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SHORT COMMUNICARION article

Amino-acid composition, essential/non-essential amino-acid contents, recommended daily allowance, and amounts that meet RDAs of Cocoyam (*Colocasia exculenta*) and Soybeans (*Glycine max*) flour and their blends

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Abstract: Nutrients are chemical substances required by the body to sustain basic functions and are optimally obtained by eating a balanced diet. This study was performed to ascertain the amino acid (AA) composition, essential/non-essential AA contents, recommended daily allowance, and the amount that meets the recommended daily allowance of *Colocasia exculenta* and *Glycine max* flour and their various blends. AA analyses were done by using a gas chromatograph: HP 6890 with Chemstation Rev. A 09.01 (1206) software. The total amounts of AAs in unblended *Glycine max* and *Colocasia exculenta* flours were 93.68 and 75.47 (g/100g protein), respectively. Essential AAs found were phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, lysine, while the non-essential AAs found were alanine, aspartic acid, glutamic acid, serine, tyrosine, cystine, glycine, proline, histidine, and arginine. The order of the essential/non-essential AAs ratio was: unblended cocoyam flour (0.659) > 30% blend (0.656) > 20% blend (0.656) > 10% blend (0.644) > 40% blend (0.618) > 50% blend (0.577) > unblended soybean (0.574). The USAD, 2015 recommended daily allowance per kg body weight for the eight essential AAs was: Isoleucine = 19 mg, leucine = 42 mg, lysine = 38 mg, methionine (+ the non-essential AA cysteine) =19 mg, phenylalanine (plus the non-essential AA tyrosine) = 33 mg, threonine = 20 mg, tryptophan = 5 mg, and valine = 24 mg. Accordingly, the AA data presented in this study can be used to calculate how much of the soybean and cocoyam samples will supply wholly or partially these recommended allowances for the various AAs.

Introduction

A nutrient is considered essential (E) if it must be obtained from an outside source either because the animal cannot synthesise it or because inadequate quantities are formed. Non-essential (NE)nutrients are nutrients that the body can produce (synthesise) on its own [1]. Micronutrients are used to form and mend tissues and control body processes, while macronutrients are transformed to smaller sugars (glucose, fructose, and galactose) from carbohydrates, protein to amino acids (AAs), and fats to fatty acids and glycerol [2]. Organic nutrients contain carbohydrates, fats, proteins (or other building blocks, AAs), and vitamins [3]. Inorganic chemical compounds such as dietary minerals, water, and oxygen may be seen as nutrients [4]. Traditionally, cocoyam is used in the preparation of different foods. Vitamin B6 and potassium are useful for controlling blood pressure and protecting the heart [5]. Cocoyam is used to produce cocoyam flour used as food and a replacement for wheat flour. The leaves are used in soups [6]. The crop is used to produce infant meals as well as foods for recuperating patients [7]. Overwhelmingly nutrient-packed food like cocoyam is vital for maintaining a healthy immune system, and aids the body to make use of carbohydrates, fats, proteins, and additional nutrients in the food [6]. Soybeans contain significant amounts of iron, zinc, selenium, and antioxidants. It contains vitamins, organic compounds, and other nutrients, including a significant amount of dietary fibre and a large amount of protein [8]. Isoflavones and isoflavone-rich soya bean prevent prostate tumours prompted by chemical carcinogens or via the establishment of prostate cancer cells [9]. Cocoyam root has more than 17 different AAs vital to maintaining good health. Omega-3 and 6 oils are useful for cancer prevention, maintaining cardiovascular health, and treating other diseases [10]. Presently, there is tremendous research interest in the health effects of soya bean foods. Future benefits include drops in the risk of several chronic diseases, including breast cancer, heart disease, and osteoporosis [11]. There is also an indication that soya beans alleviate hot flashes in menopausal women. Substantial data suggest that soya bean isoflavones are mainly accountable for many of the health benefits of soya foods [9]. AAs are the monomer units in proteins. Experimental studies showed, with few exceptions, that isoflavones and isoflavone-rich soy protein prevent prostate tumour by chemical carcinogens or via the establishment of prostate cancer cells. Remarkably, tumour inhibition occurs in spite of quite low prostate isoflavone concentration. Furthermore, isoflavone in combination with tea extracts was shown to reduce tumour growth in mice implanted with androgen-sensitive prostate cancer cells more effectively than either of them [12]. Among the objectives of this study were the calculation of E/NE ratios as an additional method of assessing the quality of protein, supplementary to existing methods such PER, NPR and BV, and also the use of chemical composition data of the products to calculate the RDA values for nutrients such as proteins, carbohydrates, lipids, vitamins and mineral elements and the chemical score values for soybean and cocoyam proteins. These data would be important in home and institutional uses of the products in managing various health conditions where necessary and applicable.

Materials and methods

Cocoyam (*Colocasia esculenta*) and soya bean (*Glycine max*) were purchased from New Benin Market in Benin City, Edo state, Nigeria, and identified at the Plant Biology and Biotechnology, also at the Crop Science Departments of the University of Benin, Benin City.

Preparation of samples: The cocoyam corms were peeled, sliced into pieces, and sun-dried for five days. Soya beans, bought from New Benin Market, were sun-dried for five days. Both the dried cocoyam and soya beans were milled. Wire whisk sieve of 25 µm. The aperture size was used to sieve the flours to obtain homogeneous flours, packaged in airtight containers, labelled before the laboratory for analyses.

GC conditions for AAs. HP 6890 powered with Chemstation Rev. A 09.01(1206) software, slit injection, slit ratio; 20.1, carrier gas; hydrogen, flow rate; 1.0 ml/min, inlet temperature; 250 ^{0}C, column type; EZ, column dimensions;10 m x 0.2 mm x 0.25 um, oven program; initial@ 110 ^{0}C, first; ramp @ 27 ^{0}C/min to 320 ^{0}C, second; constant for 5 min at 320 ^{0}C, detector; PFPD. Detector temperature; 320 ^{0}C, hydrogen pressure; 20 psi, compressed Air; 35 psi

Procedure for the analysis of AAs: The dried and pulverized sample (0.5 g) was weighed into a 250 ml conical flask. The sample was defatted by extracting the fat content of the sample with 30 ml of petroleum spirit three times with a Soxhlet extractor. The sample was completely hydrolysed for total AAs recovery. The pulverized and defatted sample was treated with 30 ml of 1.0 M potassium hydroxide solution and incubated for 48 hrs at 110 ^{0}C in a hermetically closed borosilicate glass container. After the alkaline hydrolysis, the hydrolysate was neutralized to a pH in the range of 2.5 - 5.0. The solution was purified by cation-exchange solid-phase extraction. The AAs in purified solution were derivatised with ethylchloroformate by the established procedure. The derivation of the extracted AAs to achieve volatility in the gas chromatography with ethylchlorofomate is depicted with the reaction shown in **Figure 1**.

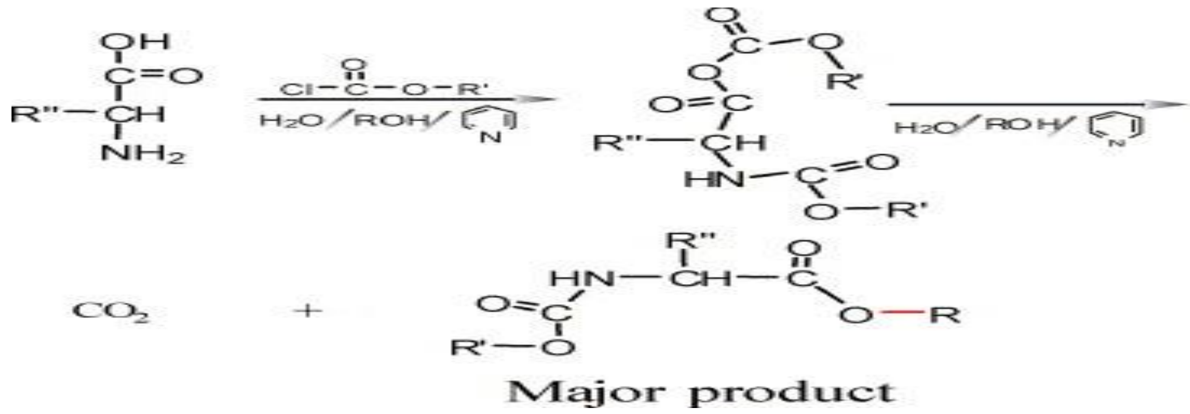

Figure 1: The derivation of the extracted AAs to achieve volatility in the gas chromatography with ethylchlorofomate

Result and discussion

The original AAs were reacted with ethyl chloroformate to produce a new compound, AA derivatives, which were more volatile, less reactive, and thus had properties that were suitable for analyses using a GC, as shown in the reaction. The derivatised AAs that were free of derivatising reagents were made up to 1.0 ml in a vial for gas chromatographic analyses using a gas chromatograph [13]. Therefore, the calculated essential to non-essential AAs ratio, the recommended daily allowance, and the amount to reach the RDA are shown in **Tables 1** and **2** [14].

Table 1: Amino acid composition (g/100 g protein) of soybean and cocoyam flours and their blends

Amino acids	Unblended cocoyam flour	Unblended soybean flour	10% blend of soybean and cocoyam flours	20% blend of soybean and cocoyam flours	30% blend of soybean and cocoyam flours	40% blend of soybean and cocoyam flours	50% blend of soybean and cocoyam flours
Essential AA							
Phenylalanine	5.16	4.10	5.05	5.09	4.75	4.60	4.13
Valine	3.56	5.13	3.72	3.90	4.18	4.49	4.48
Threonine	4.04	3.91	3.96	3.95	4.02	3.75	2.95
Tryptophan	0.97	1.20	1.06	1.04	1.23	1.40	1.74
Isoleucine	3.28	4.38	3.45	3.61	3.98	4.15	3.99
Methionine	1.11	1.28	1.15	1.21	1.25	1.38	1.42
Leucine	5.40	7.34	5.56	5.78	6.24	6.71	6.67
Lysine	6.07	6.51	6.29	6.70	6.98	7.59	7.63
Total essential AA	**29.59**	**33.85**	**30.24**	**31.28**	**32.63**	**34.07**	**33.01**
Non-essential AA							
Alanine	4.13	4.37	4.09	4.15	4.39	4.95	5.25
Aspartic acid	6.18	10.36	6.25	6.39	7.21	7.52	7.23
Glutamic acid	9.97	16.87	10.16	10.33	11.59	12.23	13.87
Serine	4.85	4.95	5.05	5.40	5.20	6.13	6.76
Tyrosine	4.39	3.27	4.24	4.34	3.18	3.47	3.41
Cystine	2.12	1.12	2.41	2.57	2.73	2.97	82.00
Glycine	4.19	4.66	4.23	4.33	4.48	5.14	5.60
Proline	1.64	4.94	1.73	1.77	1.93	2.09	2.10
Histidine	2.20	2.32	2.28	2.53	2.48	2.75	2.82
Arginine	6.22	6.09	6.48	6.37	6.53	7.86	8.11
Total non-essential amino acids	44.89	58.95	46.92	48.18	49.72	55.11	57.15
E/NE ratio	**0.659**	**0.574**	**0.644**	**0.649**	**0.656**	**0.618**	**0.577**

Table 2: Essential amino acids contents (mg/100g) of soybean and cocoyam flour, their blends, RDA's, and amounts that meet RDA'S

Essential Amino Acids	Unblended cocoyam flour (mg)	Unblended soybean flour (mg)	10% blend of soybean /cocoyam flours	20% blend of soybean /cocoyam flours	30% blend of soybean /cocoyam flours	40% blend of soybean /cocoyam flours	50% blend of soybean /cocoyam flours	RDA'S (mg/day)
Phenylalanine	5.16	4.98	5.05	5.09	4.75	4.60	4.13	33
How much to meet RDA's	639.53	666.67	653.47	648.33	694.74	717.39	799.03	
Valine	3.56	5.13	3.72	3.90	4.18	4.49	4.48	24
How much to meet RDA's	674.16	467.84	645.16	615.38	574.16	534.52	535.71	
Threonine	4.04	3.91	3.96	3.95	4.02	3.75	2.95	20
How much to meet RDA's	495.05	626.96	505.06	506.33	497.51	533.33	677.97	
Tryptophan	0.97	1.20	1.06	1.04	1.23	1.40	1.74	5
How much to meet RDA's	515.46	416.67	471.70	480.77	406.50	357.14	287.36	
Isoleucine	3.28	4.39	3.45	3.61	3.98	4.15	3.79	19
How much to meet RDA's	579.27	432.80	550.72	387.81	477.39	457.83	476.19	
Methionine	1.11	1.28	1.15	1.21	1.25	1.38	1.42	19
How much to meet RDA's	1,711.71	1,484.38	1,652.17	1,570.25	1,520.00	1,376.81	1,338.03	
Histidine	2.20	2.32	2.28	2.53	2.48	2.75	2.82	14
How much to meet RDA's	636.36	603.45	614.04	553.36	564.52	509.09	496.53	
Leucine	5.40	7.34	5.52	5.78	6.24	6.71	6.67	42
How much to meet RDA's	777.78	572.21	760.87	726.64	673.08	625.93	629.69	
Lysine	6.07	6.51	6.29	6.70	6.98	7.59	7.63	38
How much to meet RDA's	626.03	583.72	604.13	567.16	544.41	500.66	498.03	

The dominant AAs in the unblended soybean flour were glutamic acid, aspartic acid, leucine, lysine, arginine, valine, isoleucine, glycine, alanine, proline, and phenylalanine, while the AAs that occurred at lower levels were threonine, methionine, histidine, tyrosine, tryptophan, and cystine. The dominant AAs in the unblended cocoyam flour were glutamic acid, lysine, aspartic acid, phenylalanine, arginine, threonine, serine, alanine, glycine, and tyrosine, while the AAs that occurred at lower levels were proline, valine, isoleucine, methionine, histidine, tryptophan, and cystine. The total amounts of AAs in unblended soybean and cocoyam flours were 93.68 and 75.47 (g/100 g protein), respectively. It is observed that there were variations in the AA contents in the various blends of cocoyam and soybean flours, as indicated in the Tables. The values for the blended products show a relationship with the initial values of the unblended cocoyam and soybean flours, respectively. As the proportions of the blends (i.e., as the proportion of soybean flour increased, the AA contents increased. The order was: 10% blend (77.15) < 20%blend < (79.45) < 30% blend (82.36) < 40% (89.17) < 50% blend (90.16) (g/100 g protein). Also depicted was a whole range of AAs that occurred in the unblended cocoyam and soybean flours and their various blends. Essential AAs detected were phenylalanine, valine, threonine, tryptophan, isoleucine, methionine, leucine, and lysine, while the non-essential AAs detected were alanine, aspartic acid, glutamic acid, serine, tyrosine, cystine, glycine, proline, histidine, and arginine. Actually, all AAs are important in body metabolism.

The results presented in **Table 1** are therefore to be understood and appreciated within this context. In this presentation, the essential-to-non-essential AA ratios have been calculated as a novel approach to appreciating the protein values of foods. The E/NE AA ratios for unblended cocoyam and soybean flours were 0.659 and 0.574, respectively. It is suggested that E/NE ratios be compiled for all foods so that one can appreciate the relative protein value of a food sample vis-à-vis others. In agreement with the results in **Table 1**, the sample with the highest essential to non-essential AA ratio was unblended cocoyam flour (0.659). This was followed by 30% blends of soybean and cocoyam flour of 0.656, while 10% and 20% blends of soybean and cocoyam flours had values of 0.644 and 0.649, respectively. The order was: unblended cocoyam flour (0.659) > 30% blend (0.656) > 20% blend (0.656) > 10% blend (0.644) > 40% blend (0.618) > 50% blend (0.577) > unblended soybean (0.574). An interesting observation is made here. Although soybean flour has high protein content and a much higher value than the cocoyam flour used in these studies, the E/NE ratio for cocoyam flour was nevertheless higher than that of the soybean flour. In other words, the smaller amount of protein in the cocoyam is endowed with a higher proportion of essential AAs than that in the soybean flour. Herein lies the significance of the E/NE ratio captured in the present study. This is novel. Hence, for the sake of emphasis, E/NE values should be compiled for all foods, as another window to the nutritional evaluation of foods (based on protein content and AA profile). Total protein content is not the final word on the importance of a food resource as a basis of protein in human nourishment and dietetics. A food with low protein content may be loaded with high amounts of essential AAs. Two food samples equally high in protein content may be quite different in their relative quantities of essential AAs to non-essential AAs. This type of information is important in home and institutional uses of protein foods. Another implication of the results presented in **Table 1** was that the cocoyam and soybean and their various blends can, at least, meet some of the nutritional requirements for the various AAs. Since animal proteins are generally more expensive than plant proteins, any additional source of plant protein, as can be discerned in these results, is most welcome in a country like Nigeria, ravaged by rampant poverty and attendant malnutrition and under-nutrition.

The detection of these AAs in the samples is therefore of health significance, as presented above. Accordingly, the AA data presented in this study can be used to calculate how much of the soybean and cocoyam samples will supply wholly or partially these recommended allowances for the various AAs, as has been done and presented in **Table 2**. The data are presented in **Table 2**. Could find applications in the home and institutions, where critical levels of these AAs may be significant in curative and preventive medicines. Complete protein foods contain all essential AAs; examples include dairy, eggs, meat, seafood, and poultry products. It is observed from this study that soybean and cocoyam flours belong to plant-based foods having all eight vital AAs, making them nutritionally important bases of high-quality protein, although to a varying degree.

Conclusion: It has been established from the results that cocoyam and soybean flours and their various blends could be good sources of food nutrients, including proteins, which have eight essential amino acids. Quality parameters such as essential/non-essential amino acids ratio (E/N ratio), which can be used as additional quality parameters in these products and as additions to existing ones such as PER, BV, NPR, and chemical scores, have been provided and proposed.

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