysis**

### **Phytic acid**

Statistix 8.0  
11:18:14

04/04/2016,

#### **Randomized Complete Block AOV Table for parametre**

Source	DF	SS	MS	F	P
repetitio	2	13	6.6		
Traitemen	3	136526	45508.7	78014.9	0.0000
Error	6	3	0.6		
Total	11	136543			

Grand Mean 376.67 CV 0.20

**Tukey's 1 Degree of Freedom Test for Nonadditivity**

Source	DF	SS	MS	F	P
Nonadditivity	1	0.37075	0.37075	0.59	0.4763
Remainder	5	3.12925	0.62585		

Relative Efficiency, RCB 2.73

**Means of parameter for Treatment**

Treatment	Mean
T0	536.34
T1	391.53
T2	240.60
T3	340.54
Observations per Mean	3
Standard Error of a Mean	0.4410
Std Error (Diff of 2 Means)	0.6236

Statistix 8.0  
11:21:51

04/04/2016,

**LSD All-Pairwise Comparisons Test of parameter for Treatment**

Treatment	Mean	Homogeneous Groups
T0	536.34	A
T1	391.53	B
T3	340.54	C
T2	240.60	D

Alpha 0.05 Standard Error for Comparison 0.6236  
Critical T Value 2.447 Critical Value for Comparison 1.5259  
Error term used: repetitio\*Traitemen, 6 DF

**All 4 means are significantly different from one another.**

**Oxalates**

Statistix 8.0  
13:24:41

03/04/2016,

**Randomized Complete Block AOV Table for parameter**

Source	DF	SS	MS	F	P
repetitio	2	0.500	0.2500		
traitemen	3	133.583	44.5278	43.32	0.0002
Error	6	6.167	1.0278		
Total	11	140.250			

Grand Mean 9.7500 CV 10.40

**Tukey's 1 Degree of Freedom Test for Nonadditivity**

Source	DF	SS	MS	F	P
Nonadditivity	1	0.05271	0.05271	0.04	0.8437
Remainder	5	6.11395	1.22279		

Relative Efficiency, RCB 0.82

**Means of parameter for treatment**

<b>treatment</b>	<b>Mean</b>
T0	14.833
T1	12.553
T2	6.347
T3	8.337
Observations per Mean	3
Standard Error of a Mean	0.5853
Std Error (Diff of 2 Means)	0.8278

Statistix 8.0  
13:26:18

03/04/2016,

**LSD All-Pairwise Comparisons Test of parameter for treatment**

<b>treatment</b>	<b>Mean</b>	<b>Homogeneous Groups</b>
T0	14.833	A
T1	12.553	B
T3	8.337	C
T2	6.347	D

Alpha 0.05      Standard Error for Comparison 0.8278  
 Critical T Value 2.447      Critical Value for Comparison 2.0255  
 Error term used: repetitio\*traitemen, 6 DF

***There are 3 groups (A, B, etc.) in which the means are not significantly different from one another.***

**Nitrates**

Statistix 8.0  
13:28:13

03/04/2016,

**Randomized Complete Block AOV Table for parameter**

<b>Source</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
repetitio	2	2.167	1.0833		
traitemen	3	112.000	37.3333	448.00	0.0000
Error	6	0.500	0.0833		
Total	11	114.667			

Grand Mean 7.6667      CV 3.77

**Tukey's 1 Degree of Freedom Test for Nonadditivity**

<b>Source</b>	<b>DF</b>	<b>SS</b>	<b>MS</b>	<b>F</b>	<b>P</b>
Nonadditivity	1	0.00549	0.00549	0.06	0.8230
Remainder	5	0.49451	0.09890		

Relative Efficiency, RCB 3.02

**Means of parameter for treatment**

<b>traitemen</b>	<b>Mean</b>
T0	12.713
T1	8.373
T2	4.663
T3	6.197
Observations per Mean	3
Standard Error of a Mean	0.1667

Std Error (Diff of 2 Means) 0.2357

Statistix 8.0  
13:29:11

03/04/2016,

**LSD All-Pairwise Comparisons Test of parameter for treatment**

treatment	Mean	Homogeneous Groups
T0	12.713	A
T1	8.373	B
T3	6.197	C
T2	4.663	D

Alpha 0.05 Standard Error for Comparison 0.2357  
 Critical T Value 2.447 Critical Value for Comparison 0.5767  
 Error term used: repetitio\*traitemen, 6 DF

**All 4 means are significantly different from one another.**

**Nitrites**

Statistix 8.0  
13:31:23

03/04/2016,

**Randomized Complete Block AOV Table for parameter**

Source	DF	SS	MS	F	P
repetitio	2	4.17	2.083		
traitemen	3	1800.33	600.111	3086.29	0.0000
Error	6	1.17	0.194		
Total	11	1805.67			

Grand Mean 35.167 CV 1.25

**Tukey's 1 Degree of Freedom Test for Nonadditivity**

Source	DF	SS	MS	F	P
Nonadditivity	1	0.12640	0.12640	0.61	0.4710
Remainder	5	1.04027	0.20805		

Relative Efficiency, RCB 2.63

**Means of parameter for treatment**

traitemen	Mean
T0	51.153
T1	40.643
T2	18.000
T3	31.530
Observations per Mean	3
Standard Error of a Mean	0.2546
Std Error (Diff of 2 Means)	0.3600

Statistix 8.0  
13:32:12

03/04/2016,

**LSD All-Pairwise Comparisons Test of parameter for treatment**

<b>treatment</b>	<b>Mean</b>	<b>Homogeneous Groups</b>
T0	51.153	A
T1	40.643	B
T3	31.530	C
T2	18.000	D

Alpha 0.05 Standard Error for Comparison 0.3600  
Critical T Value 2.447 Critical Value for Comparison 0.8810  
Error term used: repetitio\*traitemen, 6 DF  
**All 4 means are significantly different from one another.**