Chemical and Process Engineering Research
ISSN 2224-7467 (Paper) ISSN 2225-0913 (Online)
Vol.22, 2014

Effect of Processing on Fatty Acid and Phospholipid Compositions of Harms (*Brachystegia eurycoma*) Seed Grown in Nigeria

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Abstract

A comprehensive study on the effect of processing on fatty acid and phospholipid compositions of *Brachystegia eurycoma* seed flour was conducted. Processing methods (boiling, fermentation and roasting) were adopted using standard analytical techniques. The most concentrated fatty acids (%) were linoleic acid (47.95 – 50.91) > oleic acid (26.51 – 30.91) > palmitic acid (11.51 – 14.16) > stearic acid (3.06 – 5.54). Lenoceric, erucic, and arachidic acids were present with none of them recording up to 1% while caprylic, capric and margaric acids were not at the detection limit of GC. All the processing methods increased the contents of palmitic, palmitoleic, linoleic and linolenic acids. The oleic acid content was reduced in boiled, fermented and roasted samples by 60.93, 59.97 and 63.77%, respectively. The phospholipid analysis gave result (%) of phosphatidic > phosphatidylinositol > phospatidyserine > phosphatidyethanolamine concentrations. Generally, the processing methods showed deviations in fatty acid and phospholipid components from the raw seeds. There was a clear indication that the raw and processed samples of B. eurycoma seed oils contained a high level of polyunsaturated fatty acids, making them a healthy low fat food.

Keywords: *Brachystegia eurycoma*, processing, seed oils, fatty acids, phospholipids.

1 Introduction

There has been a growing interest in African plants such as African oil bean (*Pentaclethra macrophylla*), locust bean (*Parkia clapperboniana*), sugar nut (*Irvingia gabonensis*), bread fruit (*Artocarpus altilis* Park), cashew nut (*Anarcadium occidentale*), guava (*Psidium guajava*), African star apple (*Chrysophyllum albidum* L.), akee apple (*Bilphia sapida*), mesquite bean (*Prosopis africana*), mango (*Mangifera indica*), pear (*Pyrus communis* L.), etc. which are useful as food supplements. They provide fat, protein, fibre and carbohydrate needed for the body (Adeyeye, *et al.,* 1999; Adeyeye & Agesin, 1999; Akintayo *et al.,* 2002; Omafurbe *et al.,* 2006; Aremu *et al.,* 2007). The success of utilizing plant proteins as additives depends greatly upon the favourable characteristics that they impart to foods. In the developed countries, plant proteins are now either regarded as versatile functional ingredients or as biologically active components more than essential nutrients (Marcello & Gius, 1997).

Brachystegia eurycoma (Harms) belongs to the family Leguminosae–Caesalpiniodeae and order Fabaceae. It is a dicotyledonous and woody plant found in the forest zone. The tree is about 35 m tall, with bole of 2 m diameter. It is ragingly buttressed, has low branching, large flat crown, common on river banks of the forest zone in southern Nigeria and Cameroon (Olubunmi & Oremeyi, 2011). The tree flowers between September and January each year (Keay, 1986). The seeds are dispersed by explosive mechanism (Oke & Omoh, 1978) are used in food majorly as a soup condiment and flavouring agent.

Many workers (Burkill, 1985; Eze & Ibe, 2005; Ajayi *et al.,* 2006; Friday *et al.,* 2009; Ikegwu *et al.,* 2010; Olubunmi & Oremeyi, 2011) have reported the economic importance, compositional evaluation, nutritive and anti–nutritive properties of *Brachystegia eurycoma* seeds. The present study was aimed at drawing attention to the effect of processing on the fatty acid and phospholipid compositions of *Brachystegia eurycoma* seeds with a view to providing useful information towards effective utilization of this underutilized leguminous plant seeds in various food applications..

2 Materials and Methods

2.1 Collection of samples

The seeds of *Brachystegia eurycoma* were obtained from Eke Ata market in Imo State, Nigeria. The seeds were thoroughly separated from impurities, dehulled and divided into four portions for processing methods which are raw, boiling, roasting and fermentation.

2.2 Preparation of different processing methods of the seed of *B. eurycoma*

2.2.1 Raw sample

The raw seeds were dehulled by gently roasting the seed for 5 min and then soak in water for 3 h.

2.2.2 Roasted sample

The dehulled seeds were roasted in a hot iron pan until the seed turned from green to brown.

2.2.3 Fermented sample

The dehulled seeds were fermented for three days according to the method described by Eze & Ibe (2005).

2.2.4 Boiled sample

The dehulled seeds were boiled for 45 min with distilled water.

At the end of all the different processing methods, the samples were dried in the oven at 50^{o}C until a constant weight was obtained; then ground into fine powdered with a blender. The powdered samples were stored in air tight containers prior to analyses.

2.3 Extraction of oils

The sample was oven dried and extracted in Soxhlet apparatus with redistilled n–hexane of Analar grade (British Drug Houses, London) for the recovery of undiluted oil. The crude oil extract was made to be free of water by filtering through the anhydrous sodium sulphate salt. The hexane was removed from the oil/hexane mixture by using a rotary evaporator.

2.4 Fatty acid analysis

The oil extracted was converted to the methyl ester using the method described by Akintayo and Bayer (2002). About 2 mg crude oil sample was transferred into a 5 – 10 ml glass vial and 1 ml of diazomethane ether solution added. The mixture was shaken thoroughly and allowed to stand for 1 min. Then 16 microlitre of 3.33 M CH_3CONa/CH_3OH solution was added; mixture shaken and allowed to stand for 10 min after which 10 microlitre acetic acid was added. The fatty acid methyl esters were analyzed using a HP 6890 gas chromatograph powered with HP Chemistation Rev. a 09.01 (1206) software fitted with a flame ionization detector and a computing integrator. Nitrogen was used as the carrier gas. The column initial temperature was 250^{o}C rising at 5^{o}C/min to a final temperature of 310 0C while the injection port and the detector were maintained at 310 ^{o}C and 350 ^{o}C, respectively. A polar (HP INNO Wax) capillary column (30 m x 0.53 mm x 0.25 micrometre) was used to separate the esters. The peaks were identified by comparison with standard fatty acid methyl esters obtained from Sigma Chemical Co. (St. Louis MO, USA).

2.5 Phospholipids Analysis

A modified method of Raheja *et al.* (1973) was employed in the analysis of the extracted oil phospholipids content determination. 0.01 g of the extracted fats was added to the test tube. To ensure complete dryness of the oil for phospholipids analysis, the solvent was completely removed by passing the stream of the nitrogen gas on the oil. 0.04 ml of chloroform was added to the content of the tube and it was followed by the addition of 0.10 ml of chromogenic solution. The content of the tube was heated at a temperature of 100^{o}C in a water bath for about

1 min. The content was allowed to cool, 5 ml of the hexane was added and the tube with its content shook gently several times. The solvent and the aqueous layers were recovered and allowed to be separated. The hexane layer was recovered and allowed to be concentrated to 1.0 ml for gas chromatography using flame photometric detector. The conditions for phospholipid analysis include:

H.P 5890 Powered with HP ChemStation REV. A 09.01 (1206). Injection temperature: split injection.

Split ratio: 20: 1
Carrier gas: Nitrogen
Inlet Temperature: 250^{o}C
Column type: HP5
Column dimension: 30 m x 0.25 mm x 0.25 µm
Oven program: Initial temperature at 50^{o}C
First ramping at 10^{o}C/min for 20 min,
Maintained for 4 min.
Second ramping at 15^{o}C/min for 4 min, maintained for 5 min.
Detector: PFPD
Detector temperature: 300^{0}C
Hydrogen pressure: 20 psi
Compressure air: 35 psi

2.6 Statistical evaluation

The statistical calculations included percentage value, grand mean, standard deviation and coefficient of variation.

3 Results and Discussion

The percentage fatty acid composition of *B. eurycoma* is shown in Table 1. The result shows that linoleic acid (C18:2) had the highest concentration with value ranging from 47.95 in fermented to 50.91 % in roasted sample; oleic acid (C18:1) is second with values ranging from 26.51% in roasted sample to 30.91% in raw sample. Palmitic acid (C16:1) is third in the Table, with values ranging from 11.51-14.15%, average of 12.67%. It has been reported that many lipids from legume seeds contain substantial amounts of saturated fatty acids especially palmitic acid. Stearic acid (C18.0) takes the fourth position. The observations made were not in any way comparable with the results obtained by Ajayi *et al.* (2006) on *B. eurycoma*. In their result stearic acid had 7.47%, arachidic acid 3.47%, behenic acid 4.24%, lignoceric 13.27%, although their result on oleic acid (31.51%) is comparable with the present report. The result obtained in the present study is almost comparable with the result obtained by Solomon & Owolawashe (2007) on the blend of legume cereal based complementary food. They obtained palmitic acid (11.2%), stearic acid (3.75%), behenic acid (0.67%), erucic acid (0.09 %), linolenic acid (4.04%), linoleic acid (45.7%), arachidic acid 0.06% and oleic acid (31.9%). Linoleic and oleic acids have been found to be major fatty acids in peanut, soya bean, chickpea, garden bean, broad bean and lentil (Adeyeye *et al*., 1999). In this report, the values obtained are in agreement with their result. Linoleic acid was found to be the most abundant fatty acid, followed by oleic acid. This agrees with report of some workers who showed that linoleic acid was the most concentrated fatty acid in pinto bean, 62.9 – 59.4% (Audu *et al*., 2011), safflower oil (72.6%) (Ihekoronye & Ngoddy, 1985), piggeon pea (58.8%) (Oshodi *et al.,* 1993), bambara groundnut (38.3%) and kersting's groundnut (39.5%) (Aremu *et al.,* 2007). Palmitic acid, happened to be the third largest value, this is in agreement with the observation made by Audu *et al*. (2011). A higher proportion of either linoleic or oleic acid is associated with legumes containing insignificant lipids (Salunkhe *et al*., 1985).Lignoceric, behenic, arachidonic, arachidic and erucic acids contained some percentage of fatty acid less than 1%. The coefficient of variation CV% ranged from 1.7 – 80.0%.

Table 2 displays the differences in the percentage fatty acid composition between raw and boiled, raw and fermented, lastly raw and roasted samples. Palmitic, palmitoleic and linolenic acids were enhanced by all the processing methods. Myristic, oleic, arachidic, arachidonic and lignoceric acids were reduced by all the processing methods. Stearic, linoleic, behenic and erucic acids were enhanced by one or two of the processing methods used as shown in Table 2. The coefficient of variation (CV%) ranged from 2.3 % in oleic acid to 100.1 % in lignoceric acid.

Table 3 shows the fatty acid distribution according to saturation and unsaturation. The total saturated fatty acid (TSFA) ranged from 16.71% in boiled to 19.38% with an average of 17.79± 1.21% and CV % of 6.24. Mono

unsaturated fatty acid (MUFA) ranged from 27.48 –31.08 % with mean average of 29.49± 1.32 while diunsaturated fatty acid (DUFA) ranged from 47.95 – 50.91%. Linoleic acid constituted the DUFA. The values revealed for the total unsaturated fatty acid (TUFA) were between 80.62 – 83.34 % as shown in Table 3 while total nonessential fatty acid (TNEFA) gave 45.03 to 48.87% with an average of 46.70 ± 2.23% and C.V (4.99%). The total essential fatty acid (TEFA) gave 52.66% in raw while processed samples gave 54.7, 51.13 and 54.47% in boiled, fermented and roasted, respectively. The values of total saturated fatty acid (TSFA) are higher when compared with the result obtained by Audu *et al*. (2011) on pinto bean (9.0 – 12.9%) but lower than TSFA value (54.51%) of dehulled African yam bean (Adeyeye *et al.*, 1999). The TUFA values are almost in agreement with result of pinto bean (86.9 – 90.9%) (Audu *et al.,* 2011) and *Sclerocarya birrea* kernel oil (81.35%) (Muhammad *et al*.,2012). However, the seed contained appreciable amount of saturated fatty acid of which palmitic acid and stearic acid are predominant. Linoleic acid present in the seed is used in the synthesis of prostaglandins (Eromosele & Eromosele, 2002) and reduction of blood cholestrol level (Kratz *et al.,* 2002). The high content of TUFA in this study is of good concern because report has shown that fats and oils with high unsaturation are particularly susceptible to oxidation, and intakes of food containing oxidized lipid increased the concentration of secondary proxidation products in liver (Hegsted *et al.,* 1993). The O/L ratio ranged from 0.52 – 0.61 with a mean average of 0.58 ± 0.0001% and CV of 0.01%. The O/L of the present study is lower than *Anarcadium occidentale* (12.28%) (Aremu *et al.,* 2007), but comparable with pinto bean (0.4 – 0.6 %) (Audu *et al.*, 2011) The result shows that *B. eurycoma* oil may be less stable oil and may not be useful as frying oil. Linoleic and alpha-linolenic acids which constituted the essential fatty acid are required for growth, physiological functions and body maintenance (Salunkhe *et al*., 1985). Essential fatty acids are vital for normal foetal development and infant growth (Fernandiz *et al*., 2002)

Table 4 shows the difference in the distribution of fatty acid into saturation and unsaturation in raw and processed *B. eurycoma.* TSFA, TSFA (%) had an increase in fermented and roasted samples. TEFA (%) increased by 3.87 and 3.32 % in boiled and roasted samples, respectively. TNEFA increased by 3.14%. MUFA, DUFA, TUFA and O/L ratio recorded a reduction in all the processing methods. The CV % ranged from 11% in TEFA% to 88% in DUFA.

Table 5 shows the phospholipids content of *Brachystegia eurycoma*. From the result phosphatidic acid showed a greater concentration with range values of 64.18 mg /100 g in boiled to 73.33 mg/100 g in roasted sample. Phosphatidylinsitol came second with 37.15mg/100g in boiled to 44.95 mg/100g in roasted sample. Phosphatidyserine followed phosphatidylinol with a concentration of 33.88 mg/100g to 35.07 mg/100g. The fourth most concentrated phosholipid is phosphatidyethanolmine with a concentration of 33.65 to 23.97 mg/100g. Phosphatidycholine and lysophosphatidycholine were the minor phospholipids with concentrations ranging between 4.30 to 6.01 mg/100g and 2.83 to 4.44 mg/100g. Phosphatidythanolamine is usually the most abundant phospholipid in animals and plants, often amounting to almost 50% of the total and as such they are building block of membrane bilayer (Wirtz, 1991). The phosphatidycholine values for raw and processed *Brachystegia eurycoma* were 4.30 to 6.01 mg/100g. This may be as a result of the shelf life of the seed, because researchers had found that phosphatidycholine (PC) concentration is high at infancy but slowly deplets throughout the age of life, and may drop to as low as 10% of the cellular membrane in the elderly plants and animals (Adeyeye *et al.,* 2012), As a result of this, researchers have recommended daily supplementation of PC as a way of improving brain functioning memory capacity (Chung *et al.,* 1995). The US Food and Drug Administration (USFDA) have stated that consumption of phosphodyserine (PS) may reduce the rate of dementia and cognitive dysfunction in the elderly people, in young people it reduces mental stress and increases mental accuracy and stress resistance (Alter, 2006). PS supplementation promotes a desirable hormonal balance for athletes and might reduce the physiological detorations that accompanies over training and/or overstretching (Starks *et al.,* 2008). Phosphatidic (PA) mediates cellular functions through different modes of action, such as membrane tethering, modulation of enzymatic activities and structural effects on cell membranes. The regulatory processes, in which PA plays a role, include signaling pathways in cell growth, proliferation, reproduction and responses to hormones in biotic and abiotic stress (Wang *et al*., 2006). Therefore, comsumption of B. eurycoma may participate well in these functions.

Table 6 gives the difference in phosphoplipids level (mg/100g) of *B. eurycoma*. Boiled sample was more concentrated in all the phospholipids than the raw and other processed *Brachystegia eurycoma samples while* the raw sample had a higher concentration than the processed ones with the exception of the boiled sample. The CV % varied from 7.3 to 75.9.

6. Conclusion

The study had presented data on the concentrations of phospholipids, saturated and unsaturated fatty acids of Harms (*Brachystegia eurycoma*) grown in eastern part of Nigeria. There was a clear indication that the seed oils of *B. eurycoma* contained a high level of polyunsaturated fatty acids, making them a healthy low fat food. The high concentrations of phosphatidic acid levels in the raw and processed samples of *B. eurycoma* are also revealed, and this is an indication that consumption of the seed may assist in the body regulatory processes such as production and responses to hormones in biotic and abiotic stress.

7 Acknowledgement

Authors are grateful to Tertiary Education Trust Fund (TETFund), Nigeria for financially supporting this research work.

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Table 1: Fatty Acid composition (%) of Raw and Processed Seed of *Brachystegia eurycoma*

Fatty Acid	Raw	Boiled	Fermented	Roasted	Mean	SD	CV%
Myristic acid (C14:0)	0.07	0.02	0.01	0.03	0.03	0.023	70.8
Palmitic acid (16:0)	11.51	12.74	14.15	12.28	12.67	3.56	28.1
Palmitoleic acid (C16:1)	0.08	0.68	0.74	0.86	0.59	0.30	50.9
Stearic acid (C18.0)	4.52	3.06	4.57	5.54	4.42	0.88	19.9
Oleic acid (C18:1)	30.91	28.59	29.29	26.51	28.82	1.52	5.5
Linoleic acid (C18:2)	50.85	50.65	47.95	50.91	50.09	1.23	2.5
Linolenic acid (C18:3)	0.99	3.29	2.60	2.91	2.45	0.88	35.9
Arachidic acid (C20:0)	0.82	0.76	0.58	0.65	0.70	0.008	1.1
Arachidonic acid (C20:4)	0.06	0.01	0.01	0.02	0.025	0.020	80.0
Behenic acid (C20:0)	0.05	0.07	0.04	0.10	0.065	0.58	35.4
Erucic acid (C22:1)	0.09	0.07	0.04	0.11	0.063	0.031	49.2
Lignoceric acid (C24:0)	0.09	0.06	0.03	0.08	0.065	0.58	35.4
Total	**100.4**	**100**	**100**	**100**			

Table: 2: Differences in Fatty Acid Composition (%) of Raw and Processed *B. eurycoma*

Fatty acid	I – II	I – III	I – IV	Mean	SD	CV %
Myristic acid (C14:0)	0.05(71.42%)	0.06(85.71%)	0.04(57.14%)	0.05	0.01	20.0
Palmitic acid (C16:0)	-1.23(-10.59%)	-2.54(-22.94%)	–0.77(-6.69%)	1.51	0.75	50.0
Palmitoleic acid(C16:1)	-0.6(-750%)	-0.66(-825%)	-0.78(-195%)	0.68	0.07	10.3
Stearic acid (C18:0)	1.46(32.30%)	-0.05(-1.12%)	-1.02(-22.57%)	0.84	0.59	70.2
Oleic acid (C18:1)	44.59(60.93%)	43.89(59.97%)	46.67(63.77%)	45.05	1.03	2.3
Linoleic acid(C18:2)	-41.25(-438%)	-38.55(–410%)	-41.51(-441%)	40.43	1.3	3.2
Linolenic acid (C18:3)	-2.3(-232%)	-1.61(-162%)	-1.92(-193%)	1.94	0.3	15.5
Arachidic acid (C20:0)	-0.79(–0.0%)	-0.58(–0.0%)	0.65(0.0%)	0.67	0.15	22.4
Arachidonic acid (C20:4)	0.05(83.3%)	0.05(83.3%)	0.04(65.7%)	0.5	0.000	10.2
Behenic acid(C22:0)	-0.02(-40%)	0.01(-33.3%)	-0.05(-100)	0.03	0.02	66.7
Erucic acid (C22:0)	-0.04(-133%)	-0.01(–33.3%)	0.08(266%)	0.04	0.04	100.0
Lignoceric acid (C24:0)	–0.03(-33.3%)	0.06(66.7%)	0.01(11.1%)	0.04	0.03	100.1

I = Raw sample
II = Boiled sample
III = Fermented sample
IV = Roasted sample

Table: 3: Fatty Acid Distribution According to Saturation and Unsaturation of Components (%) in Raw and Processed *B. eurycoma*

Fatty acid	Raw	Boiled	Fermented	Roasted	Mean	SD	CV %
TSFA	17.06	16.71	19.38	18.68	17.95	1.12	6.2
TSFA (%)	17.06	16.71	19.38	18.68	17.95	1.12	6.2
MUFA	31.08	29.34	30.07	27.48	29.49	1.32	4.5
DUFA	50.85	50.65	47.95	50.91	50.09	1.54	3.1
TUFA	83.34	83.29	80.62	81.32	82.14	1.44	1.8
TUFA (%)	83.34	83.29	80.62	81.32	82.14	1.44	1.8
TEFA (%)	52.66	54.70	51.13	54.47	53.24	2.11	4.0
TNEFA	47.38	45.03	48.87	45.53	46.70	2.33	5.0
O/L Ratio	0.61	0.56	0.61	0.52	0.58	0.0001	0.01

TSFA = Total Saturated Fatty acid
TUFA = Total unsaturated fatty acid
TEFA = Total Essential fatty acid
DUFA = Diunsaturated fatty acid
MUFA =Monounsaturated fatty acid
O/L =Oleic/Linoleic ratio

Table 4: Difference in the Fatty Acid Distribution According to Saturation and Unsaturation of Components (%) in Raw and Processed *B. eurycoma*

Fatty acid	I-II	I –III	I-IV	Mean	SD	CV %
TSFA	0.35(2.05%)	- 2.32 (-13.5%)	-1.62(-9-49%)	1.43	0.58	40.0
TSFA%	0.35(2.05%)	-2.32(-13.5%)	-1.62(9.49%)	1.43	0.58	40.3
MUFA	1.74(5.5%)	1.01(3.29%)	3.6(11.58%)	2.12	1.09	51.1
DUFA	0.2 (0.39%)	2.9(5.70%)	-0.6(0.12%)	1.04	1.17	88.4
TUFA	0.05 (0.067%)	2.72(3.26%)	2.02(2.41%)	1.50	1.13	75.2
TUFA (%)	0.05(0.67%)	2.72(3.26%)	2.02(2.41%)	1.50	1.13	75.0
TEFA (%)	-2.04 (-3.87%)	1.53(2.9%)	-1.81(-3.32%)	1.79	0.20	11.1
TNEFA (%)	2.35(4.9%)	-1.49(-3.14%)	1.85(3.90%)	1.89	0.35	18.2
O/L ratio	0.05(8.19%)	(0)	0.09(14.75%)	0.08	0.02	25.1

TSFA =Total saturated fatty acid; TUFA = Total unsaturated fatty acid; TEFA = Total essential fatty acid; DUFA = Diunsaturated fatty acid; MUFA =Monounsaturated fatty acid; O/L = Oleic/Linoleic ratio;
I =Raw; II = Boiled; III = fermented; IV = Roasted

Table 5: Phospholipids level (mg/100g) of *Brachystegia eurycoma*

Phospholipids	Raw	Boiled	Fermented	Roasted	Mean	SD	CV %
Phosphatidylethanolamine (PE)	30.22	33.65	27.39	23.97	28.81	3.6	12.5
Phosphatidyicholine (PC)	5.13	6.01	4.39	4.30	4.96	0.7	13.9
Phosphatidylserine (PS)	32.82	35.07	31.17	30.88	32.49	1.7	5.2
Lysoposphatidycholine (LPC)	3.4	4.44	2.85	2.83	3.38	0.4	5.0
Phosphatodylinsitol (PL)	38.86	44.95	37.55	37.15	39.70	3.1	7.8
Phosphatidic acid (PA)	68.44	73.33	66.23	64.18	68.05	1.9	2.8

Table 6: Difference in Phospholipids Level (mg/100g) of *Brachystegia eurycoma*

Phospholipids	I-II	I-III	I-IV	Mean	SD	CV %
Phosphatidylethanoline	-3.43(-II.35%)	2.83(9.36%)	6.25(20.68%)	4.17	1.3	31.1
Phosphatidylcholine	-0.88(-17.15)	0.74(14.42%)	0.83(16.18%)	0.82	0.06	7.3
Phosphatidylsenine	-2.25(-6.86)	1.65(5.03%)	1.94(5.91%)	1.9	0.2	12.6
Lysophosphatidycholine	-1.03(-30.21%)	0.56(16.42%)	0.58(17.0%)	0.7	0.2	28.0
Phosphatidy linsitol	-6.09(-15.67%)	1.01(2.59%)	1.71(4.40%)	2.9	2.2	75.9
Phosphatidic acid	-4.89(-7.14%)	2.21(3.23%)	4.26(6.22%)	3.8	1.2	31.0

I = Raw Sample
II = Boiled Sample
III = Fermented Sample
IV = Roasted Sample
SD = Standard Deviation; CV = Coefficient of Variation