Page 106 to 110

Volume 1 • Issue 3 • 2017

Research Article

Nutrition and Food Toxicology

ISSN: 2573-4946

Protein Fractionation and *In Vitro* Protein Digestibility of Green Leaves of *Cassia Obtusifolia* and Kawal

Algadi MZ[1]* and Yousif NE[2]

[1]*Arab Center for Nutrition, Muharraq, Bahrain*

[2]*Faculty of food science and technology, Khartoum University, Sudan*

***Corresponding Author:** Mutasim Zeanalabdeen Algadi, Arab Center for Nutrition, Muharraq, Kingdom of Bahrain.

Received: December 12, 2016; **Published:** July 13, 2017

Abstract

Fermentation processes play important roles in food technology in developing countries. In traditional fermentation processes, natural micro-organisms are employed in the preparation and preservation of different types of food. These processes add to the nutritive value of foods as well as enhancing flavor and other desirable qualities associated with digestibility and edibility. Cassia obtusifolia (family leguminous) is a wild African plant found in wastelands in the rainy season. Its leaves can be fermented (named kawal) and is used by people from the eastern part of Chad and the western part of Sudan as meat replacer or meat extender. The role of kawal and the like is in providing the sauces which make these staples palatable. During famine years, kawal, a protein source, probably protected many children against kwashiorkor. The objectives of this study was to assess the effect of fermentation on the protein fractionation and in vitro protein digestibility of Cassia obtusifolia leaves and kawal. The effect of fermentation on protein fractions showed a significant ($P < 0.05$) increase in globulin from 58.52 to 63.38 %, Prolamin 8.69-13.83%, glutelin 5.03-8.32%, and no significant ($P > 0.05$) change in albumin 21.59-14.43%. Insoluble protein was decreased from 17.81 to 5.41%, globulin and albumin are major fraction of Kawal protein. The in vitro protein digestibility was significant ($P < 0.05$) increased from 49.43 to 61.87%. It is recommended to use fermentation to increase the protein fractionation and in vitro protein digestibility of Cassia obtusifolia.

Keywords: *Fractionation; Fermentation; Kawal; Protein; Indigenous*

Volume 1 Issue 3 July 2017

Introduction

Fermentation is considered the second oldest method for preserving food in the world, after drying (Farnworth, 2008). For many thousands of years, people used the art of fermenting to produce different varieties of food items in order to extend the time for which they could store them or to achieve specific aromas or textures, without really knowing anything about the science behind this (Pallin, 2015). It is the process of transforming simple raw materials into different products with added value by exploiting the growth and activity of microorganisms on different substrates. People soon found other advantages of fermentation, e.g. not only could they store their food for a longer time, but they could also change the taste, texture and overall sensory sensation of that food. Other advantages regarding

health and nutritional benefits also emerged (Pallin, 2015). Until a few years ago, kawal was little known to most Sudanese, for it was a product confined to the western provinces of the country, away from populated areas and centers of influence. Kawal is a strong smelling Sudanese, protein-rich food prepared by fermenting the leaves of a wild African legume, Cassia obtusifolia and is usually cooked in stews and soups. It is used as a meat replacer or a meat extender. Its protein is of high quality, rich in sulphur amino acids which are usually obtained from either fish or meat (Fellows, 1997). The objectives of this study was to assess the effect of fermentation on the protein fractionation of green leaves of Cassia obtusifolia and kawal.

Material and Methods

Kawal preparation method

In Kawal fermentation according to Fellows (1997), the Cassia obtusifolia leaves should be collected late in the rainy season when the plant is fully grown. All the stems, pods and flowers should be removed. The leaves should not be washed. It is thought that natural micro-organisms on the leaves are important for the correct fermentation. The leaves of the leguminous plant are pounded into paste without releasing the juice. The paste is placed in an earthenware jar and covered with sorghum leaves. The whole jar is sealed with mud and buried in the ground up to the neck in a cool place. Every three days the contents are mixed by hand. Irregular balls or flattish cakes which are then sun dried for 3-4 days. The duration of the fermentation is about 25 days for the supply of an average family. *Cassia obtusifolia* leaves and kawal were obtained in dry form after been sun dried and freed from foreign materials and powdered by hummer mill with same mesh size and was kept in clean bottles at room temperature for further use.

Protein fractionation due to solubility

The Mendel and Osborne (1914) technique for protein fractionation was used in this study.

Determination of water soluble proteins (Albumins): A sample of 2.5 grams was taken from defatted seed flour for fractionation of total proteins. To this amount of the flour, 2 volumes of 50 ml distilled water was added and the mixture was shaken for 30 minutes using mechanical shaker, then centrifuged at 300 rpm for 20 minutes to separate the insoluble parte from the liquor. The extraction liquor was made up to 100 ml. ten ml were taken for protein estimation according to the micro-kjeldahal method. The following formula was used for calculating percentage of albumin:

$$\text{Total albumin} = \frac{T \times 1.4 \times DF \times 6.25}{W \times 1000}$$

T = titer reading.
DF = dilution factor.
W = weight of sample.

Determination of salt soluble proteins (Globulins): The insoluble part obtained after extraction of albumin was re-extracted with 2 volumes of 50 ml NaCl (1M) for 30 minutes with continuous shaking. The mixture was then centrifuged at 3000 rpm for 20 minutes to separate the insoluble part. The extracted liquor was collected in 100 ml volumetric flask. Ten ml of the liquor were taken for estimation of soluble protein by the micro-kjeldahl method.

Percentage of globulins as following:

$$\text{Total globulins} = \frac{T \times 1.4 \times DF \times 6.25}{W \times 1000}$$

$$\text{Globulins} = \frac{\text{Total globulins} \times 100}{\text{Total proteins}}$$

Determination of alcohol soluble proteins (Prolamins): The insoluble parts obtained after extraction of Sault soluble proteins was re-extracted with 2 volumes of 50 ml 70% ethanol added to insoluble part with continuous shaking for 30 minutes in mechanical shaker. The peptized liquor was separated from the residue by centrifugation at 3000 rpm for 20 minutes. The peptized liquor was collected in 100 ml volumetric flask. Ten mil of the lacquer were taken for protein determination by the micro-kjeldahl method. The percentage of alcohol soluble proteins was calculated from total proteins as following:

$$\text{Prolamin (\%)} = \frac{\text{Total prolamins} \times 100}{\text{Total proteins}}$$

Determination of alkali-soluble proteins (Glutelins): The insoluble part obtained after the extraction of prolamin was re-extracted with 2 volumes of 50 ml NaOH (0.2%) for 30 minutes with continuous shaking. The insoluble part was separated by centrifugation at 3000 rpm for 20 minutes. The peptized liquor was collected in 100 ml volumetric flask and 10 ml taken for nitrogen determination.

Protein content of insoluble fraction: The remaining on soluble part of the sample was digested in 10 ml sulphuric acid and used for estimation of insoluble nitrogen in digest.

***In vitro* protein digestibility**

Determination of *in vitro* protein digestibility was carried out according to Saunder., *et al.* (1973) method. Two hundred milligrams of sample were placed into a 50 ml centrifuge tube, 15 ml of 0.1M HCl containing 1.5 mg pepsin were added, and the tube was incubated at 37oC for three hours. The suspension was then neutralized with 0.5 ml of NaOH (calculated 3.3 ml), then treated with 4 mg of pancreatin in 7.5 ml of 0.2M phosphate buffer (pH 8.0) containing 0.005 sodium azide, the mixture was then gently shacked and incubated at 37°C for 24 hours. After incubation the sample was treated with 10 ml 10% trichloroacetic acid and centrifuged at 5000 × g for 20 min at room temperature .

Nitrogen is supernatant was estimated using micro-kheldhal method.

Digestibility was calculated using the formula

$$\text{Protein digestibility (\%)} = \frac{\text{N in supernatant} \times 100}{\text{N in sample}}$$

$$\text{N in supernetant} = \frac{T \times TV \times N \times 14 \times 100}{A \times B \times 1000}$$

Results and Discussion

Protein fractionation of green leaves of *Cassia obtusifolia* and kawal is shown in Table 1. Fermentation was found to highly significant increase ($p > 0.05$) in Albumin content. The Albumin content of green leaves of *Cassia obtusifolia* and kawal was increased from 12.59 to 14.43% from total protein. The value obtained in this study was higher than the value (4.12%) reported by Mahmoud (2009) Fermentation was found to highly significant increase ($p > 0.05$) in Globulin content. The Globulin content of green leaves of *Cassia obtusifolia* and kawal was increased from 58.52 to 63.38% from total protein. The value obtained in this study was higher than the value (54.61%) reported by Mahmoud (2009). Fermentation was found to highly significant increase ($p > 0.05$) in Prolamin content. The Prolamin content of green leaves of *Cassia obtusifolia* and kawal was increased from 5.03 to 8.32 % from total protein. The value obtaind in this study was same that value (5.34%) reported by Mahmoud (2009). Fermentation was found to highly significant increase ($p > 0.05$) in Glutelin content. The Glutelin content of green leaves of *Cassia obtusifolia* and kawal was increased from 5.03 to 8.32% from total protein. The value obtained in this study was lower than the value (27.64%) reported by Mahmoud (2009) Fermentation was found to highly significant decrease ($p > 0.05$) in Insoluble protein content. The Insoluble protein content of green leaves of *Cassia obtusifolia* and kawal was decreased from 17.81 to 5.41 % from total protein. Lower than the value (12.48) obtained by Mahmoud (2009)

Sample	Globulin %	Albumin %	Prolamin %	Glutelin %	Insoluble Protein %	Some
Cassia obtusifolia leaves	58.52 (± 0.325)[b]	12.59 (± 0.139)[a]	8.69 (± 0.242)[b]	5.03 (± 0.162)[b]	17.81 (± 0.315)[a]	102.64
Dry Kawal	63.38 (± 0.317)[a]	14.43 (± 0.404)[a]	13.83 (± 0.078)[a]	8.32 (± 0.081)[a]	5.41 (± 0.239)[b]	105.37

-Each value in an average of three values expressed on dry weight basis.

-Values are means (± standard deviation).

-Means not sharing a common letter in a column are significant at p > 0.05 as assessed by Duncan's multiple range tests.

Table 1: *Protein fractionation of green leaves of Cassia obtusifolia and kawal (as dry matter).*

In vitro protein digestibility

In vitro protein digestibility of green leaves of *Cassia obtusifolia* and kawal is shown on Table 2. Fermentation was found to cause highly significant (p > 0.05) increase in *in vitro* protein digestibility. It was increased from 49.433 to 61.867%.

Sample	*In vitro* protein digestibility
Cassia obtusifolia Leaves	49.433 (± 1.079)[a]
Dry Kawal	61.867 (± 1.050)[b]

-Each value in an average of three values expressed on dry weight basis.

-Values are means (± standard deviation).

-Means not sharing a common letter in a column are significant at p > 0.05 as assessed by Duncan's multiple range tests.

Table 2: *In vitro protein digestibility of green leaves of cassia obtusifolia and kawal.*

The value obtained in this study were in agreement with value obtained by Babiker., *et al*. (1998) who reported that, the *In vitro* protein digestibility of green leaves of *Cassia obtusifolia* 52.6%. Fermentation is known to cause increase in *in vitro* protein digestibility due to micro flora that may produce some proteulytic enzymes during fermentation, which may be responsible for increasing in protein digestibility. Also Monawar (1983) stated that the reduction in pH during fermentation plays an important role in enhancing native proteulitic enzymes activity and consequently promotes the breakdown of protein to smaller polypeptides which are easily digested by enzymes.

References

1. Babiker A., *et al.* "A note on the effect of tannin content on the In vitro protein digestibility of some Sudanese flora". *Journal of agricultural science* 6.2 (1998): 157-161.
2. Campbell-Platt G. "Fermented Foods of the World. A dictionary and guide". Butter Worths, London (1987).
3. Dirar HA. "Traditional fermentation technologies and food policy in Africa". *Appropriate technology* 19.3 (1992): 21-23.
4. Dirar HA. "The Indigenous Fermented food of the Sudan". A study in African Food and Nutrition CAB International, Walling ford (1993).
5. El Tinay., *et al*. "Sorghum fermented Kisra bread .1: nutritive value of Kisra". *Journal of science food and agriculture* 30.9 (1979): 859-563.
6. Farnworth ERT. Handbook of fermented functional foods: CRC press. (2008).
7. Pallin A. "Fermentation of Barley Flour with Lactobacillus reuter". Licentiate Thesis. Swedish University of Agricultural Sciences. Sweden (2015).

8. Mendel L B and Osborne TB. "Nutritional properties of protein of maize kernel". *Journal of Biological Chemistry* 18 (1914): 1-8.
9. Monawar LY. "Food value of Sudanese indigenous cereal grain". Ph.D. Thesis. University of Khartoum, Sudan. (1983).
10. Algadi M. "Effect of fermentation on nutritional value of Cassia obtusifoloia Kawal", M.Sc. Khartoum University, Sudan (2008).
11. Pirie, NW. "Leaf protein and other aspects of fodder Fraction". Cambridge press UK (1978).
12. Saunder RM., *et al.* "Measurement of digestibility of Alfa protein concentrate by in vitro methods". *Journal of nutrition* 103.4 (1973): 530-535.
13. Stanton W R. "Solid-substrate fermentation." *Global impacts in applied microbiology* 5 (1978): 180-189.
14. Steinkrause KH. "Contribution of Asian fermented foods to international food science and technology". *Global Impacts in Applied Microbiology* 5 (1978):173-179.