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Evaluation of Protein and Amino Acid Composition of Selected Spices Grown in the Middle Belt Region of Nigeria

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Abstract: A study was conducted to determine the suitability of six different spices (pepper, garlic, ginger, onion, curry leaf and tomatoes) with a view to appraising their nutritive values. For this purpose, crude protein and amino acid composition were determined using standard analytical techniques. The result gave crude protein content in percentage as; pepper (20.11), garlic (19.94), ginger (11.43), onion (10.36), curry leaf (25.67) and tomatoes (19.83). The Total Amino Acid (TAA) of pepper, garlic, ginger, onion, curry leaf and tomatoes were: 48.40, 66.25, 39.21, 40.21, 78.08 and 40.30 g/100g crude protein, respectively. The order of calculated isoelectric point (pI) was curry leaf > garlic > pepper > onion = tomatoes > ginger while that of Predicted Protein Efficiency Ratio (P-PER) was garlic > curry leaf > ginger > pepper > onion > tomatoes. The contents of Essential Amino Acid (EAA) in all the spice samples are generally lower than FAO/WHO recommended values except curry leaf which had a balance content of all the EAAs and garlic that was adequate only in Leu and Phe + Tyr based on FAO/WHO provisional pattern. So curry leaf is considered to be a valuable protein source for the human diet.

Key words: Spices, crude protein, amino acids, Nigeria

INTRODUCTION

Protein deficiency is a serious cause of ill health and death in developing countries. It plays a part in the disease known as kwashiorkor. Symptoms of kwashiorkor include apathy, diarrhea, inactivity, failure to grow, flaky skin, fatty liver and edema of the belly and legs (Kerstetter *et al*., 2005). Protein deficiency can also lead to reduced intelligence or mental retardation (Lemon, 2000). In countries that suffer from widespread protein deficiency, food is generally full of plant fibres, which makes adequate energy and protein consumption very difficult. This development has stimulated research on the utilization of some under-utilized plant protein sources. Spices are dried seed, fruit, root back, leaf or vegetable substances used nutritionally in significant quantities as food additive for the purpose of flavour, colour, or as a preservative that kills harmful bacterial or prevent their growth (Sovova and Sova, 2004). Many of these spices are also used for other purposes such as medicine, religious rituals, perfumes or eating as vegetables.

Pepper (*Capsicum* sp.) is a genus of flowering plants in the nightshade family, Solanaceae. Its species are native to the Americas, were they have been cultivated for thousands of years by the people of the tropical Americas and are now cultivated worldwide (Zamski *et al*., 1987). In Nigeria, members of *Capsicum* sp. are used as spices, vegetables and medicines. Garlic *(Allium sativum)* is a species in the onion family, Alliaceae. Its close relatives include the onion, shallot, leek and chive (Augusti, 1996). Garlic has been used throughout history for both culinary and medicinal purposes. The garlic plant's bulb is the most commonly used part of the plant. Onion (*Allium cepa*) also known as "garden onion" or "bulb onion". Above ground, the onion shows only a single vertical shoot, the bulb grows underground and is used for energy storage (Daniel and Maria, 2000). Onions can be used, usually chopped or sliced, in almost every type of food, including cooked foods and fresh salads and as spicy garnish. Ginger (*Zingiber officinale*) is a tuber that is consumed whole as a delicacy, medicine, or spice. It lends its name to its genus and family, Zingiberaceae (Daniel and Maria, 2000). Other notable members of this plant family are turmeric, cardamom and galangal. Curry leaf *(Murraya koenigii)* is from curry tree which is a tropical to sub-tropical tree in the family of Rutaceae, native to India (Arulselvan and Subramanian, 2007). Curry leaves are highly valued as seasoning in Nigeria and other parts of the world. Tomato (*Solanum lycopersicum*) is a savoury, typically red and edible fruit. It is originated in South America and was spread around the world following the Spanish colonization of the Americas and its many varieties are now widely grown, often in greenhouses in cooler climates. The tomato fruit is consumed in diverse ways, including raw, as an ingredient in many dishes and sauces and in drinks (Daniel and Maria, 2000). Tomato is used in diverse ways, including raw in salads and processed into ketchup of tomato soup.

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The present study aims at drawing attention of researchers to the crude protein content and amino acid composition of six selected spices (pepper, garlic, ginger, onion, curry leaf and tomatoes) grown in the middle belt region of Nigeria and taking comparative study on them with a view to appraising their nutritive values.

MATERIALS AND METHODS

Sample collection and preparation: Sample of spices (pepper, garlic, ginger, onion, curry leaf and tomatoes) in their fresh forms were collected directly from the farmers in different areas (Kaduna, Jos, Doma, Keffi and Agbashi) located in the middle belt region of Nigeria. Collection was done in the month of April, 2010. After the collection of the samples, due to the changes that do occur frequently in fresh spices, they were carefully cleaned with a stainless steel knife, washed with water, drained and thinly sliced with the stainless steel knife. The thinly sliced samples were oven dry at temperature of 40°C for 72h. Dry samples were ground into powder form and kept in polyethylene bags until prior to use.

Crude protein determination: Crude protein (N x 6.25) was determined in accordance with AOAC (1995) method.

Amino acid analysis: Amino acid analysis was by Ion Exchange Chromatography (IEC) (FAO/WHO), 1991) using the Technicon Sequential Multisample (TSM) amino acid analyzer (Technicon Instruments Corporation, New York). The period of analysis was 76 mins for each sample. The gas flow rate was 0.50 mL/min at 60°C with reproducibility consistent within ±3%. The net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of two determinations. Norleucine was the internal standard. Tryptophan was not determined.

Determination of isoelectric point (pI), quality of dietary protein and predicted protein efficiency ratio (P-PER): The predicted isoelectric point was evaluated according to Olaofe and Akintayo (2000):

$$pIm = \sum_{i=1}^{n=1} pliXi$$

Where:

pIm = The isoelectric point of the mixture of amino acids.
pIi = The isoelectric point of the i^{th} amino acids in the mixture.
Xi = The mass or mole fraction of the amino acids in the mixture.

The quality of dietary protein was measured by finding the ratio of available amino acids in the protein concentrate compared with needs expressed as a ratio (Oshodi *et al*., 1998). Amino Acid Score (AMSS) was then estimated by applying the FAO/WHO (1991) formula:

$$AMSS = \frac{\text{mg of amino acid in 1g of test protein}}{\text{mg of amino acid in 1g reference protein}} \times \frac{100}{1}$$

The Predicted Protein Efficiency Ratio (P-PER) of the spice samples was calculated from their amino acid composition based on the equation developed by Alsmeyer *et al*. (1974) as stated thus:

$$P\text{-}PER = -0.468 + 0.454\ (Leu) - 0.105\ (Tyr)$$

RESULTS AND DISCUSSION

Leucine was the most concentrated essential amino acid in all the samples ranging from 3.24 g/100 g crude protein, cp) in tomatoes to 8.13 g/100 g crude protein, cp) in garlic (Table 1). These values are in agreement with the observations made earlier by some researchers (Aremu *et al*., 2006a; Olaofe *et al*., 2008; Aremu *et al*., 2010) that leu is the most concentrated essential amino acid in Nigerian plant foods. Glutamic acid was the most concentrated amino acid in all the samples except ginger sample which recorded protein as the highest concentrated amino acid (6.85 g/100 cp). Tryptophan concentrations could not be determined. The calculated isoelectric point (pI) varied between 2.34 in ginger to 4.60 in curry leaf. This is useful in predicting the pI for protein in order to enhance a quick precipitation of protein isolate from biological samples (Olaofe and Akintayo, 2000). The Predicted Protein Efficiency Ratio (P-PER) is one of the quality parameters used for protein evaluation (FAO/WHO, 1991). The P-PER in this report ranged from 0.82 in tomatoes to 2.69 in curry leaf. These values (Table 1) compare favourably (except P-PER for tomatoes) with P-PER values of some legume flours/concentrates; *Prosopis africana* (Gull and Perr.) Taub. (2.3) (Aremu *et al*., 2007), *Lathyrus satirus* L. (1.03) (Salunkhe and Kakam, 1989), *Phaseolus coccineus* L. (1.91) (Aremu *et al*., 2008) and *Luffa cylindrica* (1.49) (Olaofe *et al*., 2008). However, all the spices (except tomatoes) under investigation satisfied FAO requirements (FAO/WHO/UNU, 1985). The crude protein ranged between 10.36% in onion to 25.67% in curry leaf (Table 1). The protein values of tomatoes (19.83%), garlic (19.94%), pepper (20.11%) and curry leaf (25.67%) are comparable with some commonly consumed plant proteins in Nigeria (Akobundun *et al*., 1982; Aremu *et al*., 2006b; Ihekoronye and Ngoddy, 1985) and qualified the four spices as protein-rich plant foods.

Table 1: Amino acid composition (g/100 g crude protein) of selected spices

Amino acid	Pepper	Garlic	Ginger	Onion	Curry leaf	Tomatoes
Lysine (Lys)[a]	2.52	4.48	1.61	2.26	5.32	2.04
Histidine (His)[a]	1.88	2.07	0.53	1.25	2.44	1.69
Arginine (Arg)[a]	3.57	4.59	2.72	2.89	4.94	3.40
Aspartic acid (Asp)	5.70	8.66	3.18	3.37	9.78	4.36
Threonine (Thr)[a]	2.00	3.52	2.30	1.55	3.27	1.89
Serine (Ser)	1.94	2.70	1.11	1.40	2.70	1.78
Glutamic acid (Glu)	6.26	9.33	4.13	6.87	11.12	7.49
Praline (Pro)	2.23	2.55	6.85	2.02	3.08	1.49
Glycine (Gly)	3.16	1.94	1.70	2.04	4.01	2.16
Alanine (Ala)	2.90	4.48	1.08	2.12	4.17	2.17
Cystein (Cys)	0.66	0.79	0.53	0.46	0.99	0.40
Valine (Val)[a]	3.40	3.66	2.35	2.99	5.14	2.59
Methionine (Met)[a]	0.60	0.78	0.47	0.39	1.17	0.31
Isoleucine (Ile)[a]	2.26	2.26	1.00	1.88	4.30	1.54
Leucine (Leu)[a]	5.08	8.13	5.66	4.09	769.00	3.24
Tyroseine (Tyr)	2.20	2.42	1.29	2.09	3.22	1.77
Phenylalanine (Phe)[a]	3.04	3.89	2.70	2.54	4.73	2.28
(pI)[b]	2.92	3.97	2.34	2.38	4.60	2.38
(P-PER)[c]	1.84	2.97	1.97	1.15	2.69	0.82
Crude protein (%)	20.11	19.94	11.43	10.36	25.67	19.83

[a]Essential amino acid; (pI)[b], Calculated isoelectric point; (P-PER)[c], Calculated predicted protein efficiency ratio

Table 2: Classification of amino acid composition (g/100 g crude protein) of selected spices

Amino acid	Pepper	Garlic	Ginger	Onion	Curry leaf	Tomatoes
Total Amino Acid (TAA)	48.40	66.25	39.21	40.21	78.08	40.30
Total Non-Essential Amino Acid (TNEAA)	25.05	32.89	19.87	20.07	39.07	21.63
% TNEAA	50.70	49.65	50.68	49.91	50.04	53.67
Total Essential Amino Acid (TEAA) (with His)	24.35	33.38	19.34	19.84	39.00	19.06
TEAA (without His)	22.47	31.31	18.18	18.59	36.56	17.37
% TEAA (with His)	49.29	50.38	49.32	49.34	49.95	47.30
% TEAA (without His)	45.49	47.26	47.97	46.23	46.82	43.10
Essential Alphatic Amino Acid (EAAA)	12.74	17.57	11.31	10.51	20.40	9.26
Essential Aromatic Amino Acid (EArAA)	3.04	3.89	2.70	2.54	4.73	2.28
Total Neutral Amino Acid (TNAA)	29.47	37.15	27.04	23.57	44.48	21.34
% TNAA	59.66	56.08	68.96	58.62	56.97	52.95
Total Acidic Amino Acid (TAAA)	11.96	17.96	7.31	10.24	20.90	11.85
% TAAA	24.71	27.11	18.64	25.47	26.77	29.40
Total Basic Amino Acid (TBAA)	7.97	11.14	4.86	6.40	12.70	7.13
% TBAA	16.13	16.82	12.39	15.92	16.27	17.69
Total Sulphur Amino Acid (TSAA)	1.26	1.57	1.00	0.85	2.16	0.71
% of Cystine in TSAA	52.38	50.32	53.34	54.12	45.83	56.34

The nutritive value of a protein depends primarily on the capacity to satisfy the needs for nitrogen and essential amino acids (Oshodi *et al*., 1998). The total essential amino acid (with His) ranged between 47.30% in tomatoes to 50.38% in garlic (Table 2). These are comparable with similar values obtained from soybean (Salunkhe *et al*., 1985), some under-utilized legumes (43.8-48.3%) (Aremu *et al*., 2006a) and selected oil seeds (33.3-53.6%) (Olaofe *et al*., 1994) suggesting that the spices studied which are readily available in the middle belt region of Nigeria can be used as food supplements. Essential Aliphatic Amino Acids (EAAA), Ile, Leu and Val which constitute the hydrophobic regions of proteins were more abundant in garlic (17.57 g/100 g cp) and curry leaf (20.40 g/100 g cp) compared with the other spices (Table 2). This means that better emulsification properties may be expected in the garlic and curry leaf flours. Table 2 also depicts the percent of Total Acid Amino Acids (TAAA) for all the samples (18.64-29.40%) and were found to be greater than the percent of Total Basic Amino Acids (TBAA) (12.39-19.69%) indicating that the protein is probably acidic in nature. Total Sulphur Amino Acid (TSAA) ranged between 0.71 g/100 g cp in tomatoes to 2.16 g/100 g cp in curry leaf. The TSAA for any of the spices is lower than the 5.8 g/100 g cp recommended for infants (FAO/WHO/UNU, 1985). This confirms many reports on spices that they are used as food additive for the purpose of flavour, medicine, colour or as a preservative that kills harmful bacteria or prevent their growth (Eshbaugh, 1975; Augusti, 1996; Grontved and Pittler, 2000).

Table 3: Amino acid score of the spices

		Pepper		Garlic		Ginger		Onion		Curry leaf		Tomatoes	
EAA	PAAESP[a]	EAAC	AMSS	EAAC	AMSS	EAAC	AMSS	EAAC	AMSS	EAAC	AMSS	EAAC	AMSS
Ile	4.0	2.26	0.6	2.26	0.6	1.00	0.3	1.88	0.5	4.30	1.1	1.54	0.4
Leu	7.0	5.08	0.7	8.13	1.2	5.66	0.8	4.09	0.6	7.69	1.1	3.24	0.5
Lys	5.5	2.52	0.5	4.48	0.8	1.61	0.3	2.26	0.4	5.32	1.0	2.04	0.4
Met + Cys (TSAA)	3.5	1.26	0.4	1.57	0.4	1.00	0.3	0.85	0.2	2.16	1.0	0.71	0.4
Phe + Tyr	6.6	5.24	0.8	6.31	1.0	3.99	0.6	4.63	0.7	7.95	1.2	4.05	0.6
Thr	4.0	2.00	0.5	3.52	0.9	2.30	0.6	1.55	0.4	3.27	1.3	1.89	0.7
Try	1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Val	5.0	3.40	0.7	3.66	0.7	2.35	0.5	2.99	0.6	5.14	1.0	2.59	0.5
Total	36.0	21.76	4.2	29.93	5.6	17.91	3.4	18.25	3.4	35.83	7.7	16.06	3.5

[a]Source: Belschant *et al.* (1975). EAA: Essential Amino Acid, PAAESP: Provisional Amino Acid (Egg) Scoring Patterns, EAAC: Essential Amino Acid Composition (see Table 1), AMSS: Amino Acid Scores, ND: Not Determined

The contents of essential amino acid are generally lower than FAO/WHO (1991) recommendations (Table 3) except in curry leaf which was adequate in all its EAA based on FAO/WHO provisional pattern and garlic that was adequate only in Leu and Phe + Tyr. Thus, based on our findings only curry leaf may be used as a food supplement for any food material that is not adequate in essential amino acid. It has been reported that the essential amino acids most often acting in a limiting capacity are Met (and Cys), Lys and Try (Aremu *et al.*, 2006a). However, the first limiting amino acid of the food samples in this study were: Pepper, garlic and onion (Met + Cys), ginger (Ile, Lys and Met + Cys), curry leaf (Lys, Met + Cys and Val.) and tomatoes (Ile, Lys and Met + Cys). Try could not be determined.

Conclusion: The study showed that tomatoes, garlic, pepper and curry leaf were rich in crude protein comparable with some protein-rich legumes. Although all the spices investigated were found to have contained nutritionally usefull quantities of most of the essential amino acids but only curry leaf could serve as food supplement to food materials that are not adequate in essential amino acid based on FAO/WHO provisional pattern.

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