



AGRICULTURAL AND BIOLOGICAL SCIENCES

BACTERIOLOGICAL QUALITY OF SOME FERMENTED FOOD PRODUCTS IN KEFFI, NASARAWA STATE.

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ABSTRACT

Garri, cassava and yam flours are African traditional fermented food products prepared from cassava and yam that is widely accepted by both rural and urban dwelling peoples with little concern about those microorganisms that are associated with it. The present study was undertaken to investigate the bacteria that are associated with these food flours. Total bacterial count ($\times 10^5$ cfu/g) was found to be highest in cassava (7.1), followed by garri (6.2) and yam (4.4). The total coliform count ($\times 10^5$ cfu/g) recorded 3.7 in cassava, 1.6 in garri, and 1.4 in yam flours. Similarly, total faecal coliform ($\times 10^5$ cfu/g) was highest in cassava flour (4.1), while garri had 2.4, and yam recorded 2.2; also, the Staphylococcus aureus count ($\times 10^5$ cfu/g) was highest in cassava flour (3.6), followed by yam flour (3.1), and garri flour had 2.8. However, the *Salmonella/Shigella* count ($\times 10^5$ cfu/g) was high in garri flour (3.2), compared to cassava flour (2.5), and yam flour (1.2). The percentage occurrence of the bacteria isolates showed that Staphylococcus aureus was 86.7%, followed by Klebsiella spp. (60.0%), Proteus vulgaris (66.7%), E. coli (86.7%). Similarly, percentage occurrence of Salmonella spp. was 80.0% and Bacillus spp. 66.7%. The bacteria isolated were Staphylococcus aureus, Klebsiella spp., Proteus vulgaris, Salmonella spp., E. coli and Bacillus spp. The isolates were further subjected to antibacterial susceptibility tests using Kirby-Bauer disc diffusion method. The antibiotic susceptibility test revealed that most of the bacterial isolates were resistant to Nitrofurantoin (28%), Septrin (36%), Ampicillin (39%), slightly resistant to Perfloxacin (46%), Nalidixic acid (45%), Tetracycline (46%), slightly susceptible to Augmentin (63%), Ciprofloxacin (67%), Ofloxacin (52%) and highly susceptible to Gentamicin (95%). This present work revealed high bioload and vast array of bacteria in market garri, cassava and yam flours. It is therefore recommended that these food flours be sold in well packaged bags and not as exposed in basins/ bowls. Also, personal hygiene of hawkers and sanitation of utensils are important. Hawkers should be enlightened on hygienic practices.

Keywords: Bacteriological, quality, garri, cassava, yam flour, Keffi

INTRODUCTION

Yam (*Discorea spp*) and Cassava (*Manihot esculenta*) are the most important root/tuber crops in Africa (Babajide *et al.*, 2006; Somorin *et al.*, 2011), and a significant source of calories for more than 500 million people worldwide (FAO, 2008). It is the most important crop in Nigeria in terms of food security, employment creation and income generation for cropproducing household (Ugwu and Ukpabi, 2002).

Nigeria is the largest producer of cassava in the world (FAO, 2008) with about 45 million metric tons and its cassava transformation is the most advanced in Africa (Egesi *et al.*, 2006; Adebayo-Oyetoro *et al.*, 2013). Cassava is grown throughout the tropic and could be regarded as the most important root crop in terms of area cultivated and total production (Ano, 2003; Adebayo-Oyetoro *et al.*, 2013). It is a major food in Nigeria (Ogbe *et al.*, 2007), essentially a carbohydrate food with low protein and fat (Adebayo-Oyetoro *et al.*, 2013). The major uses of cassava in Nigeria include flour, garri, fufu, livestock feed, confectionaries, monosodium glutamate processing, sweeteners, glues, textiles and pharmaceuticals (Oyeyiola *et al.*, 2014).

There are over 150 species of yam grown throughout the world. Yam contributes economically to more than 150 million people in West Africa and serves as an important source of income to the people. As of date, the age-old traditional method is still being used for the processing of yam to dried yam (Babajide *et al.*, 2006). The quality of the dried yams varies from one processor to another processor and location to location (Akissoe *et al.*, 2001). Yam can be consumed after direct cooking or fermented to produce flour which is used to prepare a dish locally called Amala (Oyeyiola *et al.*, 2014).

Gari is a granulated and dehydrated, cassava product. It is classified/ grouped based on texture, length of fermentation, region or place where it is produced and colour imparted by the addition/nonaddition of palm oil. It has a high swelling capability and can absorb up to four times its volume in water (Olopade *et al.*, 2014; Jekayinfa and Olajide, 2007).

Deterioration of flour products of yam and cassava are usually attributed to the types of packaging materials and pathogens such as bacteria and fungi (Okigbo, 2003). Flour foods can be infected by bacteria such as *Bacillus* spp, *Pseudomonas* spp, *Proteus* spp, *Klebsilla* spp and *Staphylococcus aureus* (WHO, 2001; Ogiehor and Ikenebomeh, 2006; Ijabadeniyi, 2007) some of these bacteria are pathogenic organisms which can cause certain disease if ingested beyond level of body tolerance. Also, there may be economic losses and food borne illness as a result of contamination by the bacteria. Due to variation in different methods of processing of yam and cassava into flour for consumption, the need for bacteriological assessment of these products becomes very necessary in order to ensure safety of the product for consumption.

The most economic method of processing of yam and cassava tuber is by drying. The traditional drying process is carried out by the local women who normally target the period of scarcity as the purpose for preservation. However, the drying is carried out under unhygienic environment resulting in products of low hygienic quality. In most parts of the country, drying by the road side is most economical and this method however exposes the product to dusts, insects, animals and other environmental hazards (Ogori and Gana, 2013).

Microbial contamination of this processed yam or cassava is attributed to the method of processing as well as packaging materials. Some of these contaminants are pathogens which causes foodborne illness as well as economic loss to farmers. Understanding the bacteriological quality of these products which are commonly sold in road side becomes necessary in order to ensure their safety for human consumption. The aim of the study is to assess the bacteriological quality of garri, cassava flour and yam tuber flour sold in Keffi Metropolis.

MATERIALS AND METHODS

This work was carried out within Keffi Metropolis of Nasarawa State which is located in the middle-belt of Nigeria. It is geographically situated on the latitude 8°50'N and longitude 7°52'E. Keffi town is about 850m above the sea level and it is the North-West of Lafia, the state capital. It is 53km away from Abuja (Capital of Nigeria) in the Guinea Savannah region of Nigeria (Obiekezie *et al.*, 2012; Akwa *et al.*, 2007).

A total of fifteen (15) samples were collected aseptically in a sterile polythene bag among local sellers in Keffi Main Market and transported immediately to the Microbiology Laboratory of Nasarawa State University, Keffi for analyses.The samples were prepared using the method described by Orji *et al.*, (2014) and Thoha *et al.*, (2012) with slight modification. 1g of each sample was added to 9ml of 0.1% (w/v) peptone water and homogenized by rolling between the palms at medium speed. Serial dilution into 5 fold was prepared by transfer of one millimeter of initial suspension into a tube containing 9ml of 0.1% (w/v) peptone water. These operations were repeated using a new sterile pipette to obtain 10^5 dilutions.

The bacterial load of garri, cassava and yam flourswere carried out by a method described by Adebayo-Oyetoro *et al.*, (2013). Briefly, samples of *FULafia Journal of Science & Technology Vol. 2 No.1 March*, 2016 garri, cassava flour and yam flour was serially diluted ten fold in which one gram (1g) of each sample was diluted in 9ml sterile distilled water followed by homogenization by horizontal and vertical agitation for a few minutes to obtain 10-1 dilution. Further 10fold dilution was made up to 10^{-5} for colony count. 0.1ml of volume of each dilution were spread with sterile syringe on Salmonella/Shigella Agar, Mannitol Salt Agar, MacConkey Agar, Eosine Methylene Blue Agar and Nutrient Agar for possible isolation of Salmonella/Shigella, Staphylococcus aureus, total coliform, total faecal count and total heterotrophic bacteria count respectively. These plates were incubated at 37° C for 24 - 48 hours after which the colonies that grew in each of the agar were counted and expressed as colony forming units per gramme (cfu/g).

The bacterial isolates were characterized on the bases of their morphological, cultural, Gram reaction and biochemical characteristicsas described by Cheesbrough (2006).

The antimicrobial susceptibility test was carried out using Kirby's Bauer disc diffusion method modified by Clinical and LaboratoryStandard Institute (CLSI) (CLSI, 2006). Briefly, four (4) variant colonies of the isolateswere transferred into 5ml of sterile normal saline in a tube such that the turbidity of the bacterial suspension is equivalent to 0.5 McFerland standards. A sterile swab stick was moist in the bacterial suspension and on Mueller-Hinton agar and the antibiotic disc was aseptically placed on the Muller Hinton Agar plate containing the isolates and the plate was incubated at 37°C for 24 hours. The diameter zone of inhibition was determined using meter rule and the result was interpreted in accordance with susceptibility break point earlier described by CLSI(2006).

RESULTS

Total bacterial count (×10⁵ cfu/g) was found to be highest in cassava (7.1), followed by garri (6.2) and yam (4.4). The total coliform count (×10⁵ cfu/g) recorded 3.7 in cassava, 1.6 in garri, and 1.4 in yam flours. Similarly, total faecal coliform (×10⁵ cfu/g) was highest in cassava flour (4.1), while garri had 2.4, and yam recorded 2.2. Also, the *Staphylococcus aureus* count (×10⁵ cfu/g) was highest in cassava flour (3.6), followed by yam flour (3.1), and garri flour had 2.8. However, the Salmonella/Shigella count (×10⁵ cfu/g) was high in garri flour (3.2), compared to cassava flour (2.5), and yam flour (1.2) (Table 1).

The bacteria isolated were *Staphylococcus aureus, Klebsiella* spp., *Proteus vulgaris, Salmonella* spp., *E. coli* and *Bacillus* spp. The cultural, morphological and biochemical characteristics of the isolates are in Table 2.

The percentage occurrences of bacterial

isolates from garri were found to be 86.7% for *Staphylococcus aureus* and *E. coli*, followed by 80% for *Bacillus*, 60% for *Klebsiella* and *Salmonella* spp, while *Proteus vulgaris* had 66.7%. In the same manner, percentage of bacterial isolates in cassava were 100% for S. *aureus, Proteus vulgaris, Salmonella* and *E. coli* each, followed by 80% for *Bacillus* species while *Klebsiella* recorded 60%. The percentage bacterial isolates in yam had 80% for *Salmonella*, followed by 60% for *Staphylococcus aureus, Klebsiella, Proteus vulgaris* and *E. coli* each, but *Bacillus* occurrence was 40% (Table 3).

The antibiotic susceptibility test was carried out using Kirby's Bauer disc diffusion method modified by the Clinical and Laboratory Standard Institute (CLSI). Most of the bacterial isolates were resistant to Nitrofurantoin (28%), Septrin (36%), Ampicillin (39%), slightly resistant to Nalidixic acid (45%), Perfloxacin and Tetracycline (46%), slightly susceptible to Ofloxacin (52%), Augmentin (63%), Ciprofloxacin (67%) and highly susceptible to Gentamicin (95%). The antibiotic susceptibility patterns of bacterial isolates revealed that Staphylococcus aureus was susceptible to Ciprofloxacin and Gentamicin (100%). Perfloxacin and Ofloxacin (54%), less resistant to Augmentin (46%) and resistant to Nitrofurantoin, Tetracycline, Ampicillin (23%), Nalidixic acid (15%) and Septrin (0%).*Klebsiella* spp. were susceptible to Gentamicin (100%), Tetracycline (78%), Perfloxacin, Ofloxacin and Ciprofloxacin (67%), Augmentin (56%), less resistant to Ampicillin and Nalidixic acid (44%), resistant to Septrin (22%) and Nitrofurantoin (11%). *Proteus vulgaris* on the other hand was susceptible to Gentamicin (100%), Augmentin (80%), Nalidixic acid (70%), Ciprofloxacin (60%), intermediately susceptible to Ofloxacin and Tetracycline (50%). Slightly resistant to Nitrofurantoin (40%), resistant to Ampicillin and Perfloxacin (30%) and Septrin (20%).

Susceptibility patterns of Salmonella spp. indicate that they were susceptible to Gentamicin (100%), Augmentin (83%), Tetracycline (75%), Nalidixic acid (67%), Ciprofloxacin (58%), slightly to Ampicillin (50%), but resistant to Septrin (33%), Ofloxacin (25%), Perfloxacin (17%) and Nitrofurantoin (17%). Similarly, E. coli was found to be susceptible to Gentamicin (100%), Ofloxacin (85%), Septrin and Perfloxacin (69%), Augmentin (62%), Ciprofloxacin (54%), slightly resistant to Nalidixic acid and Nitrofurantoin (46%) and resistant to Ampicillin (39%) and Tetracycline (31%). Nevertheless, Bacillus spp. were susceptible to Septrin and Gentamicin (70%), Ciprofloxacin (60%) slightly susceptible to Ampicillin and Augmentin (50%), slightly resistant to Perfloxacin (40%), but resistant to Ofloxacin (30%), Nalidixic acid and Nitrofurantoin (30%) and Tetracycline (20%) (Table 4).

4.1

2.2

3.6

3.1

Table 1: Total ba	cterial, total coliform a	and total faecal coli	form counts of garr	ri, cassava and yam flo	ours sold				
within Keffi metropolis ($\times 10^5$ cfu/g)									
Samples	ТНВС	TCC	TFCC	S. aureus	Salmonella spp				
Garri	3.5	1.6	2.4	2.8	3.2				

Key: THBC= total heterotrophic bacterial count, TCC= total coliform count, TFCC= total faecal coliform count

3.7

14

Table 2: Cultural, morphological and biochemical characteristic of bacterial isolates from garri, cassava and yam flours in Keffi metropolis

Cultural	PIG	M.P	G.S			nical te			H utilization		Probable isolates
Shape	Size			CAT	IN	V.P	MR	OX	CTGluc.	Lact.	
Circular	0.4mm	pinkish on MAC purple on EMB	straight rod	-	+	-	+	-	- +	AA	Klebsiella spp
Irregular	1mm	whitish in CLED pairs colourless on MAC	curved rod	-	+	+	-	+ -	-	А	Proteus vulgaris
Circular	1-2mm	red, black centre in SSA, colourless & transparent in MAC	straight rod	-	+	-	-	+.	- +	AG	Salmonella species
Circular	0.4mm	yellowish on MSA	cocci	+	+	-	-	- +	+ +	AA	Staphylococcus aureus
Circular	1mm	greenish on EMB	slightly	-	+	+	-	+	· -	AG	Escherichia coli
Circular	4mm	pink on MAC whitish on NA Pinkish on MAC	curved rod smooth rod	+	+	-	-		+	AAG	Bacillus spp.

Where: MP= Morphology, GS= Grams staining, CAT= Catalase, COA= Coagulase, IN= Indole, MR= Methylene red, OX= Oxidase, VP= Voges Proskauer, CT= Citrate test, + = positive, - enegative, MSA = Mannitol salt agar, EMB = Eosin methylene blue agar, NA= Nutrient agar, MAC = MacConkey agar, NA= Nutrient agar, CH= carbohydrate, Gloc= glucose, Lact= lactose, AG= acid and gas production, A= acid production

Table 3: Percentage occurrences of bacterial isolates form garri, cassava and yam flours in Keffi metropolis

Samples	Number of samples		ce				
		Staphylococcus. aureus	Klebsiella spp	Proteus vulgaris	Salmonella spp	E. coli	Bacillus spp
Garri	5	5(100)	3(60)	2(40)	3(60)	5(100)	4(80)
Cassava	5	5(100)	3(60)	5(100)	5(100)	5(100)	4(80)
Yam	5	5(100)	3(60)	3(60)	4(80)	3(60)	2(40)
Total	5	13(86.7)	9(60.0)	10(66.7)	12(80.0)	13(86.7)	10(66.7)

Table 4: Antibiotic susceptibility patterns of bacterial isolates for garri, cassava and yam flours sold

in Keffi metropolis Isolates (n) PN(30µg) AUG(30µg) SXT(30µg) CPX(10µg) CN(10µg) OFX(10µg) PFX(30µg) NA(30µg) NIT(30µg) TET(30µg) 13(100)7(54) Staph. aureus (13) 3(23)6(46)0(0)13(100)7(54) 2(15)3(23)3(23)Klebsiella spp. (9) 4(44) 5(56) 2(22) 6(67) 9(100) 6(67) 6(67) 4(44)1(11) 7(78)Proteus vulgaris(10) 3(30) 8(80) 2(20) 6(60) 10(100) 5(50) 3(30) 7(70) 4(40) 5(50) Salmonella spp. (12) 6(50) 10(83) 4(33) 7(58) 12(100) 3(25) 2(17) 2(17)9(75) 8(67) 13(100) 4(31) E. coli(13) 5(39) 8(62) 9(69) 7(54) 11(85) 9(69) 6(46) 6(46) Bacillus spp. (10) 5(50) 5(50) 7(70) 6(60) 7(70)3(30) 4(40) 3(30) 3(30)2(20)

Key: PN= Ampicillin, AUG= Augmentin; SXT= Septrin; CPX= Ciprofloxacin, PFX= Perfloxacin, NIT= Nitrofurantoin, CN= Gentamicin; NA= Nalidixic acid, TET= Tetracycline, OFX= Ofloxacin

7

DISCUSSION

Cassava

Yam

4.8

2.9

Garri, cassava and yam flours are a basic staple food in Nigeria and some African countries. An assessment of the bacteriological quality of garri, cassava and yam flours was conducted in Keffi, North-Central Nigeria. These flours are some sort of a ready-to-eat food, and ready-to-eat foods have been reported to be easily available, affordable provide diverse/variable food sources, employment and with a potential for improving food in the society and nutritional status and general social security. It is however, a viable source of food borne pathogen (Abdulsalam and Katerstain, 1993; Arambulo *et al.*, 1994). The total bacterial count, coliform count, total faecal coliform, *Staphylococcus* aureus and *Salmonella/Shigella* counts of the flours were high in the study area. The counts for garri flour ranged between $1.6-7.1 \times 10^5$ cfu/ml, while cassava flour's counts ranged between $2.5-6.2 \times 10^5$ cfu/ml. Yam flour also had a count ranged of $1.2-4.4 \times 10^5$ cfu/ml. Ready to eat foods with plate counts of $\leq 10^3$ are acceptable, counts of 10^4 to 10^5 are tolerable while counts $\geq 10^6$ are unacceptable (ICMSF, 1996).

The presence of contaminants to the order of 10⁵

2.5

1.2

in all of the flour samples however, negates the 10^3 stipulated requirements by the African Organization for Standardization. The presence of coliform could therefore be from post process contamination via food handlers and the environment (Obiekezie *et al.*, 2012, 2014, Olopade *et al.*, 2014).

Staphylococcus aureus and E. coli were the most prevalent organisms in garri, followed by Bacillus, Klebsiella and Salmonella with Proteus vulgaris as the least isolate. On the other hand Staphylococcus aureus, Proteusvulgaris, Salmonella spp. and E. coli were found to have 100% occurrence in cassava, followed by Bacillus and Klebsiella species. In yam, the most prevalent organism was Salmonella spp, followed by Staphylococcus aureus, Klebsiella, Proteus vulgaris while Bacillus spp. had the least occurrence.

Salmonella spp., Staphylococcus aureus, E. coli and Bacillus spp. were found to be the most occurring organisms in this study. The presence of B. cereus and S. aureus in high proportion calls for concern because some strains of these organisms are known to be toxigenic and have been implicated in food borne intoxication (Oranusi et al., 2007; Mensah et al., 1999).

Bacillus spp. particularly *B. cereus* is common environmental contaminants while *S. aureus* is of human origin, their presencecould therefore be from the food handlers, utensils and the environment (Olopade *et al.*, 2014).Isolation and identification of the isolates revealed the presence of *Staphylococcus aureus*, *Klebsiella* spp., *E. coli, Salmonella* spp., *Proteus vulgaris* and *Bacillus* spp. Similar isolates were reported by several authors; Orji *et al.*, (2014), Olopade *et al.*, (2014), Odetunde *et al.*, (2014), Abba-Kareem *et al.*, (1990) and Ogugbue *et al.*, (2011). Also, the fermentation of garri is by mixed microbial cultures and could have accounted for the diverse microbial population contaminating the product.

Previous reports have revealed high bioload and a vast array of microorganisms in market samples of garri and other cassava based products (Ijabadeniyi, 2007; Ogiehor et al., 2007; Omafuvbe et al., 2007; Amadi and Adebola, 2008; Ogugbue et al., 2011 and Odom et al., 2012). Proteus vulgaris has been reported to be isolated from dried cassava powder (garri) in Ogun State (Thoha et al., 2012). This is in agreement with the result obtained in this work. Salmonella causes food poisoning and typhoid fever as reported by Ekperigin and Nagaraja (1998) and Parry et al., (2002). Ekundavo and Okoroafor (2012) revealed the presence of Staphylococcus aureus and Escherichia coli in Fufu (made from cassava flour) in some communities around Umudike. The presence of Escherichia species in food samples indicate feacal contamination which could be attributed to unhygienic nature of the food handlers and food preparation areas as recorded by Abdullahi et al., (2004) and Edema et al., (2005).

The antibiogram of isolates from ready-to-eat food revealed that some of the organisms isolated in this study have become resistant to all the tested antibiotics, and this showed that they have become multi-resistant to these therapeutic agents, thus rendering these drugs ineffective as treatment of choice for infections caused by these pathogens. The presence of antibiotic resistant bacteria in food, water and the environment has been widely documented (Bolaji *et al.*, 2011; McGowan, 2006; Bawdon *et al.*, 1982; Dantas *et al.*, 2008; Zhang *et al.*, 2009).

Gentamicin (95%), Augmentin (63%), Ciprofloxacin (67%) and Ofloxacin (52%) had great efficacy against most of the bacterial isolates and this suggest that these drugs may be useful for treatment of infection cause by the bacterial isolates. Also, Tetracycline was effective against *Klebsiella* spp. (78%) and *Salmonella* spp. (75%). Nalidixic acid was effective against *Proteus vulgaris* (70%) and *Salmonella* spp. (67%).

The sensitivity pattern of the bacterial isolates to the antibiotics tested is comparable with reports of earlier researchers (Udo et al., 2001, Invang, 2009, Tagoe et al., 2011, Makut et al., 2013 and 2014). For most bacteria, there is evidence that increased usage of a particular antimicrobial correlates with increased levels of bacterial resistance (Granizo et al., 2000); perhaps this explains the high resistance to Nitrofurantoin (28%) and Septrin (36%) by the isolates because of its common and prevalent use. Resistance to some of these antibiotics is not new, as Ehinmidu (2003) as observed such effect earlier. The high susceptibility of the isolates to Gentamicin and Augmentin observed in this study might be due to their requirement for parenteral administration which hinder their misuse and abuse considered to be the major source of microbial resistance to conventional antibiotics as observed by Ngwai et al., (2010).

CONCLUSION

This present work revealed high bioload and vast array of bacteria in market garri, cassava and yam flours. These are threatening and alarming and suggest early warning signals indicating the level of safety with regards to public health in view of their acceptability and consumption. The high array and bioload of these microorganisms recorded may be associated with inadequate post processing handling practices such as spreading on the floor, mat and sometimes on high density polyethene spread on the floor after frying to allow it to cool before sieving into finer grains; and the open display in bowls and basins in the market, measurement with the aids of bare hands, coughing and sneezing while selling and the use of non microbiologically determined bags for packaging and haulage.

Optimization of the production process by using a starter culture with reduced post process handling is imperative for the realization of highly nutritious and microbial-free products.

The International Commission on Microbiological Specifications for Food (ICMSF) and the African Organization for Standardization (AOS) recommended absence of coliform in ready to eat foods. The presence of coliform in garri, cassava and yam flour samples therefore makes them of poor quality for human consumption.

The high bioload warrants renewed vigilance on the efficacies of food processing conditions, handling techniques and handlers technical knowhow, hygiene practices and safety of finished products. In addition, strict application and implementation of quality control, quality assurance, good manufacturing practice and the hazard analysis of critical control point principles will help to ensure the safety of these food flours consumed by several people in Keffi metropolis and Africa in general.

Also, adequate sanitation practice should be enforced concerning the sale of cassava based foods. Personal hygiene of hawkers and sanitation of utensils *FULafia Journal of Science & Technology Vol. 2 No.1 March, 2016* are important. Hawkers should be enlightened on hygienic practices. It is therefore recommended that these food flours be sold in well packaged bags and not as exposed in basins/

bowls. Nonetheless, in view of the antibiogram, it is therefore, necessary to intensify surveillance of isolates to detect emerging antimicrobial resistance phonotype especially Nigeria.

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