



Changes in FDNB-Available Lysine in Sprouted Cowpea

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Abstract: Cowpea a member of the legume family was sprouted to be able to improve its nutritive value especially the amino acids. Lysine, however, is highly vulnerable to process loss. One of the ways to determine the availability of the essential amino acids is to determine the available lysine. This study was geared not only in measuring the available amino acids, by determining the available lysine using the FDNB (Fluoro-di-nitro-benzene) extraction method, but to improve on the availability of these amino acids through germination. This method is also capable of removing the drudgery of animal study and faster to achieve the desired results.

1. Introduction

The first stage in germination of seeds involves the breakdown of seed reserves and their utilization by the growing root and shoots. It has been established that during germination, stored product are converted into more usable forms for both plant and man (Chen *et al.*, 1975).

The practice of sprouting legumes like mung beans and soybeans has long been known. It has been advocated as a means of improving the nutritional worth of many other seeds (Elwood, 1971; Whyte, 1973; Blanchard, 1975; Fordham *et al.*, 1975).

Many procedures using chemical methods have been applied to the evaluation of proteins: namely, chemical scores; based on the amount of the essential amino acids in protein compared to the level present in a reference protein selected for its nutritional excellence. The essential amino acid index derivation, which was based on two assumptions, that the minimum ratio of essential amino acid content relative to that of standard protein is one percent, and that the maximum is 100 percent. The biological value is correlated with essential amino acid index. The amino acid analysis, using ion exchange chromatography, has been favoured in this study. Walker (1981) regarded it as one of the most important chemical techniques for predicting protein quality.

Chemical methods for the determination of amino acid availability are almost entirely restricted to the estimation of available lysine. Most of the methods are based on the estimation of free ϵ -amino group of lysine in the intact

protein. The nutritional availability of lysine has been based on the assumption that these groups are free to react chemically. Lysine in its reactive (available) form is reported to be the first limiting essential amino acid to be rendered unavailable during processing or even prolonged storage under certain conditions (Carpenter, 1974; Concon, 1975). Thus lysine may easily deaminate, be oxidized, or undergo the browning reaction. Therefore its availability can be used to monitor processing damage and other unit operations.

A procedure for the estimation in foods of the lysine units, whose ϵ -amino group will undergo reaction with fluorodinitrobenzene (FDNB), as illustrated in Fig. 1, was developed by Carpenter and Ellinger (1955). Any lysine residue which is already bound with another group through its ϵ -amino group is not free to react with FDNB. Lysine residues with a 'free' amino group, which is available for reaction with the reagent, produce a yellow dinitrophenyl derivative (DNP), which can be estimated colorimetrically after acid hydrolysis

According to Bruno and Carpenter (1957), α -DNP-arginine, γ -DNP-ornithine and water-soluble breakdown products from the other DNP-amino acids still remained in the aqueous phase along with the ϵ -DNP-lysine. A modification was introduced by these workers to correct for this error. The colour contributed by these compounds was estimated after removal of the ϵ -DNP-lysine from the aqueous. Reaction of the yellow aqueous phase with methoxycarbonyl chloride changed the ϵ -DNP-lysine into

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an ether-soluble derivative, the interfering compounds remaining water-soluble. Colorimetric measurement of the yellow aqueous phase before and after treatment with methoxycarbonyl chloride and ether extraction accordingly gives an estimate of the colour contributed by the impurities.

The correction for the loss of ϵ -DNP-lysine produced during hydrolysis was carried out by Carpenter (1960), where he measured the recovery of ϵ -DNP-lysine added to the DNP-protein immediately before the hydrolysis stage. An average value of 92% was found. He then corrected his available lysine value on this basis, with the assumption that free ϵ -DNP-lysine reacted similarly on hydrolysis to ϵ -DNP-lysine in proteins.

Carpenter's method is well established for measuring available lysine in animal protein products; difficulties are encountered when it is applied to cereal grains and legumes. These are manifested as poor recoveries of ϵ -DNP-lysine and variable results. The reasons for the poorer performance on these materials are associated with the destruction of ϵ -DNP-lysine by carbohydrate during hydrolysis and the formation of other yellow products which are not easily separated from ϵ -DNP-lysine (Bruno and Carpenter, 1957)

Carpenter (1960) recommended that a correction factor of 1.09 should be used for animal and fish proteins to compensate for an average recovery of 92%. Rao *et al.*, (1967), on the other hand, made individual corrections based on the actual recoveries increased the standard deviation over that of the uncorrected samples, and found that the variability of the recoveries increased the standard deviation over that of the uncorrected results. This evidence, therefore, makes the use of an average correction factor recommended by Carpenter questionable.

Walker (1979b), using Carpenter, (1960) method, as modified by Booth (1971), found that the FDNB-reactive lysine was highly correlated ($r = + 0.90$) with the dye binding reactive lysine method. Dye binding using acid dyes at low pH primarily involves the formation of ionic linkages with the basic amino acids (lysine, histidine and arginine) present in protein, as well as with terminal amino groups (Walker, 1979a).

2. Materials and Methods

The fluorodinitrobenzene-reactive lysine determination was carried out by direct estimation as described by Carpenter, (1960) and modified by Booth (1971). A flow diagram method prepared by Walker (1981) for ease of systematic analysis was followed and is shown:

Flow Diagram of the Carpenter, (1960) procedure for the determination of FDNB-Reactive Lysine, as modified by Booth, (1971), prepared by Walker, (1981)

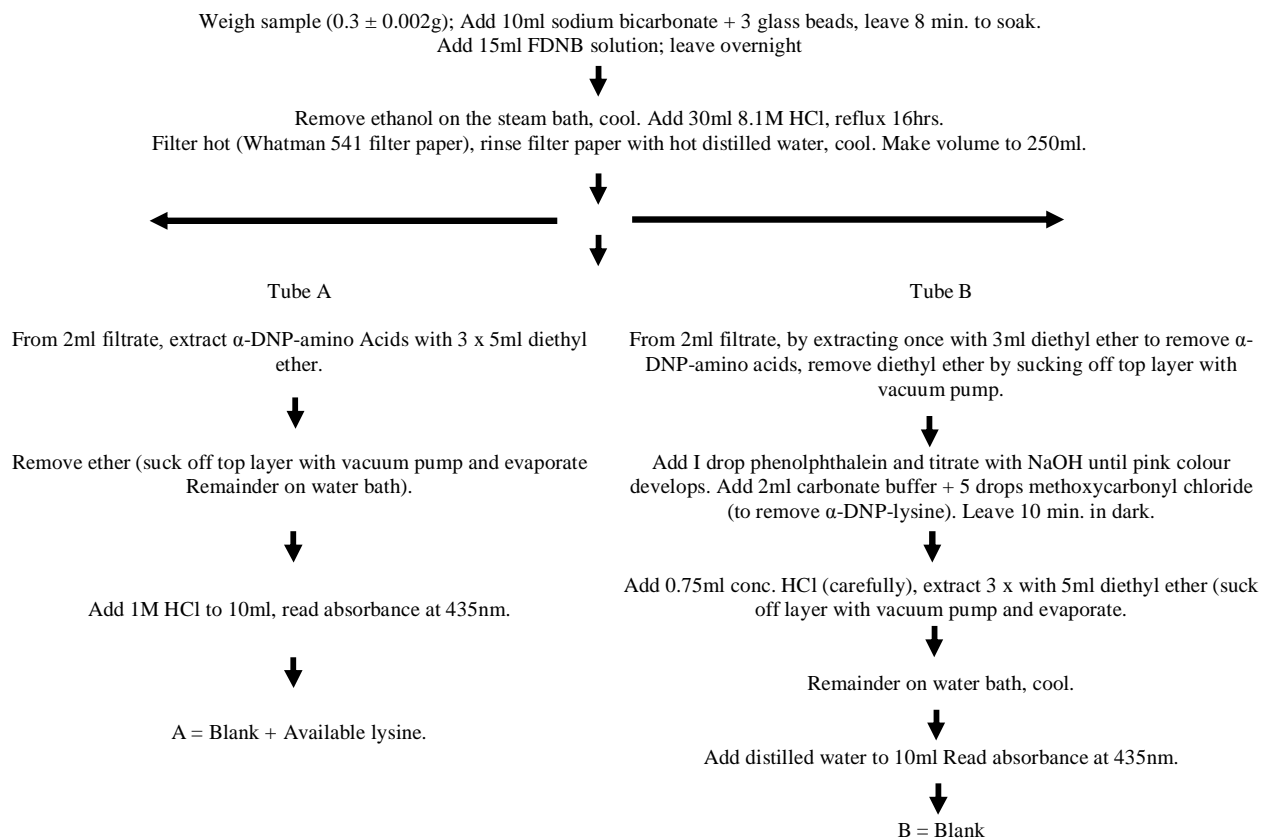


Table 1. Changes in FDNB-Available Lysine in Sprouted Cowpea Grains.

Fermentation / Germination Period (Days)	FDNB-available - lysine (uncorrected) *	Percentage Recovery *	FDNB-lysine (Corrected) *	Percentage availability of total lysine *
0	2.60	82.00	3.17	43.36
2	4.73	92.00	5.14	77.24
3	4.88	90.00	5.43	75.23
5	5.68	91.50	6.21	71.02
Standard error of difference (SED)	0.14	1.70	0.09	1.16
Variance ratio (F)	187.8	15.10	411.4	370.9

*Mean of two replicates

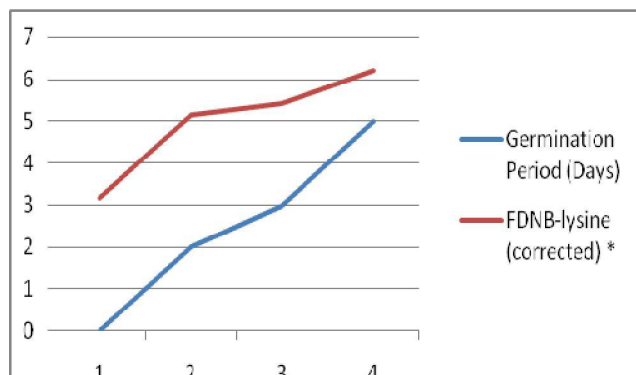


Fig. 1. Changes in FDNB-Lysine (Corrected) * During Germination.

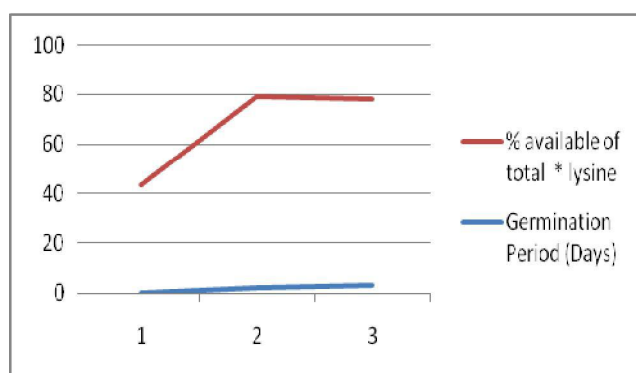


Fig. 2. Changes in % availability of total Lysine * During Germination.

Absorbance due to FDNB-reactive lysine = A - B. Sample was hydrolysed in duplicate, but each duplicate was extracted by A and B only once. Accompanying each assay batch was a standard solution of DNP-lysine, which helped in monitoring the percentage recovery.

The following reagents were used:

- 1-fluoro-2,4-dinitrobenzene (FDNB): 2.4ml of FDNB (BDH) in 96ml of ethanol, freshly prepared,
- Methoxycarbonyl chloride (methyl chloroformate, Aldrich). Toxic, to be handled with care!!
- Sodium bicarbonate solution, 80g per litre.
- Phenolphthalein solution: 40g dissolved in 100ml of 60% (v/v) ethanol.
- Sodium hydroxide solution: 120g per litre.

- Carbonate buffer: 19.5g sodium bicarbonate (NaHCO₃) and 1.0g sodium carbonate (Na₂CO₃.10H₂O) were dissolved and made up to 250ml with distilled water, the pH is adjusted to 8.5.
- Mono-N-ε-dinitrophenyl lysine hydrochloride monohydrate (DNP-lysine, BDH): 314mg of DNP-lysine were dissolved in 100ml 8.1M HCl and made up to 250ml. This was the stock solution, which was stored in the refrigerator. Stock solution (10ml) was diluted to 100ml with distilled water and used as working standard containing the equivalent of 0.1mg lysine per 2ml aliquot. The working standard was also taken through the procedures described in the figure above, as the absorbance of the standard was required for the calculation.

Apparatus used: 250ml round-bottomed flasks; stoppered glass tubes graduated to 10ml; heating mantle with 6 positions for flasks; spectrophotometer at 435nm.

Calculation: The result was calculated as follows:

$$\text{FDNB - reactive - lysine} = \frac{\text{Ws} \times \text{At} \times \text{Vx} \times 100 \times 100}{\text{Wt} \times \text{As} \times \text{a} \times \text{cP}} \times \frac{128.17}{146.19}$$

Where: Ws = Weight of standard, expressed as mg lysine in 2ml;
 Wt = Weight of test material in mg;
 As = net absorbance of standard;
 At = net absorbance of test sample;
 V = Volume of filtered hydrolysate (250ml);
 a = aliquot of filtrate taken for analysis (2ml);
 cP = Crude protein (% nitrogen x 6.25) in test sample.

This method of calculation was recommended by Booth (1971).

The molecular weight of the lysine residue was used rather than the molecular weight of the amino acid because it was used for the calculation of the total lysine in the total amino acid analysis.

The results of the recovery are calculated in a similar manner and expressed as g/16g N ε-DNP-lysine and given as a percentage of ε-DNP-lysine added to prior hydrolysis.

3. Results

Table above shows the results of the changes in FDNB-available lysine during sprouting of cowpea grains. Less than half of the lysine present in the raw cowpea is available. However, the 2-day sprouts had over 75% of the total lysine as available lysine.

Lysine availability was slightly less than this in the 3 and 5-day sprouts, but the amount of available lysine increased with the length of the germination period. Even with the two sprouting periods chosen for the process investigation, the increase in available lysine was almost 40% the first improvement in any of the nutritional parameters studied which was found to be greater than the processing loss in total dry matter.

4. Discussion

The germination process was considerably beneficial in sprouted cowpea. The highest percentage of available lysine was recorded for the 2-day cowpea sprouts (Table 1). The available lysine increased 1.78 times during two days of sprouting.

However, as the germination period was prolonged, the availability dropped slightly. This slight drop in percentage of the total lysine available appeared unimportant in view of the increased amount of FDNB-lysine present in the 3 or 5-day sprouts. The results show that germination of cowpea increased the available lysine remarkably.

The germination process was beneficial, as depicted by this increase in lysine availability. This finding is in agreement with investigation reported by Sarwar *et al.*, (1977) on amino acid available in field pea, faba bean and soybean, and also work reported by Venugopal and Rao (1978).

References

- [1]. BLANCHARD, M.P. (ED.) (1975). 'The Sprouters Cookbook'. Garden Way Publishing, Vermont.
- [2]. BOOTH, V.H. (1971). Problems in the determination of FDNB-available lysine. *J. Sci. Food Agric.*, 22: 658-66.
- [3]. BRUNO, D. and CARPENTER, K.J. (1957). Kwashiorkor in Africa. FAO Nutritional Studies No. 8. FAO: Rome.
- [4]. CARPENTER, K.J. and ELLINGER, G.M. (1955). The estimation of available lysine in protein concentrates. *Biochem. J.*, 61: XI-XII.
- [5]. CARPENTER, K.J. (1960). The estimation of available lysine in animal protein foods. *Biochem. J.*, 77: 604-610.
- [6]. CARPENTER, K.J. (1974). Chemical and microbial assays for the evaluation of processed protein foods. In: Nutrients in processed food proteins. American Medical Association (eds. P.L. White and D.C. Fletcher). Acton, Massachusetts. Pub. Sciences Group Inc.
- [7]. CHEN, L.H., WELLS, C.E. and FORDHAM, J.R. (1975). Germinated seeds for human consumption. *J. Food Sci.*, 40: 1290-1294.
- [8]. CONCON, J.M. (1975). Chemical determination of critical amino acids in cereal grains and other foodstuffs. In: Protein nutritional quality of foods and feeds. Part 1 (ed. M. Friedman). Marcel Dekker Inc., New York.
- [9]. ELWOOD, C. (1971). Feel like a million. Simon and Schuster of Canada Ltd. Richmond Hills, Ontario.
- [10]. FORDHAM, J.R., WELLS, C.E. and CHEN, L.H. (1975). Sprouting of seeds and nutrient composition of seeds and sprouts. *J. Food sci.*, 40: 552-556.
- [11]. SARWAR, G., SOSULSKI, F.W. and BELLS, J.M. (1977). Availability of amino acids in legumes and legume-wheat blends. *Can. Inst. Food Sci. Technol. J.* 10: 31-35.
- [12]. VENUGOPAL, K. and RAO, R.G. (1978). Available lysine and vitamin C in germinated black-gram (*Cicer arietinum*, L.) seed. *J. Sci. Food Agric.*, 15: 25.
- [13]. WALKER, A.F. (1979a). Determination of protein and reactive lysine in leaf-protein concentrates by dye-binding. *Br. J. Nutr.*, 42: 445-454.
- [14]. WALKER, A.F. (1979b). A comparison of the dye-binding and fluorodinitrobenzene methods for determining reactive lysine in leaf-protein concentrates. *Br. J. Nutr.*, 42: 455-465.
- [15]. WALKER, A.F. (1981). The application of in-vitro methods to the estimation of protein quality of leaf protein concentrates. Ph.D. Thesis, University of Reading, England.