

PROCESSING EFFECT ON ANTI-NUTRITIONAL AND MICROBIOLOGICAL PROPERTIES OF TRIFOLIATE YAM FLOUR VARIETIES

EFEKAT PRERADE NA ANTINUTRICIONALNA I MIKROBIOLOŠKA SVOJSTVA BRAŠNA JAMA

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ABSTRACT

This research investigated whether 72 hours of fermentation can eliminate or reduce those harmful microorganisms and chemicals in the food content of the trifoliate yam that impose several challenges in human life and exploit and improve the numerous benefits associated with trifoliate yam. Freshly harvested trifoliate yams were subjected to natural fermentation for 72 hours, dried, and milled to produce trifoliate yam flour. The anti-nutritional and microbiological properties of the processed flour were determined. Fermentation significantly reduced the anti-nutritional properties of the trifoliate yam flour at $p < 0.05$ apart from flavonoid which increased at 72 hours fermentation period. For microbiological properties mould count, yeast count, and *S.aureus* were only detected and their average values for the unfermented and fermented samples were 31.66×10^3 , 18.66×10^2 , 25.00×10^4 and 35.33×10^3 , 27.33×10^2 , 38.66×10^4 respectively. It is therefore concluded that the fermentation reduced anti-nutritional properties to an extent that is friendly to mankind, increased the basic required minerals necessary to combat malnutrition and inadequate supply of protein-based food, and has a higher level of general acceptability.

Keywords: fermentation, anti-nutritional content, microbiological properties, trifoliate yam, varieties, flour

REZIME

U ovom radu se istraživalo da li 72 sata fermentacije može eliminisati ili smanjiti štetne mikroorganizme i hemikalije u hrani jama koji nameću nekoliko izazova u ljudskom životu i iskorištavaju i poboljšavaju brojne prednosti povezane sa jamom. Sveže ubran jam podvrgnut je prirodnoj fermentaciji tokom 72 sata, osušen i mleven da bi se dobilo brašno. Utvrđena su antinutritivna i mikrobiološka svojstva prerađenog brašna. Fermentacija je značajno smanjila antinutritivna svojstva brašna jama na $p < 0,05$, osim flavonoida koji se povećao u periodu fermentacije od 72 sata. Za mikrobiološka svojstva detektovani su samo broj plesni, kvasac i *S.aureus*. Njihove prosečne vrednosti za nefermentisane i fermentisane uzorke bile su 31.66×10^3 , 18.66×10^2 , 25.00×10^4 and 35.33×10^3 , 27.33×10^2 , 38.66×10^4 , respektivno. Stoga se zaključuje da je fermentacija smanjila antinutritivna svojstva u meri koja je prijateljska za čovečanstvo, povećala osnovne potrebne minerale neophodne za suzbijanje neухranjenosti i neadekvatnog snabdevanja hranom na bazi proteina i ima viši nivo opšte prihvatljivosti.

Ključne reči: fermentacija, antinutritivni sadržaj, mikrobiološka svojstva, trolisni jam, sorte, brašno

INTRODUCTION

In the modern world, handling and processing agro-based products are the most important aspects of food and nutritional security. Due to urbanisation, most food products are required to be produced in different forms to cater for the daily nutritional requirements of the common man and to the alarming pollution in the world (Nkhata et al. 2018 and Omemu, 2011). Mostly, the processing of agricultural products is done to improve consumer acceptability, palatability and transportability. They can also have adverse effects on the nutrient profile of food products by retaining their nutritional value Chaves-Lopez et al. (2014) and Omemu, (2011). There are some techniques implored in the processing of trifoliate yams, of which most of the methods are localized to certain areas while others are being practised globally. This research intends to adopt the most used processing techniques (fermentation). Fermentations of food products are broadly used in processing of food products for production of numerous varieties of dishes in Africa. It is a prerequisite for the development of acceptable textures and flavours of food products. According to Buta and Emire (2015)

and Chaves-Lopez et al., (2014), it improves the nutritional properties, digestibility and safety of food products. Fermentation can be used to minimize the antinutritional content in a food product and improve nutrient availability (Nkhata et al., 2018; Hotz and Gibson, 2007; and Omemu, 2011). According to Ogbuji et al. (2017), the Anti-nutritional composition of food products such as phytate, oxalate, tannin, and cyanide is those food content such as chemical substances that are detrimental to human health and limit the required daily nutrient availability to the body. Agricultural plants excrete these harmful food substances to protect themselves and prevent them from being consumed. However, if the food is not processed into different bioproducts through different processing methods, some of these toxins pile up in the body to harmful levels (Ogbuji et al., 2017). According to Kabagambo, et al., (2005), anti-nutritional parameters found in food products varied though, depending on the type of food and treatment offered to them, propagation mode, herbicides and pesticides used during planting, harvesting and storage (Kabagambo, et al., 2005). Some of the anti-nutritional factors are intrinsic and extrinsic characteristics of a compound but solely depend upon the digestive process methods of

the ingesting animal. Some of the anti-nutritional content such as trypsin inhibitors, which are known to be attached to monogastric animals do not exert adverse effects in ruminants because they are degraded in the ruminants and rumen (Abulude, 2007). The benefits and knowledge of food nutrients in public health challenges led to an increase in awareness of food nutrients. The microbiological characteristics of biomaterials revealed the importance of microorganisms <https://www.sciencedirect.com/topics/earth-and-planetary-sciences/micro-organism> in foods. It also covers the area of microbial ecology in food products as well as the use of microorganisms for the production of ingredients and foods (Humberto et al., 2014). Moreover, many microorganisms impose several challenges to public health due to their ability to inflate diseases on humans and food quality. It determines the shelf life of many products as they are impactful in consumers' acceptability of taste, texture, aroma and other perceptions (Sperber, 2010). Food preservation is targeted at inactivating and controlling the excess growth of spoilage and pathogenic microorganisms by ensuring shelf-stability and healthy foods (Smith et al., 2011). Therefore, steady efforts have been inputted by different food industries, government establishments, and society to improve food products at the consumption level (Ortiz et al., 2013). *Dioscorea dumetorum* (Trifoliate yam) has proven to be the most nutritious among the eight species of yam grown and consumed in West and Central Africa (Sefa-Dedeh and Afoaka, 2002). According to Lape and Treche, (1994), the Trifoliate yam is demonstrated to be rich in protein, primarily balanced in essential amino acids with easily digested starch. According to Lyonga et al., (1982), Trifoliate yam species is a very high-yielding yam species which requires no staking like other yam species, consequently saving much labour during pre-harvest and post-harvest operations. Compared to other

yam species, it has other common names such as African bitter yam, Wild Yellow and White-Colored trifoliate (three-leaved) yam and cluster yam. In the Ibo tribe mostly in South-East Nigeria, bitter trifoliate yam is usually referred to as *ji una* or *jona* and food for the adult (Anthony and Nzewi, 2018). It cannot be processed into *fufu* like other yam species due to its soft texture which favours old people with poor teeth structure because of old age, but it can be used as a vegetable. It serves as raw material for formulations of new bioproducts and it was found to be the cheapest and high-yielding crop. Breweries and other similar companies explore the benefits of this underutilized yam species in the preparation of beer (Anthony and Nzewi, 2018). This underutilized yam species, when properly processed like other yam species can be used in making yam flakes, instant flour for the bakery sector or starch in diverse pharmaceutical applications (Ukpabi and Ndimele, 2014). It has lots of medical relevance as it has been proven to be a direct remedy for treatments of diabetes, malaria and other numerous ailments mostly in South – East Nigeria, yet it remains an underutilized tropical tuber and probably it may be driven into extinction in no distant (Clifford et al., 2013).

MATERIAL AND METHOD

Source of sample

The Deep-Yellow, Yellow and White trifoliate yam (*Dioscorea dumetorum*) varieties used for this research work, samples were harvested from a farm located at Udenu Local Government Area of Enugu State, Nigeria at physiological maturity. They were immediately transported in a heap and stored at temperature and relative humidity rates of $28^{\circ}\text{C} \pm 3^{\circ}\text{C}$ and $82 \pm 5\%$ respectively.



Fig. 1. Trifoliate yam sample used for the experiments

Preparation of the sample

The sourced trifoliate Yam tubers from each variety (White Trifoliate yam, Deep-Yellow trifoliate Yam and Yellow Trifoliate Yam) were divided into two equal batches. The first batch which represent the raw (unfermented sample) were peeled, washed and then sliced into a rectangular shape of about 30 x 20 x 10mm thickness using a stainless kitchen knife and vernier calliper. The second batch was peeled, washed and soaked in distilled water, the traditional fermentation method as reported by Oladele and Oshidi (2008) was used to ferment the sample. The micro-organisms involved in the traditional fermentation were natural inoculants from the air. The samples were collected after 72 hours. The samples were oven-dried at intervals of 60 minutes until constant moisture and weight were obtained. The processed (dried) trifoliate yam chips

were then milled into flour using a hammer mill, sieved through a standard laboratory sieve of 500-micron aperture to produce uniform particle size flour, packaged in polythene bags, sealed and then stored in airtight containers with appropriate label and then carried to the laboratory where the experiment was investigated. The sample preparation stages are shown in figure 2.

Determination of antinutritional properties of trifoliate yam flour

Antinutritional properties such as Phenols, Phytate, oxalate, Tannins, HCN, alkaloid, flavonoid and saponin were determined according to the method reported by AOAC (2005).

Determination of microbiological properties of trifoliate yam flour

The microbiological properties were determined on trifoliate yam sample flour according to the modified method of AOAC (2005).



Fig. 2. Pictorial showing procedures in preparing Trifoliate Yam Flour sample

RESULT AND DISCUSSION

Anti-nutritional properties of three cultivars of trifoliate yam (*Dioscorea dumetorum* Pax)

The data presented in Table 1 show that there was a significant ($p < 0.05$) variation in the tannin levels of fermented and

unfermented trifoliate yam. Fermented deep yellow contained the highest amount of tannin (0.91 g) than the other two fermented cultivars. While the unfermented white trifoliate had the highest levels of tannin (1.04 g) than the unfermented cultivars.

Table 1. Anti-nutritional properties of three cultivars of trifoliate yam (*Dioscorea dumetorum* Pax)

Cultivars	Tannin			Saponin			Alkaloid		
	Fermented	Un-fermented	t-test	Fermented	Un-fermented	t-test	Fermented	Un-fermented	t-test
Deep yellow trifoliate yam	0.91	1.11	0.04	2.80	1.73	0.61	1.03	2.85	0.86
Yellow trifoliate yam	0.68	0.83	0.07	0.89	0.85	0.02	0.88	0.85	0.01
White trifoliate yam	0.87	1.04	0.12	0.75	1.08	0.14	0.83	1.08	0.13
FLD (0.05) for any two cultivars	0.03	0.01		0.05	0.03		0.04	0.06	

Unfermented cultivars contained more levels of tannin than the fermented ones within each cultivar. The amount of tannin increased from 1.11 g (unfermented deep yellow) to 0.91 g (fermented deep yellow), 0.83 g (unfermented yellow) to 0.68 g (fermented yellow) and 0.87 g (unfermented white) to 1.04 g (fermented white). Fermentation for 72 hours resulted in a significant decrease in the tannin content. Tannins are due to the hydrolysis process by acids, bases or some hydrolytic enzymes (Oyarekua, 2015). Furthermore, the hydrolytic enzymes produced by the fermenting micro-organisms or acids produced during fermentation might result in the degradation of the tannin content

(Oyarekua, 2013). Tannins have proven to be heat stable and are therefore not removed by heat during processing. Saponin content was significantly ($P < 0.05$) higher in fermented deep yellow (2.80 g) and yellow (0.89 g) than the unfermented trifoliate yam respectively except in unfermented white trifoliate (1.08 g) which was higher than the fermented white trifoliate yam (0.75 g). Fermented (2.80 g) and unfermented (1.73 g) deep yellow cultivars contained more saponin content than the other two fermented and unfermented cultivars of trifoliate yam respectively. The saponin content was reduced from 1.73%, 3.84% and 2.80% for raw samples of unfermented deep yellow, unfermented white and unfermented

yellow trifoliolate yam. Fermented deep yellow yam, fermented white yam, and fermented yellow yam ranged from 0.71%, 0.75%, and 0.89% respectively. This result is in tandem with Igbabul et al., (2014) who obtained similar results on the effect of fermentation on cocoyam. Saponin is an anti-nutritional factor with foaming properties in aqueous solution, hemolytic activity and cholesterol binding characteristics and bitterness (Hassan et al., 2015). One of the most curative powers of saponin is that it contains natural antibiotics which help the body fight infections and microbial invasion (Ojokoh and Adeleke, 2019). Among the unfermented cultivars of trifoliolate yam, alkaloid content ranges significantly ($P < 0.05$) from deep yellow (2.85 g) > white (1.08 g) > yellow trifoliolate yam (0.85 g) respectively. In the fermented cultivars, alkaloid content varied significantly ($P < 0.05$) in the order of deep yellow (1.03 g) > yellow (0.88 g) > white trifoliolate yam (0.83 g) respectively. The alkaloid content increased from 2.85 g (unfermented deep yellow) and 1.08 (unfermented white) to 1.03 g (fermented deep yellow) and 0.83g (fermented white yam) respectively but fermented yellow (0.88 g) contained more alkaloids than the unfermented yellow trifoliolate yam (0.85 g). The reduction observed in alkaloid content of the trifoliolate yam species (unfermented deep yellow yam, unfermented white yam and unfermented yellow yam) ranged from 2.85%, 1.08%, 1.03% for raw and fermented deep yellow yam, fermented white yam, fermented yellow yam ranged from 0.85%, 0.83%, 0.88% respectively. This is similar to the report of Kasaye et al., (2018). The presence of alkaloid content in the trifoliolate yam species indicates that the samples cannot be eaten raw by humans or

animals. The reduction of alkaloid content in trifoliolate yam flour can be achieved by fermentation which improves the bioavailability of dietary mineral content and reduces its negative effect on human health even in animals. The alkaloids are known for their pharmacological effects rather than their toxicity which could cause gastro-intestinal upset and neurological disorders Ojokoh et al., (2015) when their concentration in a food product is unacceptable.

Furthermore, Table 2 revealed that there was a significant difference ($P < 0.05$) in the flavonoid content among the three fermented cultivars of trifoliolate yam. Fermented white yam (1.29 g) contained more flavonoids than fermented yellow (1.12 g) and fermented deep yellow (0.92 g) respectively. Unfermented white trifoliolate yam had significantly the highest flavonoid content (1.35 g) than the unfermented yellow trifoliolate yam (0.96 g) and unfermented deep yellow trifoliolate yam (0.78 g). Flavonoid content was higher in fermented deep yellow trifoliolate yam (0.92 g) and yellow trifoliolate yam (1.12 g) than in the unfermented deep yellow trifoliolate yam (0.78 g) and yellow trifoliolate yam (0.96 g) respectively except in the unfermented white trifoliolate yam (1.35 g) which had the highest alkaloid content than the fermented white trifoliolate yam (1.29 g). The flavonoid content of trifoliolate yam ranged from 0.78%, 1.35%, 0.92% unfermented deep yellow yam, unfermented white yam and unfermented yellow yam and fermented deep yellow yam, fermented white yam, fermented yellow yam ranged from 0.96%, 1.29%, 1.12% respectively. It indicated that the fermentation increased flavonoid content of all the samples apart from the unfermented white trifoliolate.

Table 2. Anti-nutritional properties of three cultivars of trifoliolate yam (*Dioscorea dumetorum* Pax)

	Flavonoid			HCN			Phenol		
Cultivars	Fermented	Un-fermented	t-test	Fermented	Un-fermented	t-test	Fermented	Un-fermented	t-test
Deep yellow trifoliolate yam	0.92	0.78	0.11	1.56	2.77	0.92	0.43	0.44	NS
Yellow trifoliolate yam	1.12	0.96	0.05	2.18	1.14	0.45	0.64	0.37	0.09
White trifoliolate yam	1.29	1.35	0.02	1.46	3.28	0.73	0.43	0.58	0.08
FLD (0.05) for any two cultivars	0.06	0.07		0.03	0.03		0.01	0.02	

Yam that decreased at the 72-hour fermentation period. Flavonoids are currently known as an important nutrient contained in a food product rather than as it is known to be antinutrients. Some flavonoids like rutin major functions are, strengthen blood capillary and other connective tissues while others like quercetins major functions are, block the sorbitol pathway that is linked with many health complications associated with diabetes (Ojokoh and Adeleke, 2019).

Table 2 results showed that there was a significant difference ($P < 0.05$) in the HCH content between the fermented and unfermented in each cultivar of trifoliolate yam respectively. Unfermented trifoliolate yams recorded significant ($P < 0.05$) HCH content (3.28 g, 2.77 g and 1.14 g in white, deep yellow and yellow trifoliolate yam respectively) when compared with the fermented (1.46 g, 1.56 g and 2.18 g in white, deep yellow and yellow trifoliolate yam respectively). Within the fermented cultivars, the yellow trifoliolate

yam significantly contained the highest amount of HCH (2.18 g) than the other two cultivars. Also in the unfermented cultivar, HCH content was observed to be the highest (3.28 g) in white trifoliolate yam than the other two cultivars. Within the fermented trifoliolate cultivars of yam, yellow trifoliolate yam (0.64 g) had significantly ($P < 0.05$) the highest amount of phenol than the other two cultivars, while in the unfermented trifoliolate cultivars of yam, white trifoliolate yam (0.58 g) was recorded to be the highest than the other two cultivars respectively. Between the fermented and unfermented cultivars of trifoliolate yam yellow trifoliolate yam (0.64 g) contained the highest level of crude phenol within each cultivar. More so in Table 3, the amount of oxalate in the fermented trifoliolate yam was observed to be significantly ($P < 0.05$) the highest in the deep yellow (1.25 g) than in the yellow (0.72 g) and white yam (0.65) respectively.

Table 3. Anti-nutritional properties of three cultivars of trifoliate yam (*Dioscorea dumetorum* Pax)

Cultivars	Oxalate		t-test	Phytate		t-test
	Fermented	Unfermented		Fermented	Unfermented	
Deep yellow trifoliate yam	1.25	0.93	0.19	1.14	1.41	0.07
Yellow trifoliate yam	0.72	0.55	0.08	0.95	1.02	0.03
White trifoliate yam	0.65	1.07	0.14	0.88	1.27	0.18
FLD (0.05) for any two cultivars	0.03	0.05		0.04	0.02	

The oxalate content of the unfermented trifoliate yam increased from 1.07 g (white) to 0.93 g in deep yellow and 0.55g yellow yam respectively). A significant difference ($P < 0.05$) was recorded between the fermented and unfermented in each cultivar of trifoliate yam. Unfermented deep yellow trifoliate yam (0.93 g) had the lowest oxalate content than the fermented (1.25 g). Fermented yellow yam (0.72 g) contained more oxalate content than the unfermented yellow trifoliate yam (0.55 g) and unfermented white yam (1.07 g) had the highest oxalate content than the fermented white yam (0.65 g). This may be because of the activities of fermenting microorganisms' presence and the adopted processing methods. The result obtained was similar to what Bello et al., (2017), Igbabul et al., (2014) reported. Also, Jacques et al., (2016) reported a decrease in the oxalate content of fermented yam flour, cocoyam flour and yam flour respectively. Oxalates are dangerous to human nutrition and health, particularly by declining calcium absorption and enhancing the formation of kidney stones. Furthermore, reducing the oxalate content of food products by fermentation could have a positive impact on the health of consumers, especially as the decrease of oxalate concentration on fermentation is expected to enhance the bioavailability of essential dietary minerals of the trifoliate yam food products, as well as limit the risk of kidney stones effect among consumers (Kasaye et al., 2018).

Furthermore, unfermented deep yellow yam and the fermented deep yellow yam had significantly ($P < 0.05$) the same levels of phytate content (1.41 g). Unfermented yellow (1.02 g) and unfermented white trifoliate yam (1.27 g) contained more phytate content than the fermented yellow trifoliate yam (0.95 g) and fermented white yam (0.88 g) respectively. Among the unfermented

cultivars of trifoliate yam, phytate content varied significantly ($P < 0.05$) in the order of deep yellow (1.41 g) > white (1.27 g) > yellow trifoliate yam (1.02 g) respectively. In the fermented cultivars, phytate content varied significantly ($P < 0.05$) in the order of yellow (1.114 g) > deep yellow (0.95 g) > white trifoliate yam (0.88 g) respectively. This variation may be attributed to the activities of endogenous phytase enzymes from raw ingredients and inherent microorganisms which can hydrolyze the phytic acid in the fermented food preparations into inositol and orthophosphate (Ojokoh and Adeleke, 2019) and (Bello et al., 2017).

Phytate has been known as an anti-nutrient due to its adverse effects on both humans and animals. It limits the bioavailability of minerals and institutes growth inhibition (Jacques et al., 2016). The residual phytate level of the processed (fermented) yam flour is similar to the FAO-recommended safe level and is not detrimental thereby making the yam flour safe for both human and animal consumption (Ojokoh and Adeleke, 2019). The result showed that fermentation reduced the phenol content in all the samples apart from unfermented yellow trifoliate yam (0.43%) with an increase in fermented yellow trifoliate yam (0.64%) after 72-hour fermenting. This reduction in the phenolic content level of the samples may be an indication that the micro-organisms had degraded the phenol contained in the samples while the increase that was observed in sample F may be attributed to the condition adopted during fermentation (Reddy and Sathe, 2002).

Microbiological properties of trifoliate yam. Table 4 presents the Microbiological analysis of fermented and unfermented trifoliate yam flours. Salmonella count, Enterococci count, Coliform count and S.aureus count were not detected.

Table 4. Microbiological properties of three species of trifoliate yam flour.

Samples	Mold count(103)	Salmonella count (104)	Enterococci count (102)	Yeast count (103)	Coliform count (MPN)	S.aureus count (104)	Streptococcus count (104)
A	35	ND	ND	21	ND	25	ND
B	32	ND	ND	18	ND	27	ND
C	39	ND	ND	32	ND	39	ND
D	28	ND	ND	15	ND	23	ND
E	38	ND	ND	24	ND	36	ND
F	29	ND	ND	26	ND	41	ND

Note: A= Unfermented Deep-Yellow Trifoliate Yam, B=Unfermented White Trifoliate Yam, C=Fermented Deep-Yellow Trifoliate Yam, D= Unfermented Yellow Trifoliate Yam, E=Fermented White Trifoliate Yam and F=Fermented Yellow Trifoliate Yam, ND=Not Detected. The microbiological of fermented and unfermented trifoliate yam samples relied on the search for potentially pathogenic microorganisms (Dogore et al., 2013). Mould counts were more prominent in fermented samples C, E and F with their values ranging from 39, 38, and 29

103 respectively while in unfermented samples A, B, D 35, 32, and 28 (103) respectively. *S. aureus* count was more noticed in fermented samples C, E and F with their values ranging from 39, 36 and 41(104) respectively. The yeast was found to be eminent in the fermented samples C, E and F with their values ranging from 32, 24 and 26 respectively. The result showed that the microbiological profiles of the samples were more pronounced in the fermented samples than unfermented samples. This could be attributed to the fact that processing (fermentation) liberated all the microbiological properties inhibited in the food material. These findings are in line with the report of (Dogore et al., 2013) on processed (fermented) cereal sourced from indigenous raw materials. The beguiled and the traditional starter are potential sources of contamination of processed composite flours. The good hygienic quality of obtained flour reveals the safety of the traditional starter and good manufacturing practices.

CONCLUSION

The effect of 72hours of fermentation on the trifoliate yam varieties flour on the antinutritional and microbiological characteristics gave mean values of tannin, saponin, alkaloid, flavonoid, phenol, oxalate and phytate are 1.02, 2.79, 1.65, 1.02, 0.86, 1.08, and 1.27 for unfermented samples while the average values of fermented sample are 0.79, 0.78, 0.86, 1.12, 0.48, 0.64, and 0.95 respectively. For microbiological properties mould count, yeast count and *S.aureus* were detected and their average values for unfermented and fermented samples were 31.66 x 10³, 18.66 x10², 25.00 x10⁴ and 35.33 x 10³, 27.33 x 10², 38.66 x 10⁴ respectively. It was observed that mould count, yeast and *S.aureus* increased throughout the fermentation period. It was found that the fermented samples were better in reducing disease risk, enhancing biological accessibility and biological availability of nutrients from numerous agricultural products and certifying organoleptic content thereby extending shelf. It makes food safe by not only limiting the growth of pathogenic bacteria because of the antimicrobial activity of lactic acid but due by detoxifying aflatoxin. With these desirable benefits of the findings, fermentation has been proven as an effective method to limit the dangers of mineral inadequate among the increasing populations, especially in developing countries like Nigeria where unrefined cereals and/or pulses are highly required and consumed.

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