

Effect of Germination and Fermentation on the Nutrient Contents, Functional and Antioxidant Activities of Pigeon pea (*Cajanus cajan*) flour

Abstract

Effect of germination and fermentation on the nutrient contents, functional and antioxidant activities of pigeon pea (*Cajanus cajan*) flour were investigated. The pigeon pea seeds were sorted, cleaned and processed into germinated, fermented and raw flours. The flour samples obtained were analyzed for proximate composition, mineral contents, antinutrient contents, functional properties and antioxidant/free radical scavenging properties using standard methods. The results of the proximate composition showed that the moisture content within the range of 9.62 ± 0.02 - 9.96 ± 0.01 , crude protein content, 16.63 ± 0.01 - 24.17 ± 0.01 , crude fibre content, 1.41 ± 0.01 - 2.59 ± 0.01 , lipid, 4.33 ± 0.01 - 5.61 ± 0.01 , ash content, 1.62 ± 0.01 - 2.17 ± 0.01 , carbohydrate, 56.80 ± 0.02 - 62.47 ± 0.01 and energy values, 366.91 - 374.40 kcal/100g. The result of elemental mineral analysis showed magnesium as the major mineral element in the sample (91.32 ± 0.02 to 123.75 ± 0.04 mg/g), calcium (96.02 ± 0.02 - 110.14 ± 0.003 mg/g), phosphorus (39.11 ± 0.02 - 46.12 ± 0.03 mg/g), sodium (8.63 ± 0.02 - 12.61 ± 0.001 mg/g), Iron (3.08 ± 0.02 - 4.11 ± 0.001 mg/g) and zinc (2.04 ± 0.01 - 3.17 mg/g) respectively. The result of the functional properties showed optimal gelatinization temperature ranged from 73.61°C - 89.41°C , emulsion capacity, 11.88 - 43.42%, foam stability, 28.22 to 65.81%, foaming capacity, 15.83 to 21.11%, bulk density, 0.53 - 0.71 g/ml, water absorption capacity, 1.11 and 1.51 ml/g while oil absorption capacity, 0.84 - 1.14 ml/g. The sensory properties of the *moi-moi* from the processed flours showed that the colour test, (6.33 - 7.53), flavour (7.63 to 7.75), taste (5.13 to 5.53), mouth-feel (7.25 to 7.36), overall acceptability, (6.93 ± 0.04 - 8.83 ± 0.04). The results of the phenolic content ranged from 20.13 mg\GAE, 53.43 mg\GAE and 42.64 mg\GAE for raw processed, malted and fermented samples respectively. The results of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant activity of ethanolic extracts showed IC_{50} values of 0.810, 1.177 and 1.014 mg/ml for the raw, malted and fermented seed extracts respectively. The moisture, crude protein and fibre contents was significantly increased ($p > 0.05$) during germination and fermentation whereas lipid, carbohydrate, ash and energy values were significantly ($p < 0.05$) decreased by the processing techniques. Germination increased the emulsion capacity, foaming capacity whereas fermentation reduced the foaming capacity of pigeon pea flour. Malting and fermentation significantly ($p > 0.05$) increased the % scavenging properties of pigeon pea extract though that of germination was higher. Malting and fermentation significantly ($p < 0.05$) decreased the sensory properties of *moi-moi* produced. Based on the result obtained, regular consumption of pigeon pea will be a proactive measure against heart diseases and other related disorders in the human body.

Keywords: Pigeon pea, processing, germination, fermentation, nutrient contents, functional, antioxidant properties

Introduction

Legumes as protein-rich crops are important source of protein for human food and animal feed. Pigeon pea (*Cajanus cajan*) is a locally available, affordable and under-utilized grain legume of the tropics and sub-tropics. Pigeon pea, is a legume commonly referred to as 'fiofio/agbogbo' in the South-East Nigeria where the seeds are boiled and eaten by the natives. Pigeon pea contained 17.9-24.3% protein, 58.7% carbohydrate, 1.2-8.10% crude fiber and 0.6-3.8% fat (Akubor, 2017). It is also a good source of calcium, phosphorus, magnesium, iron, sulphur etc. In spite of its high nutritional qualities, pigeon is not popular in the Western and Northern states of Nigeria (Uzor-Peter *et al.*, 2008). The raw seeds of pigeon pea contain antinutritional substances that affect their utilization. They also contain flatulence causing oligosaccharides and has a characteristic problem of hard-to-cook phenomenon also hinders the extensive use of pigeon peas as food. The dried seeds are hard and by the traditional processing methods, it takes 24 hours to prepare a meal of pigeon pea.

Numerous researchers have tried to improve the rate of its usage due to the need for cheaper and available plant proteins to meet the increasing demand of low-income countries. Moderate heat treatment improves the digestibility of plant proteins without developing toxic derivatives and inactivates several enzymes such as proteases, lipases, lipoxygenases, amylases and other oxidative and hydrolytic enzymes in foods (Fasoyiro *et al.*, 2006). Despite the existence of much works on the effects of a singular or combined processing methods on the nutritional and functional properties of local grains, much information has not been found in literatures on the comparative studies of the effects of malting and fermentation on the nutritional, functional, sensory and antioxidant/free radical scavenging properties of pigeon pea flour.

MATERIALS AND METHODS

A bulk of healthy mature seeds of Pigeon pea (*Cajanus cajan*) weighing 3.5kg used for this study was obtained from National Root Crops Research Institute (NRCRI), Umudike, Abia State. The seeds were sorted manually, to remove stones, damaged and immature seeds after which the seeds were divided into three equal portions of 1kg each. The first portion was processed raw while the two other portions were subjected to different processing treatments (malting and fermentation).

Preparation of Raw Pigeon pea flour.

The raw, malted and fermented Pigeon pea flour samples were prepared according to the method of Ugwu and Oranye (2006).

Sample Extraction

The method of Siddhuraju (2007) was employed to extract the samples.

Analytical Procedures

The proximate contents of the samples were determined in triplicate according to the method of AOAC (2006). Carbohydrate was determined by difference (Giami, 2005). The energy content was calculated using the Atwater factor (Atwater and Benedict, 1993). The mineral contents of the samples were determined using atomic absorption spectrophotometer (Perkin-Elmer, Model 1033, Norwalk, CT, USA)

according to the method of AOAC (2006). The functional properties were determined using the method of AOAC (2010). The antioxidant/free radical assay of the samples were determined using the method of AOAC (2010). The sensory evaluation of products from pigeon pea flours were done using the method described by Okaka (2010).

Method of Data Analysis

Mean and standard deviation were used to determine sample mean. The data generated after the analysis were subjected to Analysis of Variance (ANOVA) using special package for social sciences (SPSS version 20, 2013) to detect significant differences among the sample means at ($p \leq 0.05$). Mean value of phenols mg/g Dry Weight was compared using independent t-test. Duncan's multiple range test was used to determine significantly different means.

RESULTS AND DISCUSSION

Proximate Composition

The results of the comparative study of the effects of malting and fermentation on the proximate composition of processed pigeon pea seed was presented in Table 1. The result of the study showed that the moisture content for the different processed samples ranged from 9.62 ± 0.02 - 9.96 ± 0.01 (table 1). The moisture content of the sample was significantly ($p < 0.05$) higher in fermented sample compared to the malted and raw samples. The observation is in agreement with the report of Akubor, (2017) who stated that the moisture content of pigeon pea increases as the fermentation days increase. The low moisture content will afford a long shelf life of pigeon pea (Ali, 2012).

The result of the study showed that the crude protein content for the different processed samples ranged from 16.63 ± 0.01 - 24.17 ± 0.01 (table 1). The fermented sample had the highest protein value (24.17%). This increase in protein value during fermentation may confer nutritional advantage on the fermented pigeon pea flour. This increase in protein value with fermentation time could be attributed to net synthesis of protein by fermenting seeds which might have resulted in the production of some amino acids during protein synthesis (Akubor, 2017 and Uwagbuteet *et al.*, 2000).

The result of the study showed that the crude fibre content for the different processed samples ranged from 1.41 ± 0.01 - 2.59 ± 0.01 (table 1). The increase in the crude fiber content of the pigeon seed on germination may be attributed to the synthesis of more of the cell wall material to support the roots and rootlets (Akubor, 2017). This is in agreement with Ojukwu *et al.*, (2012) who reported increase in fibre content during germination. The presence of fibre in full fat flour and defatted flour helps in the functioning of gastro intestinal tract and aid digestion of food (Ojukwu *et al.*, 2012).

The result of the study showed that the lipid content for the different processed samples ranged from 4.33 ± 0.01 - 5.61 ± 0.01 (table 1). Results on fat content showed that values in germinated and fermented samples were slightly lower, ranging from 4.33 – 4.79% compared to the unfermented sample which was 5.61%. This is in agreement with Akubor, (2017) who reported that the fat in the seeds might have been utilized for the energy need of the microorganisms during

fermentation and germination. A study by Afify *et al.* (2012) revealed a reduction in oil content after cooking, germination and fermentation of three white sorghum varieties and attributed the reduction to biochemical and physiological changes occurring during germination and fermentation.

The result of the study showed that the ash content for the different processed samples ranged from 1.62 ± 0.01 - 2.17 ± 0.01 (table 1). Ash content significantly decreased ($p < 0.05$) from 2.17 in raw sample to 1.62% in fermented sample. An increase in ash content has been reported to possibly indicate a higher mineral content (Bassey *et al.*, 2013).

The result of the study showed that the carbohydrate content for the different processed samples ranged from 56.80 ± 0.02 to 62.47 ± 0.01 (table 1). Fermentation caused a decrease in carbohydrate content in all samples. The carbohydrate contents of the germinated and fermented seed flours were slightly lower than those of the raw sample flours. Similar decrease in carbohydrate content was reported in Akubor, (2017).

Energy values varied between 366.91 to 374.40kcal/100g with the raw pigeon pea seed flour having the highest energy value (374.40kcal/100g). This decrease in energy value could be attributed to the decrease in both fat and non-fatty energy values of the samples.

Mineral Composition

The result of elemental mineral analysis of processed pigeon pea seed flour was shown in table 2. According to the present data, mineral profile of processed pigeon pea (Table 2) showed that it contains magnesium as a major mineral in a maximum quantity (91.32 ± 0.02 to 123.75 ± 0.04 mg/g) followed by calcium (96.02 ± 0.02 to 110.14 ± 0.003 mg/g), phosphorus (39.11 ± 0.02 to 46.12 ± 0.03 mg/g), sodium (8.63 ± 0.02 to 12.61 ± 0.001 mg/g), Iron (3.08 ± 0.02 to 4.11 ± 0.001 mg/g) and zinc (2.04 ± 0.01 to 3.17 mg/g) respectively.

The processed pigeon pea contained high amount of magnesium (91.32 ± 0.02 to 123.75 ± 0.04 mg/g). The result showed that raw processed pigeon pea sample contained the high amount while malted sample contained the least. The result is in agreement with Akubor, (2017) who reported that malting and fermentation decreased the mineral content of pigeon pea.

Pigeon pea extracts contained relatively moderate amount of phosphorus (39.11 ± 0.02 to 46.12 ± 0.03 mg/g). The result showed that raw processed pigeon pea sample contained the high amount while fermented sample contained the least. The result is in agreement with Akubor, (2017) who reported that malting and fermentation decreased the mineral content of pigeon pea. Phosphorus enhances quick release of energy in the body and may combine with calcium for bone and teeth development.

Pigeon pea extracts contained relatively moderate amount of iron (3.08 ± 0.02 to 4.11 ± 0.001 mg/g). The result showed that raw processed pigeon pea sample contained the high amount while malted sample contained the least.

The result of the present study showed that zinc content of processed pigeon pea ranged from 2.04 ± 0.01 to 3.17 mg/g. Zinc deficiency causes growth retardation and hypogonadism (Nkansah and Amoako, 2010). Several mechanisms of growth retardation and hypogonadism due to zinc deficiency have been suggested.

Functional Properties

The effects of the processing treatments on the functional properties of pigeon flour are shown in Table 3. Functional properties are the intrinsic physicochemical characteristics which affect the behaviour of food systems during processing and storage (Akubor *et al.*, 2000). The optimal gelatinization temperature ranged of 73.61⁰C to 89.41⁰C for the processed pigeon pea flours was shown in (Table3). The pigeon pea grits formed strong gels as the temperature increases. It increased in the malted sample and decreased in the fermented sample.

The results of this study showed emulsion capacity of the samples were within the range of 11.88 to 43.42%. Germination increased the emulsion capacity (43.42%). The emulsion capacity of the raw pigeon pea flour was 35.12%. The high emulsion activity of pigeon pea flour could be due to its high protein content.

The results of this study showed foam stability of the samples were within the range of 28.22 to 65.81%. From the result, fermentation decreased the foam stability while malting increased it when compared with the raw sample. The results are in agreement with Akubor, (2017) who reported that the foam stability decreased with fermentation periods, which ranged between 1.97 and 2.45%.

The results of this study showed foaming capacity of the samples were within the range of 15.83 to 21.11%. Fermentation reduced the foaming capacity of pigeon pea flour (15.83%) while germination increased the foaming capacity of the flour (17.64%). The results are in agreement with Akubor, (2017) who reported that fermentation decreased the foaming capacity of processed pigeon pea flour. Foamability is related to the rate of decrease of the surface tension of air water interface caused by absorption of protein molecules (Akubor, 2017).

Bulk density ranged from 0.53 to 0.71g/ml, although, there were significant differences ($P \leq 0.05$) among the flour samples. Bulk density values decreased gradually with fermentation periods. The bulk density is a reflection of the load the flour samples can carry, if allowed to rest directly on one another. The low bulk density helps to reduce transportation and storage costs.

Water absorption capacity ranged between 1.11 to 1.51ml/g for the processed pigeon pea samples. The malted sample had the highest value while lowest value was recorded for 5day fermented sample (1.11ml/g). This result suggests that processed pigeon pea flour may find application in the production of some baked products.

Results of the present study showed the oil absorption capacity of the processed samples ranged from 0.84 to 1.14ml/g. From the result, it was observed that fermentation and malting decreased oil absorption capacity (OAC) in samples. The results are also in agreement with Udensi and Okoronkwo, (2006) who reported a decrease in oil binding capacity of Mucuna bean protein isolate after fermentation.

SENSORY PROPERTIES

The sensory properties of the *moi-moi* from the processed flours were shown in Table 6. *Moi-moi* is traditional food products in Nigeria usually prepared from fresh cowpea paste (Onimawo and Akubor, 2005). The sensory evaluation showed the result of colour, flavour, taste, mouth feel and overall acceptability. The result of the study showed that the colour test ranged from 6.33 to 7.53. From the results of the analysis of the flavour, *moi-moi* from the raw and malted samples were rated moderately like (7.63 to 7.75) while the fermented sample was rated slightly

liked (6.60). The results of the analysis of the taste showed that *moi-moi* from the raw processed sample was rated moderately liked (7.70) while and malted and fermented samples were rated 5.13 to 5.53 (neither like nor dislike). From the results of the analysis, *moi-moi* from the raw and malted samples were rated moderately like (7.25 to 7.36) while the fermented sample was rated slightly liked (6.40). The overall acceptability ratings ranged from 6.93 ± 0.04 to 8.83 ± 0.04 . It decreases gradually from raw processed to malted to fermentation samples.

ANTIOXIDANT/FREE RADICAL SCAVENGING ACTIVITY

Table 5 showed the result of the total phenolic content different processed pigeon seed samples. The phenolic content in the analysed different processed seed extracts ranged as 20.13mg\GAE, 53.43mg\GAE and 42.64mg\GAE for raw processed, malted and fermented samples respectively. The highest content of phenols was assessed in malted extract when compared to raw and fermented extracts. The result of this present study was in agreement with the work of Devi *et al.*, (2016) who reported that the phenolic content in the analysed leaf, seed and root extracts of pigeon pea ranged as 55.03mg\GAE and 57.20mg\GAE, 50.95mg\GAE and that the highest content of phenols was assessed in *Cajanus cajan* seed extract when compared to leaf and root extracts.

Antioxidant Assay (DPPH Assay)

The results of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) antioxidant activity of ethanol extract of raw, malted and fermented seed extracts of *Cajanus cajan* were reported in tables 6 and 7. The results showed that the raw, malted and fermented seed extracts of *Cajanus cajan* were able to scavenge the free radicals in different concentration in a dose dependent manner. The IC_{50} values for the raw, malted and fermented seed extracts of *Cajanus cajan* were 0.810, 1.177 and 1.014mg/ml respectively (Table 7). Results showed an increase in the % scavenging of pigeon pea extract after germination and fermentation though that of germination was higher. This result indicated that the germinated and fermented pigeon extract which contained higher amount of phenolic compound than the ungerminated one could exhibit higher antioxidant activity.

CONCLUSION

Malting and fermentation greatly improved nutritional, functional and antioxidant properties of the pigeon pea seed (*Cajanus cajan*) flour. The result of the elemental analysis showed that pigeon pea is a good source of elemental minerals requirement for normal functioning of the human body. The free radical scavenging activity of possessed pigeon pea has been demonstrated in this study. This may be associated with high content of total phenolics and increased antioxidant potential. Based on the result obtained, regular consumption of pigeon pea may help to prevent heart diseases and other related disorders in the human body.

Table 1: Result of Proximate Composition of Processed Pigeon pea

Parameter/Sample	RAW	MALTED	FERMENTED
% Moisture Content	9.62 ^a ±0.02	9.82 ^b ±0.02	9.96 ^c ±0.01
% Crude Protein	18.51 ^a ±0.01	24.02 ^b ±0.01	24.17 ^c ±0.01
% Crude Fibre	1.63 ^a ±0.01	2.59 ^b ±0.01	1.41 ^c ±0.01
% Lipid	5.61 ^a ±0.01	4.79 ^b ±0.01	4.33 ^c ±0.02
%ASH	2.17 ^a ±0.01	1.85 ^b ±0.02	1.62 ^c ±0.00
% Carbohydrate	62.47 ^a ±0.01	56.80 ^b ±0.01	58.68 ^c ±0.00
Energy equivalent (Kcal)	374.4 ^a ±0.13	366.91 ^b ±0.01	369.71 ^c ±0.16

Values are Means ± SD of duplicate determinations and values bearing different superscript in a column are significantly different (P≤0.05) from each other.

Table 2: Result of Mineral Composition of Processed Pigeon pea

Composition (mg/g)/	RAW	MALTED	FERMENTED
Calcium	110.14 ^a ±0.03	96.02 ^b ±0.02	107.22 ^c ±0.02
Sodium	12.61 ^a ±0.01	10.07 ^b ±0.00	8.63 ^c ±0.02
Magnesium	123.75 ^a ±0.04	101.51 ^b ±0.01	91.32 ^c ±0.02
Phosphorus	46.12 ^a ±0.03	44.22 ^b ±0.01	39.11 ^c ±0.02
Zinc	3.17 ^a ±0.01	2.04 ^b ±0.01	2.82 ^c ±0.01
Iron	3.875 ^a ±0.01	3.08 ^b ±0.02	4.11 ^c ±0.01

Values are Means ± SD of duplicate determinations and values bearing different superscript in a column are significantly different (P≤0.05) from each other.

Table 3: Result of Functional Properties of Processed Pigeon pea

PARAMETER/SAMPLE	RAW	MALTED	FERMENTED
Gelation Temperature (⁰C)	78.11 ^a ±0.01	89.41 ^b ±0.01	73.61 ^c ±0.01
Emulsion Capacity (%)	35.12 ^a ±0.02	43.42 ^b ±0.02	11.88 ^c ±0.02
Foaming Stability (%)	62.62 ^a ±0.02	65.81 ^b ±0.01	28.22 ^c ±0.02
Foaming Capacity (%)	21.11 ^a ±0.01	17.64 ^b ±0.02	15.83 ^c ±0.02
Bulk Density (g/ml)	0.71 ^a ±0.01	0.53 ^b ±0.02	0.70 ^c ±0.00
Water Absorption Capacity (ml/g)	1.21 ^a ±0.01	1.51 ^b ±0.01	1.11 ^c ±0.01
Oil Absorption Capacity (ml/g)	1.14 ^a ±0.01	0.84 ^b ±0.00	1.07 ^c ±0.01

Values are Means ± SD of duplicate determinations and values bearing different superscript in a column are significantly different (P≤0.05) from each other.

TABLE 4: RESULT OF SENSORY EVALUATION OF PROCESSED PIGEON PEA SEED

SAMPLE	RAW	MALTED	FERMENTED
COLOUR	7.53 ^a ±0.03	6.63 ^b ±0.03	6.33 ^c ±0.03
FLAVOUR	7.75 ^a ±0.07	7.63 ^b ±0.04	6.60 ^b ±0.00
TASTE	7.70 ^a ±0.07	5.13 ^b ± 0.04	5.53 ^c ±0.04
MOUTH FEEL	7.25 ^a ±0.07	7.36 ^b ±0.04	6.40 ^b ±0.00
OVERALL ACCEPTABILITY	8.83 ^a ±0.04	7.35 ^b ±0.07	6.93 ^c ±0.04

Means are ± SD of duplicate determinations and values bearing different superscript in a column are significantly different (P≤0.05) from each other.

Table 5: Result of Antioxidant Properties of Processed Pigeon pea

SAMPLE	mgGAE/100g TOTAL PHENOL
RAW	20.13±0.02
MALTED	53.43±0.02
FERMENTED	42.64±0.03

Values are Means ± SD of duplicate determinations and values bearing different superscript in a column are significantly different ($P \leq 0.05$) from each other.

Table 6: Result of DPPH Radical Scavenging Properties of Processed Pigeon pea

Mg/mL Concentration	% INHIBITION (VITAMIN C)	RAW	MALTED	FERMENTED
0.2	48.22±0.02	2.11±0.01	36.26±0.01	29.41±0.01
0.4	60.42±0.01	10.15±0.02	51.62±0.02	40.38±0.01
0.6	78.13±0.02	21.02±0.01	62.43±0.02	49.25±0.02
0.8	91.04±0.04	34.73±0.03	80.72±0.02	65.42±0.03
1.0	98.53±0.04	42.27±0.02	91.35±0.02	70.31±0.02

Values are Means ± SD of duplicate determinations and values bearing different superscript in a column are significantly different ($P \leq 0.05$) from each other.

Table7 In-vitro DPPH scavenging (IC₅₀) Activity of processed *C. cajan* seed extract

<i>Cajanus cajan</i>	IC₅₀ for DPPH activity
Raw	0.810
Malted	1.177
Fermented	1.184

Values are Means ± SD of duplicate determinations and values bearing different superscript in a column are significantly different ($P \leq 0.05$) from each other.

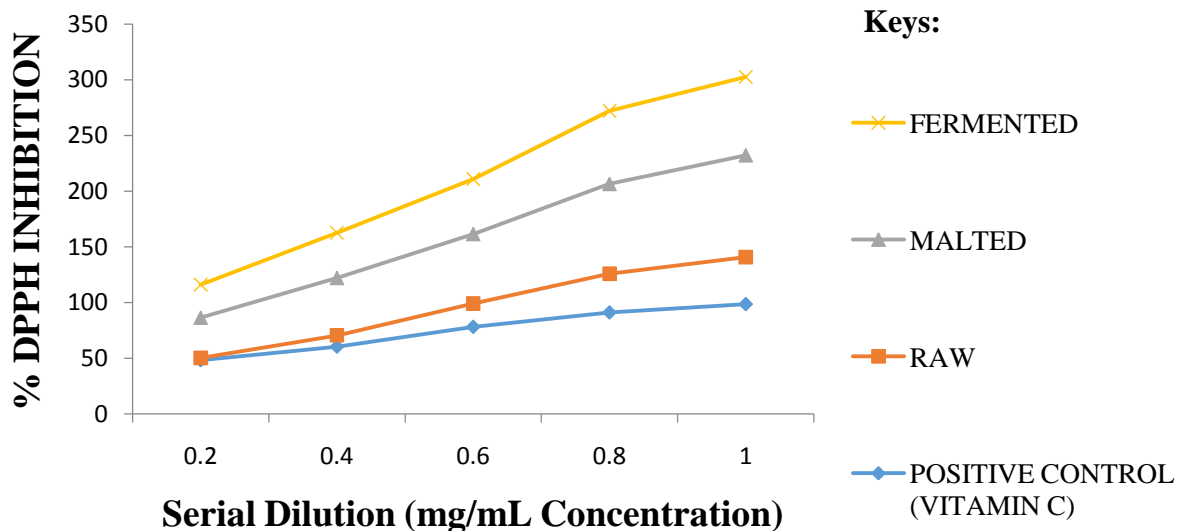


Figure 1: Result of DPPH Radical Scavenging Properties of Processed Pigeon pea

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