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Short Communication

Effect of maturity stage on protein fractionation, *in vitro* protein digestibility and anti-nutrition factors in pineapple (*Ananas comosis*) fruit grown in Southern Sudan

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The investigation shows that albumin, globulin and protein content increases with increase in the days of maturity stage. While glotulin and non-protein nitrogen decreases with increase in the days of maturity stage. The prolamine remains constant during the maturity. An *in vitro* protein digestibility improves by decreases in tannin and phytic acid with increase in the days of maturity stage because tannin and phytic acid inhibits the activity of the pepsin enzyme.

Key words: Maturity, pineapple, digestibility, anti-nutrition.

INTRODUCTION

The importance of pineapple fruit lies in their nutritive value. It is rich in protein, fat, fiber, vitamins, mineral salts and water (Atif and Hagag, 1995). It is consider as supplement to daily food (Pandey and Ajanta, 1993). The physical, chemical and sensorial characters of pineapple show significant difference at several maturation stage (Guerra and Livera, 1999). Pineapple contains 25 - 50 mg/g ellagic acid (Amakura et al., 2000). The juice of pineapple fruit contains five proteolytic enzymes collectively known as bromelain (William, 2000). Bromelain is natural blood thinner because it prevents excessive blood platelet stickiness. It also reduces the thickness of mucus, which may benefit the patient with asthma or chronic bronchitis. In addition, bromelain in combination with trypsin may enhance the effect of antibiotic in people with urinary tract infection (Manhart, 2002). Bromelain has some remarkable characters such as its ability to reduce inflammation pain, swelling and speed the healing of injuries, trauma, surgery, sinusitis or arthritis (Mackay and Miller, 2003). The pineapple fruit contained tannin, phenolic acid and flavonoids (William, 1991). Guerra and Livera (1999) reported that fruit of pineapple reached the maturation at 105 days after flowering stage.

The objectives of this research are to study the effect of maturity stage on the protein fractionation, *in vitro* protein digestibility and antinutrition factors (Tannin, phytic acid).

MATERIAL AND METHODS

Preparation of sample

The ripe and unripe pineapple fruit were collected from Southern Sudan (Yambio Research Station, Agriculture Research Organization, Ministry of Agriculture, Sudan) in 2009 and this is on basis of maturity stage (105, 90 and 75 days). The preparations of samples was carry out according to the method describes by AOAC (1984). The samples were mixed mechanically till homogenous samples was obtained and kept in glass bottle in refrigerator (-2°C) before the analysis.

Protein fractionation

The sequential extractions of protein was carried out according to Mendel and Osborne (1924). It is on the basis of the solubility of protein in different solvents: Water soluble protein (Albumins), salt

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Table 1. Protein fractionation of ripe and unripe pineapple fruit, grown in Southern Sudan.

Sample	Albumin	Globulin	Prolamin	Glotulin	Non protein N_2
105 days maturity	4.4^b (± 0.03)	1.2^a (± 0.6)	0.7^a (± 0.13)	0.9^a (± 0.03)	92.9^b (± 0.01)
90 days maturity	3.2^a (± 0.09)	0.6^a (± 0.05)	0.7^a (± 0.13)	1.0^a (± 0.12)	94.6^a (± 0.01)
75 days maturity	3.0^a (± 0.09)	0.4^a (± 0.05)	0.7^a (± 0.13)	1.8^a (± 0.12)	94.2^a (± 0.01)

Mean values of same letters within the column are significantly difference at ($P \leq 0.05$).

soluble protein (Globulins), alcohol soluble protein (Glotulin), alkali soluble protein (Prolamin) and residual proteins (None – protein nitrogen). The residues remaining after those successive extractions with four solvents are determine by semi micro-Kjeldhal method according to AOCA (1990).The Percentage of protein extracted was calculated as follows:

$$\text{Total soluble protein \%} = \frac{T \times N \times TV \times 14 \times 6.25}{1000 \times A} \times 100$$

Where: T = Titer reading (ml of HCl); N = Normality of the HCl (0.02 N); TV =Total Volume of the aliquot extracted (100 ml); A = Number of (ml) of sample extracted (2.0 g), 14 = each ml of HCl is equivalent to 14 mg; N = Nitrogen; 1000 = Factor for convert volume into number of mg and 6.25 = Conversion factor from nitrogen into protein %.

The soluble protein for each type of protein (according to its solvent soluble) was calculated as follows

$$\text{Protein solubility \%} = \frac{\text{Soluble protein}}{\text{Total protein}} \times 100$$

Anti-nutrition factors

Tannin content

Quantitative estimation of tannins for each sample was carried out by using modified vanillin- HCl methanol method as described by Price and Butler (1987).

There is no useful standards curve for tannin in food. The standard curve of tannic acid prepared according to AOAC (1990) was used for the measurement of the concentration of tannin in our samples [plotting the concentration of tanninic acid (mg) against the corresponding reading of Spectrophotometer in Absorbance].

Phytic acid content

The phytic acid content was determined according to the method described by Wheeler and Ferrel (1971). Preparations of standard curve for phytic acid was done as follows: Standard curve of different Fe $(NO_3)_3$ concentrations was plotted against the corresponding readings of the Spectrophotometer for the calculation of the ferric iron concentration. The phytate phosphorus was calculated from the concentration of ferric iron assuming 4:6 is irons: phosphorus molar ratio.

In vitro protein digestibility

It was carried out according to Maliwal method (1983) in the manner described by Monjula and John (1991) with minor modification. A known weight of the sample containing 16 mg nitrogen was taken in triplicates and digested with 1 mg pepsin in 15 ml of 0.1 M HCL at 37°C for 2 h. The reaction was stopped by the addition of 15 ml (10%) trichoroacetic acid. The mixture was separated with the use of Whatman No.1 filter paper. Trichoroacetic acid soluble fraction was then assayed for nitrogen by semi-micro Kjeldhal method.

$$\text{Protein digestibility \%} = \frac{N_2 \text{ in supernat} - N_2 \text{ in pepsin}}{N_2 \text{ in sample}} \times 100$$

Statistical analysis

Three separate sub samples from each origin sample was analyzed. The mean values was taken. Data was then assess by analysis of variance (ANOVA) (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Protein fractionation

Table 1 showed that the albumin content of 105, 90 and 75 days maturity of pineapple are 4.4, 3.2 and 3.0%, respectively. The results revealed that albumin content increases with increase in the ripening period of the fruit. Globulin content of 105, 90 and 75 days maturity of pineapple are 1.2 and 0.6, 0.4%, respectively. The finding indicated that globulin content of fruit increases with increase in the period of maturity. The Prolamin content of 105, 90 and 75 days maturity of pineapple is the same (0.7%). These results revealed that the Prolamin of pineapple is not affected by period of maturity. The Glotulin content of 105, 90 and 75 days maturity of fruit are 0.9, 1.0 and 1.8%, respectively. The findings revealed that Glotulin content decreases with increase in the period of maturity. non protein N_2 of 105, 90 and 75 days maturity of fruit are 92.9, 94.6 and 94.2%, respectively. The results indicated that non protein N_2 is affected by the maturity stage.

In vitro protein digestibility and Anti-nutrition factors

Table 2 illustrated that protein content of 105, 90 and 75 days maturity are 4.11, 3.75 and 3.70%, respectively. The findings are higher than those given by Ahmed (2001). The net protein utilization refer to the percentage of ingested and utilized protein for growth and

Table 2. *In vitro* protein digestibility and Anti-nutrition factors of ripe and unripe pineapple, grown in Southern Sudan.

Sample (days maturity)	Protein (%)	Protein digestibility (%)	Tannin (%)	Phytic acid (mg/g)
105	4.1^{a} (± 0.01)	39.4^{a} (± 1.1)	4.5^{b} (± 0.07)	0.40^{b} (± 0.02)
90	3.8^{a} (± 0.05)	36.2^{a} (±1.2)	22.2^{a} (± 0.13)	0.20^{a} (± 0.01)
75	3.7^{a} (± 0.05)	32.2^{a} (± 1.2)	20.2^{a} (± 0.13)	0.15^{a} (± 0.01)

Mean values of same letters within the column are significantly difference at (P ≤ 0.05).

maintenance (Osborne et al., 1978).

The protein digestibility of 105, 90 and 75 days maturity are 39.4, 36.2 and 32.2%, respectively. Those results are lower than protein digestibility of sorghum (46%), rice (66%), maize (73%) and wheat (81%) reported by MacLean (1981).

This finding indicates the protein digestibility of pineapple fruit is extremely poor compared with sorghum, rice, maize and wheat. Tannin content of 105, 90 and 75 days maturity are 4.5, 22.2 and 20.2%, respectively. The results reveal the high tannin content reduces the digestibility of the protein because tannin acts as anti-enzymatic activity. In addition, tannin reacts with protein to form insoluble complex compound. The phytic acid of 105, 90 and 75 days maturity are 0.40, 0.20 and 0.15 mg/g, respectively. Phytic acid interacts with protein forming complex compound and reduces the bioavailability of protein and inhibits the action of pepsin, trypsin and α-amylase. These results explain the lowering of protein digestibility in the pineapple fruit.

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