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Amit Kumar Singh
Department of Horticulture
Rani Lakshmi Bai Central Agricultural University, Jhansi, Uttar Pradesh, India

Arvind Kumar Chaurasiya
Department of Horticulture
North Eastern Hills University
Tura, Meghalaya, India

Surajit Mitra
Department of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India

Correspondence
Amit Kumar Singh
Department of Horticulture
Rani Lakshmi Bai Central Agricultural University, Jhansi, Uttar Pradesh, India

Evaluation of antioxidant properties in elephant foot yam (*Amorphophallus paeoniifolius* Dennst-Nicolson) pickles

Amit Kumar Singh, Arvind Kumar Chaurasiya and Surajit Mitra

Abstract
Elephant foot yams are perishable foods; they required processing technologies that preserves chemical and nutritional characteristics of fresh forms. The principle objective of this research was to analyze the antioxidant properties to contribute the diversification of food. Therefore, two improved cultivars *viz.* BCA-1 and IGMA-1 have been selected for antioxidant rich pickle preparation. The tubers were made into cubes, blanched (20 minutes at 8 kg/cm^2) and subjected to lactic acid fermentation in pre-standardized brine solutions (20% common salt). The final product evaluated for various nutritional and organoleptic evaluations and found to have a titrable acidity (0.50-0.64%), ascorbic acid (2.56-3.82 mg/100 g), β-carotene (337.34-370.71 μg/100g), total phenol (43.15-53.51 mg/100g), microbial load of bacterial population (9.17-90.83 cfu/g) and microbial load of yeast population (0.00-19.67 cfu/g) during storage period. The pickling process of elephant foot yam caused a significant decrease in their antioxidant component and activity.

Keywords: *Amorphophallus paeoniifolius*, antioxidant, cultivar, pickle

Introduction
Pickling, also known as brining or corning is the process of preserving food by anaerobic fermentation in brine (a solution of salt in water) to produce lactic acid, or marinating and storing it in an acid solution, usually vinegar (acetic acid). The resulting food is called a pickle. Food fermentation is one of the earliest technologies developed by humans. Littoral foragers in Asia during the primitive pottery age (8000 to 3000 b. c.) are believed to have fermented vegetables prior to the development of crop-based agriculture (Lee, 2009) [1]. It is believed that cucumbers were first fermented around 2000 b. c. in the Middle East. Early written records of cucumber pickles come from paper fragment remains of a play (The Taxiarchs) by the Greek writer Eupolis (429-412 b. c.), and pickles are also mentioned several times in the Christian Bible. Korean-style fermented cabbage, kimchi, is thought to have originated in the primitive pottery age from the natural fermentation of withered vegetables stored in seawater (Lee, 2001; Breidt *et al*. 2013) [2, 3].
The fermentation process for elephant foot yam can result in nutritious foods that may be stored for extended periods, more than a year, without refrigeration. Prior to fermentation, fresh corms were difficult to store in healthy condition due to perishable in nature (Nguyen and Carlin, 1994) [4]. The pickling is basically, conversion of sugar to acid by microorganisms that are lactic acid bacteria (Nurul and Asmah, 2012) [5]. Brining vegetables for fermentation results in the production by lactic acid bacteria of organic acids and a variety of antimicrobial compounds (Caplice and Fitzgerald 1999; De Vuyst and Vandamme, 1994) [6, 7]. During fermentation, diffusion of organic acids into the brine, and the low pH that results, influences microbial growth across the surface of the vegetable material. As sugar convert to the lactic acid the condition become acidic and inhibits the growth of pathogens and other nonacidic tolerant microorganisms' especially aerobic spoilage microorganisms. As a result from pickling, the vegetable will have a longer shelf life, translucent appearance, firm texture and pickle flavour. Fermentation of fruits and vegetables can occur spontaneously by the natural lactic acid bacteria that placed surface of vegetable, such as *Lactobacillus spp*, *Leuconostoc spp*, and *Pediococcus spp*. (Karovicova *et al*. 1999) [8]. In India, pickling is an important way of consumes vegetable. It is preferred not only as a good way to keep vegetables fresh but also it is a popular taste in Indian cuisine.

Although there is an industrial production of vegetable pickles mostly pickling still a domestic process. There are studies demonstrating that fermentation increased the antioxidant capacity of vegetables like soybean (Moktan *et al.* 2008) [9] but some plant foods showed decrease in antioxidant capacity like olive (Othman *et al*. 2009) [10]. Lactic acid bacteria fermented vegetables helps to enhance human nutrition with the attainment of balanced nutrition, providing vitamins, minerals, and carbohydrates (Yamano *et al*. 2006) [11], besides; phytochemicals act as antioxidants in the body by scavenging harmful free radicals implicated in degenerative diseases like cancer, arthritis, and ageing (Kaur and Kapoor, 2001) [12]. Fruit and vegetables are good sources of natural antioxidants such as vitamins, carotenoids, flavonoids and other phenolic compounds (Takebayashi *et al*. 2013; Isabelle *et al*. 2010) [13, 14]. Protecting these nutrition values of plant foods is a growing scientific field. Then a common way to maintain and improve the nutrition as well as antioxidant in vegetables through pickling or lactic acid fermentation (Demir *et al*. 2006) [15].

In recent year, lactic acid products gained popularity due to its probiotic nature and shelf stability, without causing too much increase in processing costs (Panda *et al*. 2017) [16]. Elephant foot yam corms are consumed by many people as a food and widely used in many ayurvedic preparations because it contains different bioactive components like alkaloids, flavonoids, phenols, vitamins, minerals etc (Singh *et al*. 2016; Singh *et al*. 2017; Angayarkanni *et al*. 2007) [17, 18, 19]. It is eaten in varied manners-boiled like potatoes and eaten with mustard, as curry, as pickle after boiling with tamarind leaves, as preserve after cooking in syrup. It can also be cooked with salt, chilly, tamarind and turmeric powder and is used as curry (Yesodharan and Sujana, 2000) [20]. In Assam (India), farmers consume a special dish made of elephant foot yam in the month of Bhadoh, which they perceive to be strength giving (Borah *et al*. 2008) [21]. With living developments, fast food and instant food have become more popular than ever before; thus, there is need for elephant foot yam processing to convert them into delicious products (Singh *et al*. 2017) [18]. The research was focused on the development of a product *viz.* pickles are favoured as a side dish with a main meal or appetizer in the world (Khaskheli *et al*. 2017) [22]. This study is aimed to evaluate the quality of elephant foot yam for pickle preparation, which may be important to increase the levels of antioxidant as elephant foot yam are easily available in a huge quantity.

Materials and Methods

The experiment was carried out in the laboratory of All India Coordinated Research Project on Tuber Crops, Research Complex, Kalyani (Bidhan Chandra Krishi Viswavidyalaya) West Bengal (India) with a view to prepare antioxidant rich lactic acid fermented pickle. Two cultivars (BCA-1 & IGAM-1) were selected on nutritional point of view at maturity stage, and after peeling it has been sliced into suitable size of pieces (2.5x2.5x2.5cm) for preparing cubes with the help of knife. The pieces were blanched (20 minutes at 8kg/cm^2), and then by adding 50% of total salt and turmeric allow storing for 6 days. Remaining salt and all roasted spices with oil mixed in cubes, and then filling in glass jars and finally stored at room Temperature for their monthly nutritional evaluation.

Physico-chemical analysis

The physico-chemical attributes of elephant foot yam cubes were analyzed by mentioned methods like titrable acidity by titration method (AOAC, 1990) [23], ascorbic acid by (2, 6-dichlorophenol indophenols-Dye) titration method, β-carotene analyzed with the help of ELICO Bio-spectrophotometer at 452 nm by Moorthy and Padmaja (2002) [24] and total phenol was estimated by ELICO Bio-spectrophotometer by Swain and Hills (1959) [25]; Walter (1979) [26] at monthly intervals.

Statistical procedure

The nutritional data were evaluated by Completely Randomized Design (CRD) as suggested by Raghuramula (1983) [27]. The critical difference (CD) value at 5% level of probability was used for comparing the treatments and to find out the significant difference in between them. Each treatment was replicated for three times.

Results and Discussion

There were variations in titrable acidity, ascorbic acid, β-carotene, total phenol, population of bacteria and yeast in pickles within the year of two selected cultivars that could be clinically important.

Ascorbic acid

Ascorbic acid of elephant foot yam corm pickle also varied significantly among the different cultivars (Table 1) at all stages of storage. At the early stage of storage *i. e.* at 0 month of storage and 15 month of storage, the cultivar IGAM-1 showed highest (4.78 and 2.37 mg/100g) amount of ascorbic acid, while cultivar BCA-1 contained the least (3.82 and 2.13 mg/100g) amount of ascorbic acid at 0 and 15 months of storage, and it was also observed that the ascorbic acid content decreased in both cultivars during the storage period. Taking all the stages during storage into consideration, it was observed that there was significant variation in mean ascorbic acid with the value ranging from 2.13 to 4.78 mg/100g. Variability that may influence ascorbic acid content includes cultivars (genetic conditions), harvesting time, growing location, environmental conditions, storage and processing conditions (time, temperature, humidity) (Franke *et al*. 2004; Wall, 2006) [28, 29]. These determinants of ascorbic acid content were in part considered in some reports on ascorbic acid concentrations. For instance, longer day lengths and higher light intensities in summer months can increase the concentrations of ascorbic acid and glucose, the precursor to ascorbic acid in fruits (Lee and Kader, 2000) [30].

Titrable Acidity

Titrable acidity of elephant foot yam corm pickle was significantly influenced by cultivars and storage stages (Table 1). The cultivar IGAM-1 pickle contained highest (0.57 and 0.65 %, respectively) acidity at 0 and 15 months of storage, while cultivar BCA-1 contained lowest (0.50 and 0.61 %, respectively) amount of acidity at 0 and 15 months of storage. The mean value of pickle was also varied significantly among the cultivars. Increase in acidity content of corm pickle with the advancement of storage might be due higher accumulation of photosynthates to the corms.

Table 1: Changes in Titrable acidity (%) and Ascorbic acid content (mg/100g) in elephant foot yam corms pickle during storage

MAS/cv.	Titrable Acidity						Ascorbic Acid					
	BCA-1			IGAM-1			BCA-1			IGAM-1		
	2011-12	2012-13	Pooled	2011-12	2012-13	Pooled	2011-12	2012-13	Pooled	2011-12	2012-13	Pooled
0	0.512	0.487	0.500	0.563	0.576	0.570	3.900	3.750	3.825	4.181	5.371	4.776
1	0.512	0.507	0.510	0.572	0.583	0.578	3.653	3.412	3.533	3.846	4.950	4.398
2	0.521	0.513	0.517	0.579	0.587	0.583	3.387	3.199	3.293	3.711	4.473	4.092
3	0.538	0.527	0.533	0.584	0.592	0.588	3.230	2.686	2.958	3.509	4.125	3.817
4	0.546	0.538	0.542	0.588	0.598	0.593	2.990	2.498	2.744	3.279	3.836	3.558
5	0.555	0.546	0.551	0.593	0.604	0.599	2.821	2.389	2.605	3.125	3.667	3.396
6	0.566	0.557	0.562	0.599	0.610	0.605	2.609	2.368	2.488	3.008	3.489	3.249
7	0.571	0.561	0.566	0.608	0.614	0.611	2.400	2.345	2.373	2.901	3.321	3.111
8	0.576	0.565	0.571	0.616	0.618	0.617	2.220	2.311	2.266	2.797	3.211	3.004
9	0.579	0.569	0.574	0.625	0.622	0.624	2.121	2.289	2.205	2.645	3.131	2.888
10	0.585	0.573	0.579	0.630	0.626	0.628	2.107	2.275	2.191	2.500	2.994	2.747
11	0.591	0.586	0.589	0.634	0.631	0.633	2.091	2.261	2.176	2.412	2.813	2.613
12	0.594	0.592	0.593	0.641	0.635	0.638	2.078	2.248	2.163	2.302	2.745	2.524
13	0.599	0.595	0.597	0.645	0.639	0.642	2.059	2.233	2.146	2.221	2.619	2.420
14	0.606	0.604	0.605	0.649	0.642	0.646	2.051	2.227	2.139	2.199	2.593	2.396
15	0.608	0.607	0.608	0.652	0.644	0.648	2.047	2.222	2.135	2.174	2.574	2.374
Mean	0.566	0.558	0.562	0.611	0.614	0.612	2.610	2.545	2.577	2.926	3.495	3.210
	CD 0.05	CD 0.01	S Ed	CD 0.05	CD 0.01	S Ed	CD 0.05	CD 0.01	S Ed	CD 0.05	CD 0.01	S Ed
M	0.046	0.062	0.023	0.048	0.064	0.024	1.621	2.154	0.811	1.984	2.636	0.993
Y	0.016	0.022	0.008	0.016	0.022	0.008	0.573	0.762	0.287	0.701	0.932	0.932
MY	0.066	0.088	0.033	0.067	0.089	0.034	2.292	3.047	1.147	2.805	3.728	1.404

B-Carotene

B-carotene content of elephant foot yam corm pickle also varied significantly among both cultivars (Table 2) at all stages of storage. At the early stage of storage *i. e.* at 0 months of storage, the cultivar BCA-1 showed the highest (370.71 and 362.85 μg/100g, respectively) amount of β-Carotene at 0 and 15 months of storage, while the cultivar IGAM-1 contained lowest (349.42 and 327.93 μg/100g, respectively) amount of β-Carotene at 0 and 15 months of storage. Taking into consideration all the stage during storage, it was observed that there was significant variation in β-carotene content with the value ranging from 327.93 to 370.71 μg/100g. B-carotene is one of the better-known carotenes because of its high vitamin A activity and its wide distribution in nature (Silalahi, 2002) [31]. The protective effects of β-carotene occur through one or several actions, which include singlet oxygen quenching (photo protection), antioxidant protection, and enhancement of the immune response (Siddiqui *et al*. 2015) [32]. B-carotene may also function as a redox reagent, an immunological regulator, or by increasing cell-to-cell communications (Burri, 1997) [33].

Total Phenol

The total phenol contents of pickle found to vary significantly ($p<0.05$) among all treatments as well as between both years (Table 2). The cultivar BCA-1 contained highest and lowest (53.51 and 37.00 mg/100g, respectively) amount of total phenol at 0 and 15 months of storage, while cultivar IGAM-1 contained lowest and highest (51.85 and 38.19 mg/100g, respectively) amount of total phenol at 0 and 15 months of storage, and it was also found that the total phenol content was in decreasing order during the storage period in both pickles in both years. The mean value at different storage stages was also varied significantly among the cultivars. A similar finding was observed by Nurul and Asmah (2012) [5] in papaya pickle.

Table 2: Changes in β-carotene content (μg/100g) and total phenol content (mg/100g) in elephant foot yam corms pickle during storage

MAS/cv.	β-carotene						Total Phenol					
	BCA-1			IGAM-1			BCA-1			IGAM-1		
	2011-12	2012-13	Pooled	2011-12	2012-13	Pooled	2011-12	2012-13	Pooled	2011-12	2012-13	Pooled
0	373.190	368.231	370.711	347.100	351.745	349.423	52.345	54.673	53.509	52.781	50.921	51.851
1	372.783	367.115	369.949	345.007	349.214	347.111	50.256	51.782	51.019	50.551	48.983	49.767
2	372.392	366.578	369.485	343.115	347.103	345.109	48.189	49.543	48.866	48.992	47.438	48.215
3	371.887	366.009	368.948	341.079	344.789	342.934	46.893	47.984	47.439	47.667	46.008	46.838
4	371.413	365.512	368.463	339.783	343.425	341.604	45.132	46.791	45.962	46.321	44.763	45.542
5	370.956	365.011	367.984	338.491	342.007	340.249	44.023	45.685	44.854	44.983	43.452	44.218
6	370.491	364.541	367.516	336.895	340.653	338.774	43.009	44.791	43.900	43.798	42.331	43.065
7	370.002	364.104	367.053	335.591	339.351	337.471	41.993	43.831	42.912	43.013	41.643	42.328
8	367.574	363.667	365.621	334.132	338.003	336.068	41.102	42.996	42.049	42.412	40.943	41.678
9	367.189	363.225	365.207	333.006	336.785	334.896	40.563	42.131	41.347	41.818	40.343	41.081
10	366.771	362.782	364.777	331.879	335.441	333.660	39.745	41.021	40.383	41.347	39.783	40.565
11	366.299	362.391	364.345	330.593	334.103	332.348	38.913	40.201	39.557	40.743	39.211	39.977
12	365.865	362.001	363.933	329.199	332.984	331.092	38.208	39.472	38.840	40.101	38.625	39.363
13	365.412	361.675	363.544	328.008	331.874	329.941	37.510	38.889	38.200	39.632	38.514	39.073
14	365.002	361.391	363.197	326.887	330.673	328.780	36.903	38.241	37.572	39.177	38.067	38.622
15	364.712	360.995	362.854	325.965	329.895	327.930	36.221	37.779	37.000	38.785	37.598	38.192
Mean	368.871	364.077	366.474	335.421	339.253	337.337	42.563	44.113	43.338	43.883	42.414	43.148
	CD 0.05	CD 0.01	S Ed	CD 0.05	CD 0.01	S Ed	CD 0.05	CD 0.01	S Ed	CD 0.05	CD 0.01	S Ed

M	12.928	17.184	6.471	13.623	18.106	6.819	10.890	14.474	5.451	11.224	14.917	5.618
Y	4.571	6.075	2.288	4.816	6.401	2.411	3.850	5.117	1.927	3.968	5.274	1.986
MY	18.284	24.301	9.152	19.265	25.606	9.644	15.401	20.470	7.709	15.873	21.097	7.945

Changes in microbial status of elephant foot yam corm pickles during storage

Changes in bacterial population during storage

The bacterial colonies of elephant foot yam corm pickle was found in decreasing trend with increase in storage duration (Table 3) and the observed value came down from 60.42 to 18.17, 47.83 to 19.17 in cultivar BCA-1 at $x10^2$ and $x10^3$ cfu/g, respectively at 0 to 13 months of storage, while in cultivar IGAM-1, it was 90.83 to 35.50, 4.25 to 12.17 $x10^2$ and $x10^3$ cfu/g, respectively at 0 to 13 months of storage. However total population of bacteria was high as compared to yeast during storage.

Table 3: Bacterial population (Microbial load cfu/g) of Elephant foot Yam Corm pickle during storage

Cv.	BCA-1						IGAM-1					
	Cfu (X 10^{-2}) per ml product			Cfu (X 10^{-3}) per ml product			Cfu (X 10^{-2}) per ml product			Cfu (X 10^{-3}) per ml product		
Month/year	**2011-12**	**2012-13**	**Pooled**	**2011-12**	**2012-13**	**Pooled**	**2011-12**	**2012-13**	**Pooled**	**2011-12**	**2012-13**	**Pooled**
0	61.33	59.50	60.42	46.00	49.67	47.83	78.00	103.67	90.83	43.50	49.00	46.25
1	56.67	54.50	55.58	46.00	43.00	44.50	74.50	98.00	86.25	40.00	46.00	43.00
2	53.00	53.00	53.00	42.33	42.67	42.50	73.50	91.00	82.25	36.50	42.33	39.42
3	49.33	49.50	49.42	39.00	37.33	38.17	69.50	81.00	75.25	35.50	39.00	37.25
4	48.67	43.50	46.08	36.33	35.33	35.83	63.50	80.67	72.08	31.50	35.00	33.25
5	43.00	43.50	43.25	34.67	30.67	32.67	63.50	75.33	69.42	28.50	32.33	30.42
6	40.67	36.50	38.58	30.67	28.67	29.67	57.00	72.33	64.67	24.00	29.67	26.83
7	36.33	35.50	35.92	28.67	24.00	26.33	54.50	68.67	61.58	20.50	24.67	22.58
8	33.67	33.50	33.58	21.67	22.67	22.17	49.50	61.67	55.58	18.50	23.67	21.08
9	29.67	26.50	28.08	20.67	19.67	20.17	47.50	59.33	53.42	15.50	22.33	18.92
10	24.67	25.50	25.08	17.67	15.33	16.50	41.00	55.00	48.00	11.50	19.00	15.25
11	22.00	23.50	22.75	14.67	13.67	14.17	39.50	51.00	45.25	10.50	18.00	14.25
12	18.33	23.50	20.92	10.00	11.33	10.67	33.00	48.00	40.50	9.00	18.67	13.83
13	15.34	21.00	18.17	8.67	9.67	9.17	24.00	47.00	35.50	6.00	18.33	12.17
Mean	38.05	37.79	37.92	28.36	27.40	27.88	54.89	70.90	62.90	23.64	29.86	26.75
MY	Y=60.885 -3.513M R^2=0.997	Y=57.657 -3.057M R^2=0.976		Y=48.342 -3.074M R^2=0.991	Y=47.268- 3.056 R^2=0.99		Y=80.443 -3.931 R^2=0.982	Y=99.191 -4.352 R^2=0.979		Y=42.843 -2.954 R^2=0.992	Y=46.323 -2.533 R^2=0.95	

Yeast grown on yeast extract dextrose medium incubated at 28 ^{O}C for 2 days

Table 4: Yeast population (Microbial load cfu/g) of Elephant foot Yam Corm pickle during storage

cv.	BCA-1						IGAM-1					
	Cfu (X 10^{-2}) per ml product			Cfu (X 10^{-3}) per ml product			Cfu (X 10^{-2}) per ml product			Cfu (X 10^{-3}) per ml product		
Month/year	**2011-12**	**2012-13**	**Pooled**	**2011-12**	**2012-13**	**Pooled**	**2011-12**	**2012-13**	**Pooled**	**2011-12**	**2012-13**	**Pooled**
0	18.00	15.67	16.83	5.33	7.67	6.50	20.00	19.33	19.67	7.33	8.33	7.83
1	17.00	14.00	15.50	3.67	7.00	5.33	18.67	18.00	18.33	5.67	7.33	6.50
2	15.33	11.67	13.50	2.67	5.67	4.17	17.33	16.67	17.00	4.00	5.67	4.83
3	14.33	11.33	12.83	3.00	4.00	3.50	16.33	15.33	15.83	3.00	3.33	3.17
4	12.33	9.33	10.83	2.33	2.67	2.50	15.00	14.00	14.50	1.67	2.67	2.17
5	10.67	7.00	8.83	1.33	2.00	1.67	12.67	12.33	12.50	1.33	1.33	1.33
6	9.00	5.33	7.17	1.00	1.67	1.33	11.33	11.00	11.17	1.33	0.33	0.83
7	5.67	4.00	4.83	0.67	1.33	1.00	10.67	8.33	9.50	1.00	0.33	0.67
8	3.67	2.00	2.83	0.33	0.67	0.50	9.00	5.33	7.17	0.33	0.33	0.33
9	1.00	1.33	1.17	0.00	0.33	0.17	6.67	3.67	5.17	0.00	0.00	0.00
10	0.00	0.33	0.17	0.00	0.00	0.00	4.33	0.67	2.50	0.00	0.00	0.00
11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.17	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	7.64	5.86	6.75	1.45	2.36	1.90	10.14	8.93	9.54	1.83	2.12	1.98
MY	Y=18.218 -1.627 R^2=0.957	Y=14.327 -1.31 R^2=0.947		Y=3.876- 0.373 R^2=0.849	Y=6.240- 0.597 R^2=0.860		Y=20.981- 1.667 R^2=0.977	Y=19.98 9-1.702 R^2=0.975		Y=5.066- 0.497 R^2=0.790	Y=6.112- 0.615 R^2=0.762	

Bacteria grown on nutrient agar medium incubated for 4 days at 34 ^{O}C

Changes in yeast population during storage

The bacterial colonies of elephant foot yam corm pickle was found in decreasing trend with increase in storage duration (Table 4) and the observed value came down from 16.83 to 1.17, 6.50 to 0.17 in cultivar BCA-1 at $x10^{-2}$ and $x10^{-3}$ cfu/g, respectively at 0 to 9 months of storage, while in cultivar IGAM-1, it was 19.67 to 7.17, 7.83 to 0.33 $x10^{-2}$ and $x10^{-3}$cfu/g, respectively at 0 to 8 months of storage, but after 13 month of storage yeast population at $x10^{-2}$ and $x10^{-3}$ was found 0.00 cfu/g. However total population of yeast was low as compared to bacteria during storage.

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