IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 9, Issue 4 Ver. III (Apr. 2015), PP 50-55
www.iosrjournals.org

Effect of Processing on Antioxidant Activity of Conventional Mango (*Mangifera indica*) Seed

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Abstract: *Conventional mango (Mangifera indica) seed was analyzed for the antioxidant activity of its kernel, singly or in combination with testa and, before and after defatting. Two in-vitro assay techniques namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP) were employed for the assessment. Results of the processed samples were compared with that of reference vitamin C (L-ascorbic acid). The powdery undefatted kernel with testa (mkt) showed the highest radical scavenging potential and the defatted kernel without testa (dmk), the least. Undefatted kernel without testa (mk) ranked next to mkt sample but with 2-fold loss in antioxidant activity. However, the activity level of mk sample was comparable to that of reference vitamin C in DPPH assay. Equivalent 2-fold loss in activity was observed when mk sample was defatted (dmk), and 3 folds when mkt was defatted (dmkt). The results revealed that the processing operations of removing testa and/or defatting, and the choice of solvent for defatting (absolute ethanol in this study), had profound effect ($p < 0.05$) on antioxidant capacity of mango seed. While the processed forms could find useful application as preservatives in food systems, raw mango seed is a recommendable source of antimicrobial agents.*
Key words: *antioxidant activity, IC_{50}, mango, processing, kernel, testa.*

I. Introduction

Conventional mango (*Mangifera indica*) is of *Anacardiaceae* family. The tree grows and flourishes in tropical countries of the world while the fruit mesocarp is relished for vitamins A and C (L-ascorbic acid). However, the discarded seed which is composed of shell, testa, and kernel has not enjoyed worldwide utilization as the mesocarp for several decades, due to dearth of research details. The high astringency of the kernel had scared animals and man from its consumption. Other limiting factors have been the limited knowledge on the functional properties of the kernel flour and appropriate processing technology [1]. Early studies in the eighties by Asian scholars [2-6] witnessed the potential application of the kernel oil extract in confectionery industry, without reporting possible implications of the phytochemicals of phenolic origin. The composition of tannic substances in mango kernel was identified and analyzed in our laboratory, in the nineties [7]. Correspondingly, properties of the related polyphenol oxidase in the unprocessed kernel were characterized [8]. Furthermore, we had developed and characterized model biscuit samples with residues of the soaked/blanched kernel [9-11]. The processed kernel flour was also successfully incorporated in some indigenous food formulations which had principal ingredients of maize or cowpea, and subjected to sensory evaluation by panelists [Unpublished]. In-vitro and in-vivo studies are on-going to assess the antioxidant activity of the extracted mango oil [12] and the defatted kernel respectively, and their effects on the lipid [12] and haematological profiles of wistar rats.

Given emulsified environments, phenolic compounds exhibit amphipathic properties. They have known pharmacological and ethno-medicinal applications as phytochemicals [13-15], for their capability in scavenging free radicals and reactive oxygen species that aggravate oxidative stress in living organisms. Antioxidant enzyme system [16] in the living tissues constitutes an endogenous defense mechanism by catalyzing reactions, to help protect against free-radical-induced cell damage. The enzymes involved include superoxide dismutase, glutathione peroxidase, glutathione reductase, catalases, and polyphenol oxidase (ppo). They are activated by dietary minerals such as selenium, copper, manganese and zinc [17]. Low concentrations of phenolic compounds in food systems, when catalyzed by these enzymes, play preservative roles as primary antioxidants in lipid media.

The relative natural abundance of polyphenolic substances in the testa and kernel of wild mango (*Irvingia gabonensis*) had been reported [18] but that of the conventional mango (*Mangifera indica*) is lacking. However, records of the phenolic constituents of raw *M. indica* kernel and the antioxidant activity of the blanched kernel are available [7, 19, 20]. Since the raw kernel is inedible, its application as neutraceutical or antimicrobial could be influenced by processing methodologies. Therefore, processing *M. indica* seed for human consumption is of critical concern to estimate residual levels of phenolics that could be innocuous and yet present a preservative function as antioxidant. Literature information is unavailable in this respect. Processing, for example, could involve removal or retention of testa, and/or defatting of the combined kernel and testa which could influence the relative distribution of these phenolic substances between the lipid extract and the residual kernel.

Hence the present study was primarily undertaken to assess the effects of testa and defatting on the antioxidant activity of conventional mango (*M. indica*) kernel/testa. A secondary objective was to collaborate results of two popular antioxidant assay techniques namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Power (FRAP). The use of a single method to assess antioxidant activity has merits and limitations, more-so for the fact that radical scavenging activity of materials depends not only on the total phenolic content but also, on types and relative proportions of the individual components present [21-23]. Combination of methods, therefore, is recommended to affirm total antioxidant capacity (TAC) of materials of interest.

II. Materials And Methods

2.1 Procurement and Processing of sample

Mango (*M. indica*) fruits were plucked directly from trees at Ochadamu, Kogi State, Nigeria during the fruiting season of March to April 2014.

Stainless steel knife was used to peel-off the mesocarp. The shell was bisected to reveal the testa and kernel. The latter (100g) were dried at ambient temperature of 30 $\pm$ 2^{o}C to constant weight, hand-milled to powdery form, from which analytical samples were dried in an air-driven oven at 105 $\pm$ 0.5^{o}C to constant weight in order to estimate dry matter content. The dried samples (20g) were divided into two portions, one of which retained the testa (mkt) while the testa was removed from the other portion (mk).

Each portion (10g) of mkt and mk was further divided into two equal portions of 5g respectively. Samples were then labeled as mkt, dmkt, mk, and dmk.

2.2 Ethanolic extraction of sample for antioxidant activity assay

A cold extraction of dmkt and dmk samples was conducted when 10% (w/v) of each sample was soaked in absolute ethanol (99.9%) contained in a beaker. The mixture was kept in the dark at ambient temperature of 30 $\pm$ 2^{o}C and shaken periodically for 24 h, before filtration, and concentration. The extracted fat was weighed and expressed in percentage (w/w).

The defatted residue samples (dmkt, dmk) and the undefatted samples (mkt, mk) were each subjected to determination of antioxidant activity by the DPPH and FRAP techniques.

2.3 Antioxidant assay (DPPH technique)

The stable 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical was used for the determination of free radical-scavenging activity of the extracts as reported [24].

The ethanolic extract (residue) was diluted with ethanol to obtain serial dilutions of 1000, 500, 250, 125, and 62.5 µg/ml. To 1 ml of each dilution, was added 5ml ethanol, 1ml of DPPH and kept in the dark at ambient temperature of 30 $\pm$ 2^{o}C for 30minutes. The control was without the sample but replaced with an extra 1ml of ethanol. The absorbance was recorded at 517 nm. The reference for this assay was vitamin C which was treated similarly. The percentage antioxidant activity (AOA) or percentage inhibition was calculated as follows:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance sample}}{\text{Absorbance of control}} \text{X} \frac{100}{1} \quad \ldots\ldots\ldots\ldots(1)$$

From the serial dilutions (µg/ml) and percentage inhibition of the samples, linear regression curve was plotted. The IC_{50} was calculated as $\log^{-1}x$ value (µg/ml) from the equation: $y = mx + c$ when $y = 50$ (2)

2.4 Antioxidant assay (FRAP technique)

The method described [25] was modified in this study thus: The ethanolic extract (residue, 100mg) was diluted with 20ml ethanol from which 1ml was mixed with 1ml FRAP reagent (10:1:1 ratio of acetate buffer 300mM pH 3.6 : TPTZ [2,4,6,-tripyridyl-s-triazine] 10mM in 40mM HCl : $FeCl_3.6H_2O$). The absorbance (593 nm) was taken at both 0 and 4 min. Reference sample of Vitamin C was prepared in the same manner, given a FRAP value of 2.0 (equivalent to 28.39 mM in this study):

FRAP value of sample= (Change in sample absorbance/Change in Reference absorbance) x 2 (3)

III. Results And Discussion

The conventional mango (*Mangifera indica*) kernel was analyzed for antioxidant activity before and after defatting. The DPPH and FRAP techniques were employed for a collaborative study of the antioxidant activity.

Though the testa is normally loosely attached to mango kernel, the study was also conducted with, and without testa respectively. Accordingly, dry matter contents were 89.7 and 90.4% (w/w). On defatting, the

ethanolic extract gave oily residues of 22.2 and 20.0% (w/w) (Fig 2). The observed yield in this study was averagely 7% higher than the 14% reported [1] for Ikanekpo mango kernel variety from similar region. The difference was not unexpected as even lower values varying to 6% have been reported for Asian mango kernels [2, 5, 6, 26, 27] due to geographical location and variety.

From the DPPH experiment, results in TABLE 1 were transformed to linear regression curves, TABLE 2. The mean inhibitory concentration (IC_{50}) values of undefatted kernel with testa (mkt), and the defatted kernel without testa (dmk) were the lowest and highest respectively. Besides the obvious large difference in values, separation of means affirmed a significant difference ($p < 0.05$) between the samples. Similarly, paired comparison between mkt and when undefatted without testa (mk), as well as between dmkt and when defatted without testa (dmk) showed significant differences ($p < 0.05$). By the inverse relationship [20], it implied that the mkt and dmk had the highest and lowest antioxidant activity respectively. Furthermore, the mkt sample would have the highest ranking as antimicrobial while dmk would be the preferred choice as a nutraceutical.

The reference vitamin C sample had comparable antioxidant activity with mk ($p > 0.05$). Where this level of activity by reducing substances is tolerable physiologically, then removal of testa (as with mk), and an additional unit operation in processing such as defatting (comparing with lower activity in dmkt and dmk samples) could valuably justify the application of processed mango (*M. indica*) seed in food systems.

About two-fold loss in activity was observed between mkt and mk, and even between mk and dmk, resulting from removal of testa and defatting respectively. The observed three-fold loss of activity between mkt and dmkt appeared to identify the additional effect of solvent choice. Absolute ethanol was employed in this study. A wide range of solvents have been experimented with, though excluding ethanol, to assess antimicrobial and antioxidant activity [28]. In a sequential extraction with several solvents, the polarity of the solvents was implicated in the extent of solubilization of phenolics during the extraction process and consequently, the antioxidant activity evaluation [29, 30]. Further supportive evidence [1] also indicated that about 48% of tannin materials were leached when mango kernels alone were soaked in sulphited water for a period of time, rinsed and blanched. The magnitude of loss recorded in this study by removing testa alone, was so pronounced to infer that the relative thickness of testa to that of kernel was insignificant compared with their contents of phenolic compounds. The composition of phenolics in the kernel without testa was previously analysed [7, 19] to be 74.5% gallotannins, 11% ellagic acid, 7% gallocatechin, 5% n-butyl-cyanidin. Similar literature report on the testa alone is unavailable. A comparable record, however, exists on the concentration of phenolics in the testa of wild mango (Irvingia gabonensis) seed [18].

Figs. 1 and 2 graphically illustrate the relationship between the samples and the reference vitamin C in respect of their relative radical scavenging capability. The reference vitamin C curve intersected both undefatted samples with parallel curves and similar gradients (Fig. 1). On defatting (Fig. 2), the sample curves became steeper by similar magnitude of gradient but dropped below that of the reference vitamin C, affirming the loss of substantial amounts of reducing substances from the kernel and hence, antioxidant activity. Similarly, the difference in the relative gradients of the samples before and after defatting, depicts the complementary contribution of testa to antioxidant activity.

The FRAP values in TABLE 3 showed again that the mkt sample had higher antioxidant potential than the reference vitamin C, implying that constituents of the kernel/testa samples were amphipathic in character, and possibly with more lipophilic compounds in the testa (compare mkt with mk). The FRAP results also identified with the significant effect of defatting; dmkt and dmk activity were significantly different ($p < 0.05$). However, FRAP technique poorly discriminated between the activity of mk and dmkt, compared with the DPPH technique (compare TABLEs 2 and 3).

IV. Conclusion

The study has shown that by two techniques for assessing antioxidant activity, processing operations involving removal of testa and defatting of conventional mango (*M. indica*) kernel singly and synergistically led to profound loss ($p < 0.05$) of radical scavenging capability. The residual kernel/testa appeared to be favorably disposed for application in food systems while the raw kernel/testa could serve useful purposes in the pharmacological sector as an antimicrobial.

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Table 1: Percentage Inhibition Of DPPH In Different Concentrations Of Processed *Mangifera indica* Kernel/Testa.

Concentration	Undefatted		Defatted		Reference
(µg/ml)	(+ testa)	(- testa)	(+ testa)	(- testa)	(Vitamin C)
1000	82.2	75.4	72.8	66.0	83.0
500	73.3	65.2	64.6	60.5	78.0
250	64.5	56.5	46.9	42.9	60.6
125	55.6	47.5	38.8	34.2	43.7
62.5	46.1	37.8	29.9	25.6	34.5

Table 2: Fat Content, And Antioxidant Activity Of *Mangifera indica* Kernel Using DPPH Technique

Sample type	% Fat	Regression equation y = mx + c	Activity as IC_{50} (µg/ml)
Undefatted (+ testa)	*	y = 29.86x - 7.268	82.77[a]
Undefatted (- testa)	*	y = 30.76x - 17.22	153.22[b]
Defatted (+ testa)	22.2	y = 37.07x - 38.29	240.83[c]
Defatted (- testa)	20.0	y = 35.57x - 39.46	327.37[d]
Reference (vitamin C)	*	y = 49.46x - 56.88	289.72[d]
SEM			44.781

* = Not available. SEM = Standard Error of Mean. Values with different alphabets differ significantly at $p < 0.05$. IC_{50} is $\log^{-1}$ x value (µg/ml) in a linear regression equation when y = 50

Table 3: Antioxidant Activity Of *Mangifera indica* Kernel/Testa Using FRAP Technique

Sample type	FRAP value	FRAP value (Vit. C eqv. in mM)
Undefatted (+ testa)	1.255	17.81[a]
Undefatted (- testa)	2.808	39.85[c]
Defatted (+ testa)	2.759	39.17[c]
Defatted (- testa)	3.676	52.18[d]
Reference (vitamin C)	2.000	28.39[b]
SEM		5.805

SEM = Standard Error of Mean. Values with different alphabets differ significantly at $p < 0.05$.

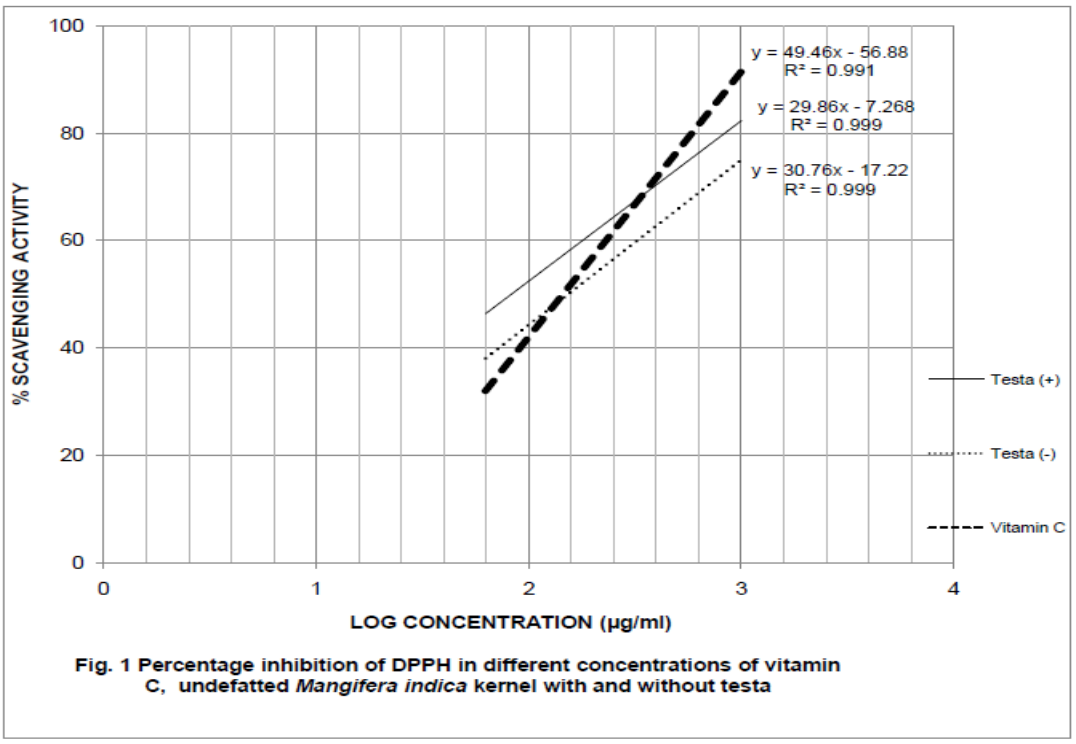

Fig. 1 Percentage inhibition of DPPH in different concentrations of vitamin C, undefatted *Mangifera indica* kernel with and without testa

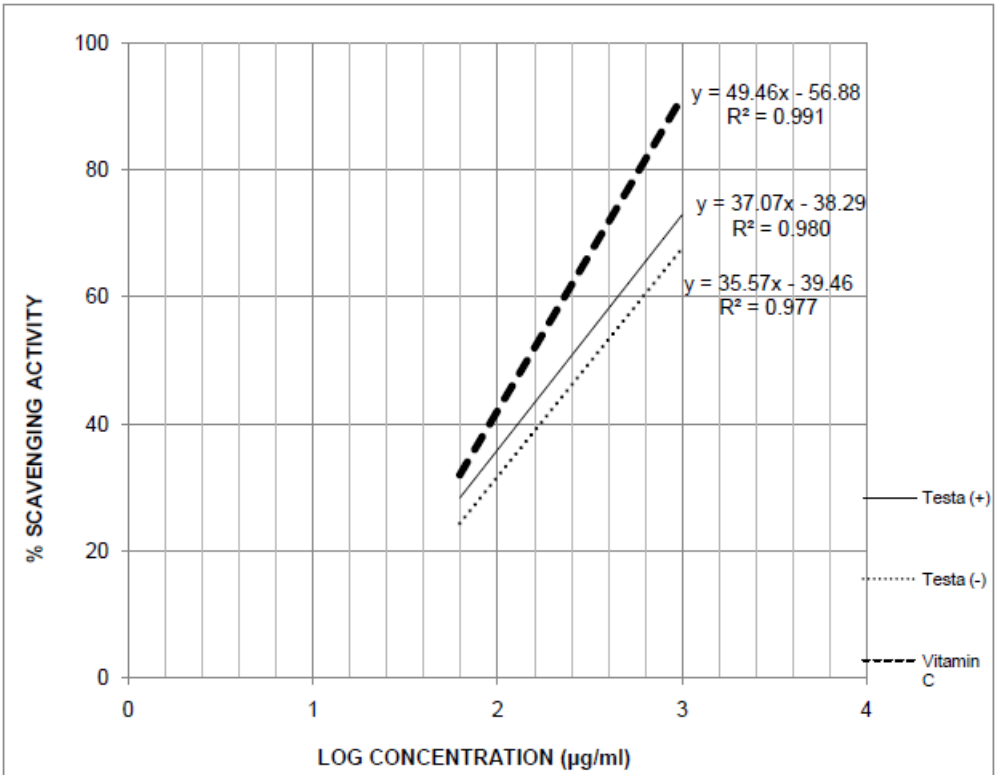

Fig. 2 Percentage inhibition of DPPH in different concentrations of vitamin C, defatted *Mangifera indica* kernel with and without testa