

Macronutrients and Micronutrients profile of some underutilized beans in South Western Nigeria

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ABSTRACT

Beans are a rich source of nutrients in human diet. However a number of edible bean varieties are largely underutilized in developing countries due to little or no information on their nutritional composition. The present study investigated the nutritional content of mung beans, African yam beans, soybeans, black eyed peas and pigeon peas from parts of South Western Nigeria. Samples were collected randomly in duplicates across the six South-western states of Nigeria. Common beans, was included as a reference for comparison. Standard methods were used to determine the proximate composition of all bean samples. Mineral nutrients and phaseolin protein fractions (albumin, globulin and prolamine) of bean samples (excluding pigeon pea varieties) was also determined. The proximate, mineral and phaseolin protein contents differed significantly ($P < .05$) between bean types. Highest protein, fat, carbohydrate, crude fibre and ash content was in African yam bean (24.96%), mung beans (6.60%), soybean (62.81%), mung beans (15.24%) and African yam beans (4.30%) respectively. The beans compared fairly with common beans in proximate composition. Mineral nutrients differed significantly ($P < .05$) between bean types. Calcium, magnesium, phosphorus and potassium (particularly in Mung beans) were in high amounts. Black eyed peas had the least total of mineral content while mung beans had the highest. The phaseolin protein fractions were significantly different ($P < .05$) among bean types. African yam bean had the highest albumin and globulin content (%/mg protein) of 41.89 and 35.70 respectively, while prolamine was highest in soybeans. These results indicated that these underutilized beans compares favourably in terms of nutritional composition with widely consumed common beans in Nigeria. In addition, African yam beans and Mung beans are equally suitable alternatives from a protein-rich standpoint.

Keywords: *underutilized beans, legumes, nutrients, phaseolin protein, Nigeria*

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30 1. INTRODUCTION

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32 Beans are a rich source of essential nutrients in human diet, providing proteins, complex
33 carbohydrates, vitamins, mineral elements, dietary fibres and complex carbohydrates, while
34 being low in fats, sodium and cholesterol-free [1-6]. On a global scale, beans is the most
35 important legume staple for human consumption [4, 7]. Their high protein content makes
36 them relatively cheap alternative protein source compared to the highly priced animal or
37 meat-protein sources for households in developing countries [8]. In Nigeria, a number of
38 beans and beans varieties are cultivated. These include varieties of the common beans
39 (*Phaseolus vulgaris*); African yam bean (*Sphenostylis stenocarpa*); mung beans (*Vigna*
40 *radiata*); soybeans (*Glycine max*); pigeon pea (*Cajanus cajan*); African oil bean
41 (*Pentaclethra marcophylla*) and the black eyed pea (cowpea) (*Vigna unguiculata*) [2, 8-12].
42 With the exception of common beans and cowpea, a number of these bean types are largely
43 underutilized, especially for human consumption. At present a number of these bean seeds
44 are processed into flours and utilized as food or feed [8, 13]. Their uses include, the
45 fortification of indigenous starchy meals as composites [14-18]; production of flavouring
46 fermented food condiments used in the preparation of traditional dishes [19-21]; beverage
47 (soy-milk) and cake (soy-cake, tofu) [22, 23].

48 As a specific example, the utilization of Mung bean for human consumption in Nigeria is still
49 very low when compared to its uses and wide adoption rate in other parts of the world,
50 including India and Asia [24]. This is due to the fact that it was recently introduced into the
51 agro-ecological sphere of Southern Nigeria [25-27]. Nevertheless, the mung bean is an
52 important legume cultivated for human consumption (as either fresh sprouts or dry beans)
53 [24]. However, awareness of its nutritive potentials are required in order to increase its
54 current low adoption rate and consumption in Nigeria [27]. Similarly, the African yam bean,
55 Pigeon pea and black eyed pea are all under exploited despite their rich nutritional resource
56 [28-32]. Diversification in the use of the beans can stimulate an increased cultivation and
57 supply of these bean types. Furthermore, the increased utilization of these beans in human
58 nutrition may contribute to achieving the targeted daily dietary protein intake recommended
59 by the World health Organization [33], especially in poor developing nations. This is
60 essential to attaining sustainable food security in terms of nutritional quality.

61 The poor utilization of these beans can be attributable to a lack of adequate characterization
62 and documentation of the nutritional profile of these bean seeds, as well as a lack of
63 documentation of the genotypic accessions available in the Nigerian agricultural sphere [11,
64 34, 35]. Hence, we set out to characterize some of the underutilized beans types, including
65 mung beans, pigeon pea, African yam bean and soybean in parts of South Western Nigeria
66 in terms of nutritional, chemical and genotypic content. However, the first two aims are the
67 focus of the current article. Here, we determined the macronutrients, micro nutrients or
68 minerals and the phaseolin protein fractions (major seed storage protein) content of the bean
69 types.

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71 2. MATERIAL AND METHODS

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73 2.1. Sample collection

74 Six Bean varieties including the African yam bean (*Sphenostylis stenocarpa*), mung bean
75 (*Vigna radiata*), red and grey colour varieties of the pigeon pea (*Cajanus cajan*), black eye
76 pea, pigeon pea (greyish colour variety) and soybean (*Glycine max*) were collected from

77 parts of South Western Nigeria. Samples were collected in clean polythene bags and stored
78 at 4°C prior to analysis.

79 **2.2. Determination of proximate composition**

80 The proximate compositions: ash, moisture, fat, carbohydrate, crude protein (Kjeldahl
81 method) and were determined by standard methods of the A.O.A.C [36]. Carbohydrate was
82 obtained by subtracting the sum of other proximate parameters from 100.

83 **2.2.2 Determination of reducing sugar**

84 Reducing sugar was determined according to the method of Somogi-Nelson [37]. To 1 ml of
85 bean sample powder solution in a test tube, of a mixture of alkaline copper reagent A, 4
86 volumes of stock A reagent (NaCO₃ 12g, NaK tartarate 6g, NaHCO₃ 8g, NaSO₄ 72g, and
87 dH₂O 400ml) and alkaline copper reagent B, 1 volume of stock reagent B (CuSO₄ 2g, NaSO₄
88 18g and dH₂O 100ml) were added. Mixture was then boiled for 20mins and allowed to cool
89 on ice. 1ml of arseno-molybdate (see appendix for preparation) was added and mixed by
90 gentle agitation. Seven millilitres (7 ml) of distilled water was added to each tube and mixed
91 thoroughly. The absorbance of the solution mixture was measured at 540 nm in a
92 spectrophotometer and subtracted from a blank reading.

93 **2.3. Determination of Micronutrients**

94 Bean samples (with exception of pigeon peas varieties) used in this study were analysed for
95 nutrients including potassium, sodium, calcium, magnesium, iron, zinc, copper and cobalt by
96 following the methods of the AOAC [36] method. Samples were first acid-digested and
97 analysed using the atomic absorption spectrophotometer as described below. To 5.0g of
98 powdered samples was added 30ml of concentrated Nitric acid (HNO₃).The flask were
99 placed in the dark overnight. Afterwards, 40ml of perchloric acid (HClO₄) was added. The
100 mixture was then initially heated on a hot plate at 50°C for 15mins. Afterwards the
101 temperature of heating was gradually increased to 200°C. Heating was continued until the
102 white dense fumes of perchloric acid disappeared. After digestion, the contents were cooled,
103 filtered through Whatman filter paper (No. 2), filtrate transferred to a 100ml volumetric flask
104 and then made up to the 100 ml mark with deionized water.

105 An Atomic Absorption Spectrophotometer (model 1233, Beckmann, US) equipped with a
106 hollow cathode lamp and a fuel rich flame (air acetylene) with 20pa pressure and air 45Pa
107 pressure was used for the mineral content analyses. Parameters were adjusted according to
108 manufacturer's instruction. The hollow cathode lamps for selected minerals with their
109 wavelengths were used as a light source for each analyte. Lamp current was also set. The
110 instrument was calibrated with standard solutions and the sample introduced to it by means
111 of a capillary tube or aspiration. The mean signal responses were recorded at the elements
112 respective wavelength (Potassium, 766 nm; Calcium, 422 nm; Magnesium, 205 nm; Sodium,
113 509 nm, Copper, 324 nm; Iron, 243 nm; Manganese, 279 nm; Zinc, 213 nm; Cobalt, 240 nm)

114 The concentration of each element was calculated as follows:

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$$\frac{[\text{Standard Concentration} \times \text{Sample Absorbance} \times 100 \times \text{df}]}{[\text{Standard Absorbance} \times \text{Weight} \\ \text{of sample}]}$$

117 Where df= dilution factor.

118 **2.3.2. Determination of Phosphorus**

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119 The A.O.A.C [36] method was used for the determination of phosphorus. Four millilitres of
120 concentrated sulphuric acid (H_2SO_4) was added to 2 g of sample in a beaker. The mixture
121 was left for 1 h to allow digestion of the sample. The sample digest was neutralized with 10
122 ml of 5 N NaOH solution, allowed to sit for a further 20 min and thereafter filtered with
123 Whatman filter paper (Whatman No. 1). The filtrate was then made up to 50 ml with distilled
124 water in a standard volumetric flask. To 5.0 ml aliquot of the diluted filtrate was added 35 ml
125 of distilled water and 2.5 ml of vanado molybdate reagent (1.25 g common vanadate
126 dissolved in 500ml water with 10 ml HNO_3 , 0.5 ml of molybdate; 13 g of sodium molybdate
127 in 200ml of water) to produce a yellow-orange complex. The solution was mixed and the
128 absorbance measured at 520 nm wavelength with a spectrophotometer (PTUV/Vis
129 spectrophotometer). The phosphorus content of the sample was calculated using
130 absorbance of 1.0 mg potassium dehydrate phosphate (KH_2PO_4) as follows:

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132 Phosphorus = [Sample Absorbance x Standard Concentration x100 x df(10)]/
133 [Standard Absorbance x Weight of sample]

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135 2.4. Determination of phaseolin content

136 The solubility fractionation of seed protein was carried out according to the method of
137 Gheyasuddin *et al.* [38]. The protein, phaseolin was extracted by a sequence of successive
138 extractions at room temperature for 1 hour at milled sample to solvent ratio of (1:10 w/v) on a
139 magnetic stirrer. Firstly, distilled water was used to extract albumin at 1:10 w/v; then,
140 40g/litre Sodium Chloride solution was used to extract globulins at 1:10 w/v. Thereafter, 600
141 g/l Isopropanol was used to extract prolamine at 1:10 w/v. A 4g/Litre sodium hydroxide
142 solution was then added to extracted sample. After extraction, the slurry was centrifuged at
143 1500 x g for 10 min. The supernatant was collected and the extraction steps repeated three
144 times successively on the residue. Supernatants from each extraction batch were pooled
145 together. The nitrogen content in each extract (i.e. supernatant) was determined by a micro-
146 kjedahl method.

147 %Nitrogen = [0.014 x (sample titre - blank titre) x 0.1N x 100]/ [Sample weight]

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149 2.5. Statistical analysis

150 Data obtained were subjected to one-way analysis of variance (ANOVA) to determine
151 significance ($P \leq .05$). Were significant, means were separated using Duncan multiple range
152 test. Statistical package for the Social Sciences (SPSS) (IBM, Version21) was used for
153 statistical analyses.

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155 3. RESULTS AND DISCUSSION

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157 Beans are important legumes in human nutrition [1]. They have been reported to contain
158 essential nutrients and minerals required for Human growth and body functioning [1, 4, 6]. In
159 the present study, the chemical and nutritional composition of six “underutilized” beans were
160 determined. The proximate composition of beans are shown in Table 1. Overall, there were

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significant differences ($P \leq .05$) between the proximate compositions of bean types. The proximate compositions are also comparable to values reported in literature elsewhere [32, 39-42]. Proteins which are essential building blocks of body tissues was highest (24.96%) in African yam bean (AYB) (*Sphenostylis stenocarpa*). Mung beans (*Vigna radiata*) had the second highest crude protein content of 22.07%. AYB and mung beans were the only bean types which had higher protein content than the "control" (common beans, *Phaseolus vulgaris*, 21.07%). Soybean (*Glycine max*) had the lowest crude protein content of all bean types. The high crude protein content of these beans make them qualify as suitable alternatives and relatively affordable protein source [8, 19] in comparison to the rather expensive price of animal-meat protein in developing countries. Some of these beans are currently being used as protein-rich condiments to enhance the flavour of traditional soups in Nigeria [8, 19, 20]. Ash content is an indication of the mineral nutrients present in a food material. The ash contents of the beans were significantly different ($P < .05$) among bean types. The highest mineral composition was in the African yam bean (4.3%), followed by Soybean (4.01%). The ash content of both pigeon pea varieties and black eyed peas did not differ significantly ($P > .05$). All the beans had higher ash content than common beans (3.26%). These observation shows that these underutilized beans are quite richer in minerals than common beans. Beans have been reported to be a good source of essential minerals and vitamins in Human diet [1, 6]. It is important to note that mineral content data does not translate to absolute bioavailability of these minerals when beans are consumed; the presence of anti-nutritional factors following bean processing are part of factors to be taken into consideration in determining absolute nutritive value of bean seeds [6, 39, 43].

The mean carbohydrate (CHO) content of black eyed pea and soybean were similar (62.81%) and were the highest values obtained. Mung beans had the lowest CHO content (47.47%). Reducing sugars were highest in common beans and lowest in pigeon pea (grey variety). Crude fibre content was highest in Mung beans (15.24%) and lowest (excluding the control, common beans) in AYB (6.26%). Beans have been known to be a source of complex carbohydrates, including dietary fibre and resistant starch [1, 44]. Fat content of the beans were generally low. Beans and legumes in general are low in fat and cholesterol [6]. Mung beans had the highest fat content (6.60%), while the lowest was pigeon pea (grey variety) (0.79%).

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Table 1. Proximate composition of bean samples

Parameters (%)	*Common beans	African Yam beans	Mung beans	Pigeon pea (red)	Pigeon pea (grey)	Black eyed pea	Soybean
Moisture	7.09±0.01 ^b	1.93±0.01 ^f	4.74±0.01 ^d	5.81±0.01 ^c	1.24±0.03 ^g	7.48±0.03 ^a	3.58±0.03 ^e
Ash	3.26±0.03 ^e	4.30±0.14 ^a	3.82±0.03 ^c	3.55±0.01 ^d	3.48±0.01 ^d	3.41±0.01 ^d	4.01±0.01 ^d
Fat	2.52±0.14 ^d	2.70±0.01 ^c	6.60±0.01 ^a	2.57±0.01 ^d	0.79±0.01 ^f	1.89±0.01 ^e	5.89±0.01 ^b
Crude protein	21.07±0.01 ^c	24.96±0.01 ^a	22.07±0.03 ^b	18.67±0.01 ^d	17.98±0.01 ^e	16.59±0.03 ^f	15.45±0.01 ^e
CHO	60.36±0.31 ^c	59.85±0.04 ^d	47.47±0.01 ^e	60.12±0.03 ^{cd}	61.52±0.01 ^b	62.81±0.03 ^a	62.81±0.03 ^a
Crude fibre	5.71±0.20 ^g	6.26±0.14 ^f	15.24±0.04 ^a	9.28±0.06 ^c	15.00±0.06 ^b	7.82±0.06 ^e	8.26±0.06 ^d
Reducing sugar	37.90±0.04 ^b	38.37±0.01 ^a	27.53±0.01 ^d	28.46±0.03 ^c	18.58±0.01 ^g	21.92±0.01 ^f	23.36±0.01 ^e
⁺ Calories (Kcal/100 g)	348.38±0.06 ^c	363.54±0.01 ^b	338.1±0.18 ^d	338.29±0.30 ^d	325.07±0.05 ^f	334.61±0.35 ^e	366.05±0.05 ^a

*Common beans (*Phaseolus vulgaris*) was included in the analysis for the purpose of comparison since it is more widely utilized in Nigeria. +Calculated by summing the product of the crude protein, fat and carbohydrate content with the respective Atwater's general energy

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conversion factors (4, 9, 4) [45]. Values are mean $\pm$ standard deviation (n=2). Values with different superscript on the same row are statistically different (P < .05).

The macro and micro mineral elements of bean types/varieties are shown in Table 2. Nutrient composition of the bean was significantly different among bean sample types. All elements determined in this study was present in all bean types. These results reaffirm the mineral richness of beans as stated earlier in the introduction. Overall, calcium, magnesium, phosphorus and potassium (in mung beans) were present in high concentrations in the beans. While trace amounts of micro nutrients, copper and manganese were present. The total minerals composition was highest in mung beans and least in black eyed peas (Fig. 1). The importance of these mineral elements in humans have been published elsewhere [46-49]. Going by the assumption that ash content indicates the amount of mineral nutrients in beans, then it will be expected that the bean type with highest mineral elements in fig 1 ought to have the highest ash content in Table 1. However the data obtained in this study does not seem to suggest that assumption. The discrepancy may be attributable to the presence of other minerals which were not assayed for in the present study (at least one of these minerals would be expected to be higher in African yam bean than the mung beans)

Table 2. Microelements of bean samples

Mineral Nutrients (mg/100g)	*Common beans	African yam bean	Mung beans	Black eyed pea	Soybeans
Potassium	5.91 $\pm$ 0.01 ^e	11.35 $\pm$ 0.1 ^d	154.7 $\pm$ 0.42 ^a	13.77 $\pm$ 0.03 ^c	15.06 $\pm$ 0.01 ^b
Calcium	108.55 $\pm$ 0.64 ^d	203.96 $\pm$ 0.01 ^a	194.27 $\pm$ 0.01 ^b	71.56 $\pm$ 0.01 ^e	135.48 $\pm$ 0.01 ^c
Magnesium	60.5 $\pm$ 0.28 ^e	120.60 $\pm$ 0.42 ^b	186.05 $\pm$ 0.02 ^a	71.28 $\pm$ 0.1 ^d	80.19 $\pm$ 0.03 ^c
Sodium	12.33 $\pm$ 0.18 ^e	30.04 $\pm$ 0.01 ^b	18.44 $\pm$ 0.01 ^c	40.20 $\pm$ 0.14 ^a	14.99 $\pm$ 0.01 ^d
Iron	0.91 $\pm$ 0.00 ^b	19.91 $\pm$ 0.01 ^a	11.01 $\pm$ 0.01 ^b	5.86 $\pm$ 0.03 ^c	2.54 $\pm$ 0.1 ^d
Zinc	6.67 $\pm$ 0.03 ^c	10.13 $\pm$ 0.01 ^b	18.33 $\pm$ 0.01 ^a	4.68 $\pm$ 0.01 ^d	3.99 $\pm$ 0.01 ^d
Phosphorus	151.38 $\pm$ 0.03 ^d	244.63 $\pm$ 0.01 ^c	357.52 $\pm$ 0.03 ^a	121.91 $\pm$ 0.03 ^e	286.61 $\pm$ 0.01 ^b
Cobalt	0.135 $\pm$ 0.00 ^a	0.06 $\pm$ 0.04 ^b	0.13 $\pm$ 0.01 ^a	0.11 $\pm$ 0.00 ^a	0.02 $\pm$ 0.00 ^b
Copper	0.90 $\pm$ 0.00 ^b	2.31 $\pm$ 0.00 ^a	0.33 $\pm$ 0.00 ^e	0.64 $\pm$ 0.03 ^c	0.46 $\pm$ 0.00 ^d
Manganese	0.99 $\pm$ 0.1 ^e	8.38 $\pm$ 0.00 ^a	1.40 $\pm$ 0.14 ^d	6.23 $\pm$ 0.03 ^b	4.53 $\pm$ 0.01 ^c

*Common beans (*Phaseolus vulgaris*) was included in the analysis for the purpose of comparison since it is more widely utilized in Nigeria. Values are means $\pm$ Standard error of mean (n=2). Values with the same superscript on the same row are statistically the same (P< .05).

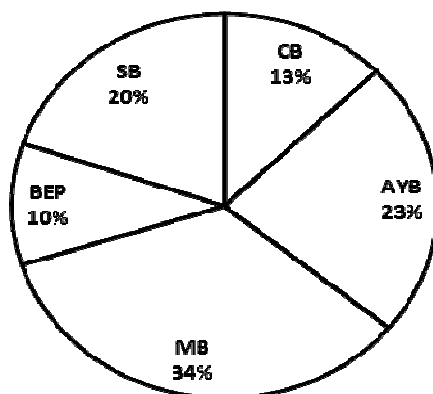


Fig. 1. Relative mineral composition of beans

CB, common beans; AYB, African yam beans; MB, Mung beans; BEP, Black eyed peas; SB, soybean.

Presented in table 3 are the phaseolin protein fractions of the beans. Phaseolin is a major storage protein in bean seeds [7]. The different fractions of phaseolin protein differed significantly ($P<0.05$) in between seeds. Overall, highest phaseolin fractions were present in African yam beans (AYB) and lowest in black eyed peas (BEP). The albumin fraction was highest in AYB and least in soybean, while the globulin fraction was highest in AYB and lowest in BEP. On the other hand, prolamine was highest (not taking into account common beans which served as control) in soybean and lowest in mung beans. Similar observation was reported on velvet beans. Among the seed proteins studied, globulins (9% to 62%) were higher, followed by albumin (4% to 21%), glutelin (1.3% to 2.9%), and prolamin (0.8% to 2%) [50-52]. Seeds of *Pachyrhizus erosus* (Yam beans) has been analyzed and reported glutelins constituting highest protein fraction followed by globulins. Compared to the globulins, albumins and protamins were low and the ratios were 28.8%, 16.3%, and 7%, respectively (53).

Table 3. Phaseolin protein fraction of beans

Protein (%/mg)	*Common beans	African yam beans	Mung beans	Black eyed pea	soybean
Albumin	34.38±0.53 ^c	41.89±1.57 ^a	37.19±0.69 ^b	31.69±0.72 ^d	29.96±1.21 ^d
Globulin	28.59±0.72 ^c	35.70±0.71 ^a	31.85±0.49 ^b	26.69±0.43 ^d	27.79±0.55 ^{cd}
Prolamine	16.10±0.42 ^a	11.45±0.14 ^c	9.13±0.74 ^d	8.92±0.40 ^d	12.90±0.42 ^b
Total protein	77.61±0.30 ^b	83.07±1.49 ^a	72.23±2.16 ^c	66.12±0.00 ^d	67.36±1.75 ^d

*Common beans (*Phaseolus vulgaris*) was included in the analysis for the purpose of comparison since it is more widely utilized in Nigeria. Values are means ± Standard error of mean ($n=2$). Values with the same superscript on the same row are statistically the same ($P<0.05$).

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4. CONCLUSION

The underutilized bean seeds are rich in nutrients, especially protein and mineral elements. Their nutritional composition are comparably to widely adopted and consumed beans—common beans—in Nigeria. With the exception of anti-nutritional factors and other undesirably factors which were not the subject of the present study, these underutilized beans, especially African yam bean and mung beans appear to be suitable alternatives to common beans for human consumption. Hence, the diversification of their uses is encouraged.

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COMPETING INTERESTS

Authors have no competing interests whatsoever

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between all authors. Authors DAO, AGOL and AOBO conceptualized and designed the study. Author AGOL carried out the experiments and managed data generated. Author EOT did the statistical analyses and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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434 APPENDIX

436 Preparation of arsenomolybdate reagent.

437 100g of ammonium molybdate was dissolved in 1800mls (1.8 liters) of distilled water. To this,
438 84ml of concentrated hydrogen sulphate was added to the solution. 12g of bisodium
439 hydrogen arsenate (orthoarsenate, $\text{Na}_2\text{H}_2\text{O}_4 \cdot 7\text{H}_2\text{O}$) was dissolved in 10ml of distilled water.
440 This solution was mixed with ammonium molybdate, placed in the incubator between 24-
441 84hrs at 37°C and stored in amber bottle.
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