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Enhancement of nutritional quality of rice flour with mucuna beans (*Mucuna pruriens*)

Ojokoh A. O.[1], Fayemi O. E.[2*] and Akinseye O. A.[1]

[1]Department of Microbiology, School of Sciences, Federal University of Technology, P. M. B. 704, Akure, Ondo State, Nigeria.
[2]Department of Biological Sciences, College of Basic and Applied Sciences, Mountain Top University, Kilometre 12, Lagos-Ibadan Expressway, Ogun State, Nigeria.

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The effect of fermentation on the proximate and anti-nutritional properties of rice flour enriched with mucuna beans was investigated in this study. The rice-mucuna beans flour blends were formulated in various ratios and fermented for 72 h. The predominant moulds isolated during the fermentation were *Aspergillus flavus, Aspergillus fumigatus, Geotrichum candidum* and *Rhizopus stolonifera.* After fermentation, the protein and ash contents of the flour blends increased while carbohydrate, lipid and fibre contents reduced. However, there was no notable difference in the moisture content of the fermented flour blends. Fermentation also caused a significant reduction in the tannin, phytate and oxalate contents of the rice-mucuna beans flour blends. Nutritional benefits of rice flour can be enhanced through enrichment with mucuna beans and fermentation process. Therefore, fermented rice-mucuna beans flour blends have potential application in managing protein-energy malnutrition (PEM) in developing countries.

Key words: Fermentation, improvement, anti-nutrient, rice flour, mucuna beans.

INTRODUCTION

Malnutrition is a major health problem in developing countries and has contributed to infant and maternal mortality, poor physical and intellectual development in infants, and lower resistance to diseases among infants, pregnant women and adolescents (Rizwana et al., 2015). There are widespread problems with infant and other vulnerable groups' malnutrition in the developing nations of the world most especially protein-energy malnutrition (PEM) (Temba et al., 2016). Formulation of nutritious foods using locally available raw materials has received a lot of attention in many developing countries (Adebayo-Oyetoro et al., 2017). There is encouraging evidence of progress in reducing the prevalence of protein-energy malnutrition (PEM) through production and consumption of flours from locally available and abundantly grown grains and pulses (Temba et al., 2016). One of the main and affordable raw materials used in the formulation of many traditional food products in developing countries are flours from cereals (Mensah et al., 1991). Cereals provide more than 60% of the total energy supply of most Africans diet with little or no consumption of protein of animal origin (Galati et al., 2014). However, cereals have high carbohydrate but relatively low protein content (Bouis, 2000) while legumes on the other hands are nutritious foods, and a potential substitute for animal protein (Muzquiz et al., 2012).

*Corresponding author. E-mail: oefayemi@mtu.edu.ng.

Table 1. Sample code and formulated ratio of the rice flour enriched with mucuna beans.

Sample code	Rice flour (g)	Mucuna beans flour (g)
RAA	100	0
RBB	0	100
RCC	90	10
RDD	80	20
REE	70	30
RFF	60	40
RGG	50	50

The prevalence of malnutrition in Africa is due to unaffordability of animal protein which calls for alternative sources of protein from under-utilized legumes in lieu of costly and scarce animal protein (Adebowale and Lawal, 2004). Furthermore, legumes are good sources of affordable protein most especially for rural communities that are susceptible to high risks of malnutrition due to total reliance on cereal-based diets (Temba et al., 2016). Therefore, the utilization of legumes in food formulations will lead to a significant drop in total dependency on animal sources for nutritious foods (Ryan et al., 2014).

Although, legumes are rich in protein, fibre, vitamins and minerals, they also contain anti-nutrient compounds, which interfered with nutrient absorption (Rehman and Shah, 2005). Fermentation is one of the strategies utilized in reducing the amounts of anti-nutrients in legumes (Adeyeye et al., 2017). Food fermentation by microorganisms played a major role in enrichment of food substrate with proteins, essential amino acids, fatty acids, and vitamins. This enhanced the digestibility and acceptability of foods, detoxification of anti-nutritional compounds in foods (Obadina et al., 2013).

It has been proposed that compositing cereal with legume is a crucial solution to malnutrition in Africa (Temba et al., 2016). This is because the protein quality of the consequent products is enhanced through a mutual complementation of their limiting amino acids and possess better nutritional and calorific value than those produced either from cereal or legume (Annan and Plahar, 2005; Igbabul et al., 2015). Enrichment of cereal staple foods with legumes is a common practice in many developing countries. However, mucuna beans are an underutilize legumes in cereal-legume food fortifications (Balogun and Olatidoye, 2012).

Since the consumption of these cereals based food is very popular among children and adults in Nigeria, it imperative to study the impact of fermentation on nutritional quality and anti-nutritional factors in flour from cereals enriched with legumes with the aim of improving the nutritive value of these important indigenous food products. This study, therefore, investigate the potential for improving the nutritional and reducing the anti-nutritional properties of rice flour through fermentation and enrichment with mucuna beans.

MATERIALS AND METHODS

Rice (parboiled) *Oryza glaberrima* was purchased from a local retail outlet in Oja-Oba market at Akure in Ondo State, Nigeria while the mucuna beans (*Mucuna pruciens*) was supplied by the International Institute of Tropical Agriculture (IITA), Ibadan in Oyo State, Nigeria. The mucuna beans were soaked in water for 48 h and boiled for 1 h to inactivate trypsin inhibitor activity (Mang et al., 2015). The boiled beans were manually dehulled in cold water, washed and rinsed thoroughly with potable water, sundried and grounded into fine flour to pass a 0.4 mm screen.

Formulation of samples

The rice-mucuna beans flour blends were formulated in the following ratios: 100:0, 90:10, 80:20, 70:30, 60:40 and 50:50 (rice:mucuna beans in grams) and were labelled appropriately (Table 1).

Fermentation of samples

Fifty millilitres (50 mL) of potable water was added to the rice-mucuna formulated flour samples and properly mixed to form a paste. The formulated flour blends were then fermented in a covered sterile container for 72 h at 25±2°C. Physico-chemical characteristics of both the fermented and unfermented samples were determined at 24 h intervals.

Microbiological analysis

The dominant moulds involved in the fermentation process were determined on Potatoes Dextrose Agar (PDA) (Merck, Darmstadt, Germany) supplemented with 50 mg/L of streptomycin. Ten grams (10 g) of fermenting rice-mucuna beans flour beans were taken at 48 and 72 h of fermentation and aseptically homogenized with 90 mL sterile 0.1% buffered peptone water (Merck). After platting the appropriate dilutions, the PDA plates were incubated at 25°C for 3 to 5 days. After incubation and purification, the mould isolates were identified microscopically as previously described by Barnett and Hunter (1972).

Physicochemical analyses

pH and total titrable acidity

The pH of the samples was determined using pH meter (Model 3520, Bibby Scientific Limited Dumow Essex, UK) while the titratable acidity (% lactic acid equivalent) was determined

Table 2. Morphological and microscopic characteristics of the fungi isolated from the rice-mucuna beans flour blends during fermentation.

Fungi	Microscopic description
Aspergillus flavus	Pin like green growth, Non-branched conidiophore with bulb end which carries conidia.
Aspergillus fumigatus	Pin like black growth. Non-branched conidiophore with bulb end carries conidia like sun rays.
Geotrichum candidum	Colonies are fast growing, flat, white to cream, dry and finely suede-like with no reverse pigment. Chains of hyaline, smooth, one-celled, sub-globose to cylindrical, slimy arthroconidia (ameroconidia) by the holoarthric fragmentation of undifferentiated hyphae.
Rhizopus stolonifer	Cotton like white growth spotted with black color on plate. Sporangia contain spores, have rhizoids.

according to AOAC (1990).

Proximate composition

The moisture, crude fibre, fat, protein (N×6.25), and ash contents of both the fermented and unfermented samples were determined using the methods of the Association of Official Analytical Chemists (2000). The carbohydrate content was determined by calculating the difference between 100 and total sum of the percentage of moisture, protein, fat and ash.

Analyses of the anti-nutrient contents

Determination of tannin

One gram (1 g) of each sample was soaked with solvent mixture (80 mL of acetone and 20 mL of glacial acetic acid). After 5 h of soaking, the samples were filtered with a double layer filter paper to obtain a clear filtrate. The absorbance of the standard solution and the filtrates were read on a Spectronic 20, England spectrophotometer (AOAC, 1990) at 500 nm. A tannic acid solution (10 ppm to 30 ppm) was used as standard.

Determination of phytate and oxalate

Phytate and the oxalate contents were determined as previously described by AOAC (1998).

Statistical analysis

All experiments were performed in triplicate and the data were analyzed using one-way analysis of variance (ANOVA) to determine whether fermentation has an effect on the nutritional and anti-nutritional properties of rice flour enriched with mucuna beans. Fisher's Least Significant Difference Test (LSD) was used to determine significant differences between the treatments at $p \leq 0.05$ using Statistica software for Windows, version 12 (Stat-soft, Tulsa, OK).

RESULTS AND DISCUSSION

The predominant moulds identified in the fermented rice flour enriched with mucuna beans were *Aspergillus flavus, Aspergillus fumigatus, Geotrichum candidum* and *Rhizopus stolonifera* (Table 2). Several studies have reported the involvement of these microorganisms in cereal fermented food products (Mbata et al., 2006; Nwokoro, and Chukwu, 2012; Nsofor et al., 2014). The pH decreased rapidly as the fermentation progressed with concomitant increase in the titratable acidity after fermentation (Figure 1). The decrease in pH with corresponding increase in titratable acidity could be due to the production of organic acids by the fermenting microorganisms during degradation of carbohydrates resulting in acidification (Edema and Sanni, 2008; Fayemi and Ojokoh, 2014).

The proximate contents of the unfermented and fermented rice-mucuna beans flour blend samples is presented in Table 3. In the proximate analyses of all the samples, there was no significant increase in moisture content after fermentation and this suggested that the fermented flour blends may have a good keeping quality as high moisture content has been associated with rapid spoilage of food products (Rawat, 2015). There was increase in protein content of the fermented samples which is probably due to the fact that the microorganisms in the course of fermentation may have secreted extra cellular enzymes which subsequently increases the protein content as well as the microbial biomass (Odedokun, 2000). The increase in the protein contents after fermentation is an indication that fermentation may be a useful means of improving the functionality and protein content of rice-mucuna beans flour blends (Mang et al., 2015).

The decrease in the fat contents of all the samples after fermentation can be attributed to the metabolic activities of the lipolytic enzymes produced by the fermenting microorganisms which were able to utilise the lipids in the flour during fermentation thereby causing a drop in the final contents (Onweluzo and Nnamuchi, 2009). There was an increase in the ash contents after fermentation. Ash content is a measure of the total amount of minerals present within a food sample, therefore, an increase in its level during fermentation may be attributed to incomplete utilization of available minerals by fermenting organisms during metabolism (Balogun and Olatidoye, 2012). The crude fibre content of the rice-mucuna beans flour samples decreased as the fermentation progressed and this may be due to the enzymatic breakdown of the fibre during fermentation by the microorganisms (Ojokoh et al., 2013). Similarly, the carbohydrate levels of all the rice-mucuna beans flour samples decreased significantly (p ≤

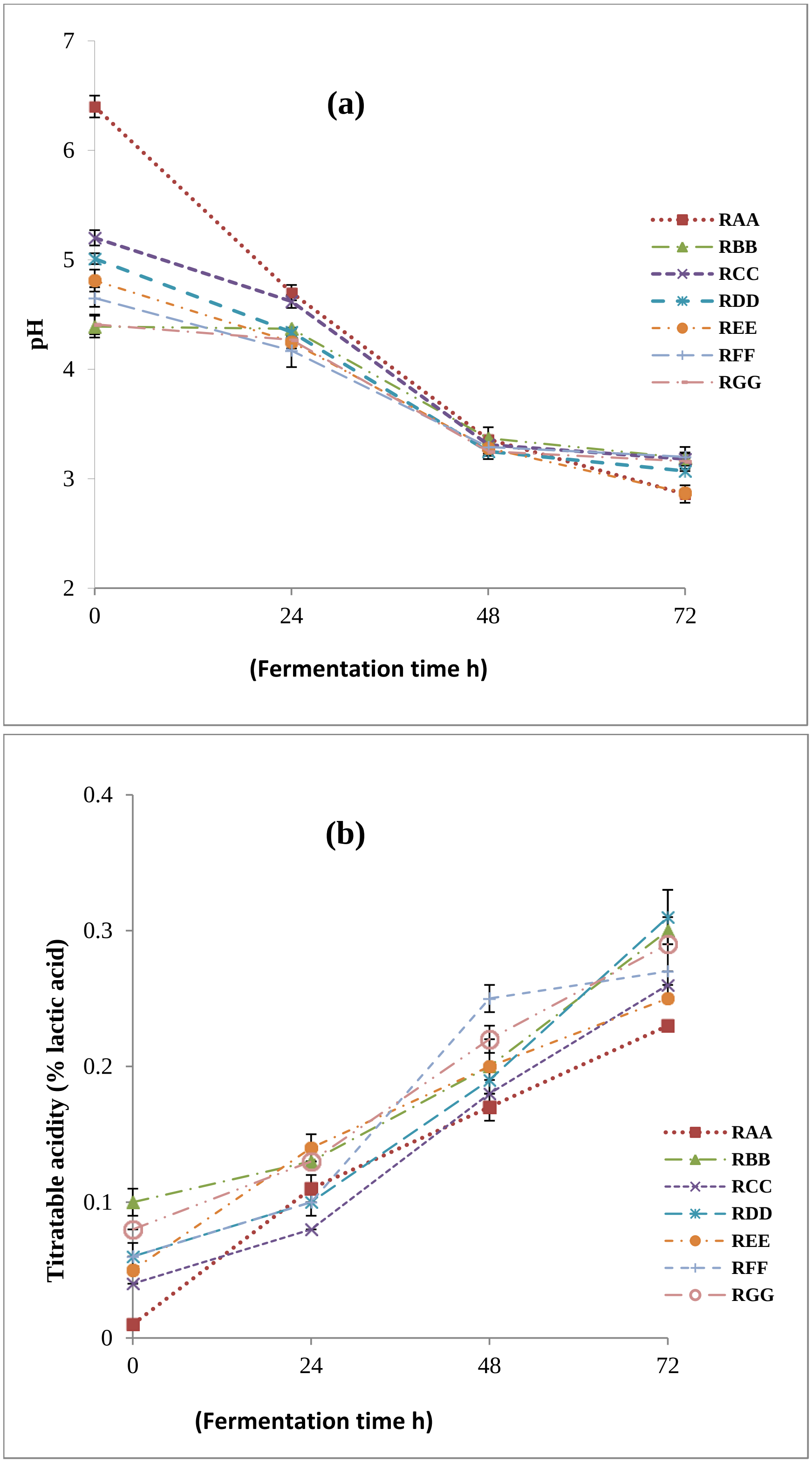

Figure 1. Changes in (**a**) pH and (**b**) titratable acidity of the Rice-mucuna beans flour blends during fermentation period. RAA, 100 g of Rice flour; RBB, 100 g of mucuna beans flour; RCC, Rice flour (90 g) + mucuna beans flour (10 g); RDD, Rice flour (80 g) + mucuca beans flour (20 g); REE, Rice flour (70 g) + mucuna beans flour (30 g); RFF, Rice flour (60 g) + mucuna beans flour (40 g); RGG, Rice flour (50 g) + mucuna beans flour (50 g).

Table 3. Effect of fermentation on the proximate composition of the Rice-mucuna beans flour blends.

Proximate (%)		RAA	RBB	RCC	RDD	REE	RFF	RGG
Moisture	Unfermented	13.00±0.10[a]	12.20±0.10[a]	11.39±0.15[a]	12.12±0.30[a]	12.19±0.30[a]	12.20±0.10[a]	12.60±0.10[a]
	Fermented	13.42±0.00[a]	12.61±0.01[a]	12.02±0.02[a]	12.62±0.08[a]	12.52±0.00[a]	12.77±0.02[a]	12.92±0.00[a]
Protein	Unfermented	12.70±0.10[a]	30.10±0.20[a]	13.89±0.10[a]	15.22±0.15[b]	16.45±0.10[b]	17.42±0.10[b]	24.90±0.20[c]
	Fermented	13.90±0.02[a]	33.81±0.00[e]	15.91±0.01[a]	16.98±0.03[a]	18.70±0.02[b]	21.40±0.10[c]	26.47±1.00[d]
Lipid	Unfermented	1.66±0.00[a]	6.50±0.01[d]	2.59±0.02[b]	3.89±0.02[b]	4.59±0.00[c]	8.43±0.10[e]	10.43±1.00[f]
	Fermented	1.11±0.00[a]	5.64±0.01[c]	2.54±0.10[b]	2.80±0.01[b]	3.28±0.00[b]	7.22±0.01[d]	7.89±0.11[d]
Ash	Unfermented	2.73±0.01[a]	4.43±0.03[a]	3.98±0.05[b]	2.70±0.00[a]	2.72±0.01[a]	3.01±0.02[a]	4.02±0.02[b]
	Fermented	4.00±0.01[b]	4.73±1.00[c]	5.51±1.00[d]	3.56±0.01[b]	2.90±0.00[a]	3.84±0.02[b]	5.70±1.00[d]
Fibre	Unfermented	1.00±0.00[a]	3.00±0.05[c]	1.88±0.01[b]	1.87±0.00[b]	1.85±0.01[b]	2.08±0.00[b]	3.61±0.20[c]
	Fermented	0.80±0.00[a]	2.79±1.00[c]	1.17±0.01[a]	1.81±0.10[b]	1.36±0.10[ab]	2.04±1.00[b]	2.79±0.00[c]
Carbohydrate	Unfermented	68.93±0.04[cd]	43.78±0.10[a]	65.68±0.10[c]	64.22±0.10[c]	62.21±0.01[c]	56.87±0.10[b]	44.45±0.10[a]
	Fermented	61.92±0.10[c]	41.78±0.00[a]	62.05±0.10[cd]	63.11±0.02[cd]	61.24±0.01[c]	51.62±0.01[b]	41.93±0.11[a]

Values are the means and standard deviations of three replicate experiments (n = 3). Means with different superscripts in the same row are significantly different at P ≤ 0.05. RAA, 100 g of Rice flour; RBB, 100 g of mucuna beans flour; RCC, Rice flour (90 g) + mucuna beans flour (10 g); RDD, Rice flour (80 g) + mucuca beans flour (20 g); REE, Rice flour (70 g) + mucuna beans flour (30 g); RFF, Rice flour (60 g) + mucuna beans flour (40 g); RGG, Rice flour (50 g) + mucuna beans flour (50 g).

0.05) after fermentation. Balogun and Olatidoye (2012) attributed such reduction in carbohydrate content during fermentation to metabolic activities of the microorganisms associated with the fermentation.

Fermentation significantly (p ≤ 0.05) reduced the anti-nutrients contents of the rice-mucuna beans flour (Table 4). The presence of anti-nutrients compounds in food product can affect its nutritive value such as oxalate, tannin and phytate (Adeyeye et al., 2017). Phytate is naturally found in cereals and legumes and has potential of reducing the availability of minerals and solubility, functionality and digestibility of proteins (Akubor and Badifu, 2004). The significant (p ≤ 0.05) reduction in the phytate level of all the rice-mucuna beans flour samples after fermentation is in accordance with the findings of Gabriel and Akharaiyi (2007) who reported a reduction in the phytic acid contents in cereal and legumes based products during fermentation. The low pH of the fermented rice-mucuna beans flour blends and the temperature of fermentation would have provided a favourable condition for the phytase activity (Odedokun, 2000).

Tannin and oxalate decreased significantly (p ≤ 0.05) in the rice-mucuna beans flour blends after 72 h of fermentation. Odedokun (2000) attributed the reduction in the tannin contents of fermented cereals to the hydrolysis during fermentation. Fermentation has been proposed as one of the approaches that can be applied in reducing the anti-nutritional factors in food, thereby enhancing the application of legumes and cereals in the production of nutritionally balanced foods with potential of alleviating the prevalent problem of PEM in the developing countries (Temba et al., 2016). Therefore, the 72 h fermentation employed in this study appears to be optimal for the production of low tannin flour from rice-mucuna beans blends. Furthermore, the breaking down of tannin indigestible complexes such as tannin-protein, tannic acid-starch and tannin-iron to release free nutrients can be attributed to tannase activity by lactobacillus

Table 4. Effect of fermentation on the anti-nutrient contents (%) of the rice-mucuna beans flour blends.

Sample	Unfermented flour blends			Fermented flour blends		
	Phytate	Oxalate	Tannin	Phytate	Oxalate	Tannin
RAA	8.02±1.00^{d}	0.46±0.01^{a}	3.45±0.07^{ab}	0.65±0.01^{a}	0.35±0.00^{a}	3.69±0.01^{c}
RBB	3.71±0.00^{b}	1.62±0.10^{c}	5.52±0.00^{b}	0.65±0.10^{b}	1.45±0.01^{c}	4.07±0.02^{d}
RCC	5.03±0.20^{c}	0.40±0.00^{a}	3.47±0.20^{b}	0.65±0.00^{b}	0.65±0.03^{b}	1.83±0.01^{b}
RDD	4.19±0.01^{b}	0.77±0.01^{b}	4.02±0.01^{ab}	0.80±0.01^{c}	0.75±0.01^{b}	0.45±0.01^{a}
REE	4.18±0.00^{b}	0.81±0.02^{b}	4.45±0.10^{a}	0.50±0.10^{b}	0.70±0.01^{b}	2.03±0.00^{b}
RFF	3.96±0.10^{b}	1.19±0.01^{bc}	4.51±0.00^{a}	0.45±0.01^{a}	0.70±0.00^{b}	2.90±0.00^{c}
RGG	2.07±0.00^{a}	1.84±0.10^{c}	4.58±0.01^{a}	0.40±0.10^{a}	0.35±0.00^{a}	1.77±0.10^{b}

Values are the means and standard deviations of three replicate experiments (n = 3). Means with different superscripts in the same row are significantly different at P ≤ 0.05. RAA, 100 g of Rice flour; RBB, 100 g of mucuna beans flour; RCC, Rice flour (90 g) + mucuna beans flour (10 g); RDD, Rice flour (80 g) + mucuca beans flour (20 g); REE, Rice flour (70 g) + mucuna beans flour (30 g); RFF, Rice flour (60 g) + mucuna beans flour (40 g); RGG, Rice flour (50 g) + mucuna beans flour (50 g).

during fermentation (Simwaka et al., 2017).

Conclusions

This study demonstrated that the nutritional benefits of rice can be enhanced when enriched with mucuna beans but rice-mucuna beans flour blends contain some naturally occurring anti-nutrients. However, fermentation of such flour blends decreases its anti-nutrient contents with corresponding increase in the protein content and could be used in enhancing protein availability to the consumers in developing countries. Therefore, fermented rice-mucuna beans flour blends have potential application in formulation of complementary food for the management of Protein-Energy Malnutrition (PEM). However, further studies are required on the functional properties and health benefits of the fermented flour blends.

Practical applications of the study

Rice has become an important staple food in many developing countries, Nigeria inclusive. Rice is consumed in many homes as boiled, fried or jollof rice. The use of rice has been limited and its utilization confine to family menu. However, there is need to improve nutrition properties and industrial utilization of rice as government intervention in most developing nations has led to increase in rice production. Several locally produced cereal and tuber crops had been adopted in composite flour production and rice could come in handy as a substitute for some of these cereal crops. Rice composite flour could be used in the production of baked products such as bread, doughnut, egg burns, and meat pies based on the nutritional requirements of the people in the developing nations. Application of available local cereals and legumes in producing novel and cheap food products with health benefits could be an alternative to the high cost of wheat importation by the developing nations. Fermented rice-mucuna flour blends could be a ready substitute and this product could be produced commercially to meet the nutritional need of consumers and can also serve as complementary food for infants and children in developing countries.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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