Bacteriology quality of traditionally processed peanut butter sold in Port Harcourt metropolis, Rivers State, Nigeria

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ABSTRACT: The assessment of the quality and safety of food is important in human health. This study evaluated the bacteriology quality of traditionally processed peanut butter sold in Port Harcourt metropolis, Rivers State, Nigeria. A total of four samples of processed and packaged (plastic and nylon) peanut butter were purchased from two local markets (Mile 1 and Mile 3 markets) and analyzed for their bacteriological quality. Eighteen isolates belonging to seven genera of bacteria were isolated from the samples. These include Staphylococcus sp., Escherichia coli, Salmonella sp., Bacillus sp., Serratia sp., Proteus sp. and Micrococcus sp. Staphylococcus sp. and Escherichia coli had equal and highest percentage occurrence of 22.2%. This was followed by Salmonella and Bacillus species (16.7%), Serratia sp. was (11.1%) while Proteus and Micrococcus species had the lowest percentage occurrence (5.6%). The total viable counts ranged from 3.5 x 10^2 cfu/g – 2.3 x 10^3 cfu/g. The Staphylococcus count ranged from 1.2 x 10^2 cfu/g - 2.1 x 10^3 cfu/g, Bacillus count ranged from 1.5 x 10^2 cfu/g - 2.5 x 10^3 cfu/g and the total coliform count 2.0 x 10^2 cfu/g - 1.75 x 10^3 cfu/g. A total of 12 bacteria isolate were detected from mile1 peanut butter samples (MIN and MIP) and a total of 6 bacteria isolates were also recovered in mile 3 peanut butter samples (M3N and M3P). Whereas, Bacillus sp. Proteus sp. Staphylococcus sp. Salmonella sp. Escherichia coli, and Serratia sp, were isolated from MIP samples, Proteus sp and Staphylococcus sp were not isolated from MIP samples. Bacillus sp and Escherichia coli, only was isolated from M3N while Staphylococcus and Micrococcus sp. were isolated from M3P samples. Also, MIP had more gram negative bacteria (GNB) compared to other samples while the numbers of gram positive bacteria (GPB) isolates were equal in the MIP samples. This study showed that the bacteriological quality of traditionally processed peanut butter creates a potential danger with regard to public health. Therefore, there is need for systematic and universally applicable approach to food safety control. The substantial presence of pathogenic bacteria in retailed peanut butter samples indicates the need for appropriate hygienic handling of the product from the raw materials through processing stages to storages and/or retailing and to protect indigenous consumers visitor exposed to consumption of such peanut butter from potential health hazards.

Keywords: Bacteriological quality, Staphylococcus sp., Escherichia coli, peanut butter, Salmonella sp., Bacillus sp., Serratia sp., Proteus sp., Micrococcus sp.

1. INTRODUCTION

Peanut (Groundnut) (Arachis Hypogaea L.) Occupies an important position in the developing countries, the major groundnut producing countries are India, China and the United States. It was introduced in to Nigeria in the 16th century and it has been estimates that about 1.4 million hectare is cultivated for groundnut in Nigeria. Peanut butter is a food paste made primarily from ground dry roasted peanut (groundnut) and is popular in the Philippines, North America, the Netherlands and the United Kingdom. It is mainly used as a sandwich spread, sometimes in combination (peanut butter and jelly sandwich). Peanut butter has been frequently associated with food illness in which initial contamination is traceable to food handlers. Numerous epidemiological reports and studies have implicated foods of ready to eat origin as the major vehicles associated with illness caused by food-borne pathogens. Person to person transmission has also been described (Sokari, 1991). Escherichia coli, Staphylococcus aureus, Yesinia enter-colititia, Salmonella sp, Yeasts and moulds have been used to assess the microbiological safety and sanitation conditions during processing and keeping quality of peanut butter product (Consumer report, 2009).

In Nigeria, peanut butter is produced traditionally on a small scale and as such has been given little or no attention on the microbiological quality and safety of traditionally processed Nigerian peanut butter is lacking in literature. The aim of the present study was to evaluate the bacteriological quality of Nigerian traditionally processed peanut butter.
processed and packaged (nylon and plastic) peanut butter sold in Port Harcourt metropolis.

2. MATERIALS AND METHODS

2.1. SOURCE OF SAMPLES

Traditionally processed and packaged (nylon and plastic) samples of peanut butter used for this study were purchased from two local markets (mile 1 and mile 3 markets) in Port Harcourt, Rivers State, Nigeria. Samples were collected and transported in previously alcohol-sterilized food flasks and analyzed within two hours of arrival at the microbiology laboratory of University Port Harcourt, Nigeria.

2.2. MICROBIOLOGICAL ANALYSIS

For the purpose of this study, four forms of samples were used, mile 1 nylon sample (MIN), mile 1 plastic sample (MIP), mile 3 nylon sample (M3N), mile 3 plastic samples (M3P). MIN and M3N are traditionally processed peanut butters, wrapped in nylon and procure from retail points from mile 1 and mile 3 markets respectively whereas, MIP and M3P are peanut butters packaged in plastic containers from the same location respectively.

2.3. SAMPLE PREPARATION

A 25g representative sample was removed aseptically from the samples (MIN, MIP, M3N and M3P) in to a sterile stomacher bag and 225ml 0.1% peptone water (ph 7.0) was added and blended for 2 minutes using a stomacher laboratory blender 400 (Seward, London) to obtain a 1:10 dilution. Further 10-fold dilutions were prepared using the same diluents.

2.4. ENUMERATION OF MICROBIAL POPULATION

2.4.1. Total Viable Aerobic Counts

Appropriate dilutions of the homogenate samples (MIN, MIP, M3N and M3P) were made and aerobic plate counts determined using the pour plate method (1.0ml) on nutrient agar (Unipath Ltd, Basingstoke, UK). Plates (in duplicate) were incubated at 37°C for 24 hours. Representative typical colonies from well-isolated plates showing 30-300 colonies (Efiuvwewere and Amadi, 1992) were picked at random, sub-cultural on selective growth media for purification, stored in slopes at refrigeration temperature and used for morphological and biochemical tests.

2.4.2. Enumeration of Total Coli forms and Escherichia coli

Coliform contamination level (CCL) was used as an index of sanitary quality of peanut butter. A liquid media repair method (Speck, 1984) was employed for enumeration of sub-lethally injured coli-forms. Appropriate dilutions of the homogenate samples were inoculated into tubes of lactose broth. The tubes were incubated at 30°C for 1 hour to allow metabolic recovery of the coli-forms; Inoculums from the tubes were then spread-plated onto plates of MacConkey agar in duplicates and incubates at 37°C for 24 hours. For enumeration of Escherichia coli, inoculums from tubes of appropriate dilutions were spread-plated onto plates of Eosin Ethylene Blue agar in duplicate (Oxide CM 69) and incubated at 37°C for 24 hours.

2.4.3. Enumeration of Salmonella and Shigella sp

A liquid media repair method (Speck, 1984) was adopted for stressed Salmonellas and Shigella. Appropriate dilution of the homogenate samples were inoculated into tubes of tetrathionate broth (culture A.), incubated at 42-43°C for 24 hours and tubes of serenity (culture B), incubated at 37°C for 24 hours. After 24 hours incubation in tetrathionate broth (culture A) and selenite broth (culture B), samples (0.1ml) of each culture were then spread-plated onto Bismuth sulphite agar plus Brilliant green after 48 hours incubation at 37°C.

2.4.4. Enumeration of Bacillus sp

Appropriate dilutions were heat-shocked in water bath at 80°C for 10 minutes, then 0.1ml of the dilutions were spread-plated on Mussel agar (Bacillus cereus selective agar) supplemented with polymyxin-egg Yolk-mannitol-bromothymol blue agar (PEMBA) (Unipath Ltd.). The plates were incubated at 37°C for 24 hours (Speck, 1984).

2.4.5. Enumeration of Staphylococci

A liquid repair method (Speck, 1984) was employed for the enumeration of stressed Staphylococci. Appropriate dilutions were made in nutrient broth and then incubated for 24 hours at 37°C. Aliquots (0.1ml) of dilutions were spread-plated onto plates of mannitol salt agar in duplicates and incubated for 24-48 hours at 37°C. The plates were examined for Staphylococcus aureus colonies.

2.5. IDENTIFICATION OF BACTERIAL ISOLATES

The morphological tests included Gram-staining and motility while biochemical characteristics of the bacteria isolates were based on in dole, citrate utilization, cataloese, methyl red, coagulate, starch hydrolysis, sugar fermentation Voges Proskauer and production of hydrogen sulphide, following these tests, the isolates were identified.
3. RESULTS ANALYSIS

3.1. BACTERIOLOGICAL QUALITY OF PEANUT BUTTER

A total of four samples of peanut butter from two local markets were analyzed to assess their bacteriological quality. The total viable counts ranged from $3.5 \times 10^2$ cfu/g – $2.3 \times 10^3$ cfu/g. The *Staphylococcus* count ranged from $1.2 \times 10^2$ cfu/g - $2.1 \times 10^3$ cfu/g, *Bacillus* count ranged from $1.5 \times 10^2$cfu/g - $2.5 \times 10^3$ cfu/g and the total coliform count $2.0 \times 10^2$ cfu/g - $1.75 \times 10^3$ cfu/g (Table 1).

<table>
<thead>
<tr>
<th>Samples</th>
<th>TVC (cfu/g)</th>
<th><em>Staphylococcus</em> count (cfu/g)</th>
<th>Total coliform count (cfu/g)</th>
<th><em>Salmonella-Shigella</em> count (cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP</td>
<td>$3.5 \times 10^2$</td>
<td>$2.6 \times 10^2$</td>
<td>$2.5 \times 10^2$</td>
<td>$2.75 \times 10^2$</td>
</tr>
<tr>
<td>M3P</td>
<td>$3.8 \times 10^2$</td>
<td>$1.2 \times 10^3$</td>
<td>$1.5 \times 10^2$</td>
<td>$2.0 \times 10^2$</td>
</tr>
<tr>
<td>MIN</td>
<td>$1.5 \times 10^3$</td>
<td>$2.0 \times 10^3$</td>
<td>$1.2 \times 10^3$</td>
<td>$1.75 \times 10^3$</td>
</tr>
<tr>
<td>M3N</td>
<td>$2.3 \times 10^3$</td>
<td>$2.1 \times 10^3$</td>
<td>$2.5 \times 10^3$</td>
<td>$1.70 \times 10^3$</td>
</tr>
</tbody>
</table>

Key: MIN = mile 1 Nylon sample; MIP = mile 1 Plastic sample; M3N = mile 3 Nylon Sample; and M3P = mile 3 Plastic sample

3.2. IDENTIFICATION OF ISOLATES FROM PEANUT BUTTER SAMPLES

On the whole, 18 isolates belonging to seven genera were identified (Table 2). The bacteria genera were *Proteus*, *Serratia*, *Micrococcus*, *Bacillus*, *Staphylococcus*, *Salmonella* and *Escherichia* species. A total of 12 bacteria isolate were detected from mile1 peanut butter samples (MIN and MIP) and a total of 6 bacteria isolates were also recovered in mile 3 peanut butter samples (M3N and M3P) (Table 2). Whereas, *Bacillus* sp, *Proteus* sp, *Staphylococcus* sp, *Salmonella* sp, *Escherichia coli*, and *Serratia* sp were isolated from MIP samples, *Proteus* sp and *Staphylococcus* sp were not isolated from MIP samples. *Bacillus* sp and *Escherichia coli*, only was isolated from M3N while *Staphylococcus* and *Micrococcus* sp were isolated from M3P samples. Also, MIP had more gram negative bacteria (GNB) compared to other samples while the numbers of gram positive bacteria (GPB) isolates were equal in the MIP samples (Table 2).

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Present or absent in samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1N</td>
</tr>
<tr>
<td><em>Bacillus</em> sp.</td>
<td>+</td>
</tr>
<tr>
<td><em>Proteus</em> sp.</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus</em> sp.</td>
<td>-</td>
</tr>
<tr>
<td><em>Micrococcus</em> sp.</td>
<td>-</td>
</tr>
<tr>
<td><em>Serratia</em> sp.</td>
<td>+</td>
</tr>
<tr>
<td><em>Salmonella</em> sp.</td>
<td>+</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>+</td>
</tr>
</tbody>
</table>

Key: MIN = mile 1 Nylon sample; MIP = mile 1 Plastic sample; M3N= mile 3 Nylon Sample; and M3P = mile 3 Plastic sample; + = present; - = absent.

3.3. PERCENTAGE OCCURRENCE OF BACTERIAL ISOLATES

The percentage frequency of occurrence of bacterial isolates is shown in Table 3. Of the seven genera of bacteria identified *Staphylococcus* sp and *Escherichia coli* had the highest percentage of occurrence (22.2% each). This was followed by *Bacillus* sp and *Salmonella* sp which had 16.7% occurrence each. The frequency of occurrence of *Serratia* sp was 11.1% while *Proteus* and *Micrococcus* species had equal occurrence, 5.6% each. Other details are shown in Table 3.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Number (%)</th>
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<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td><em>Bacillus</em> sp.</td>
<td>3(16.7)</td>
</tr>
<tr>
<td><em>Proteus</em> sp.</td>
<td>1(5.6)</td>
</tr>
<tr>
<td><em>Staphylococcus</em> sp.</td>
<td>4(22.2)</td>
</tr>
<tr>
<td><em>Micrococcus</em> sp.</td>
<td>1(5.6)</td>
</tr>
</tbody>
</table>
from mile 1 peanut butter samples and sp, Staphylococcus markets (Mile 1 and mile 3). The results revealed that processed peanut butter at retail points in two local evaluated the bacteriological quality of traditionally materials (Newsome, 1988). The present study hygiene during processing and/or poor quality raw materials (Okonko et al., 2008a,b,c, 2009a,b) and Adebayo-Tayo et al. (2012a). These organisms were also reported in a previous study on ready-to-eat seafood and palms of food handlers by these organisms was also reported in a previous study (Okonko et al., 2009a,b). The presence of these organisms over others are dependent on the environment (Okonko et al., 2009a,b) and Adebayo-Tayo et al., 2012c).

The presence of *Escherichia coli*, *Proteus* sp, and *Salmonella* sp in peanut butter samples indicates the possibility of a microbial hazard and fecal contamination. Coliforms are considered as normal flora of the intestinal tract of humans and animals. They have been used as indicator organisms for bacteriological quality of food and water. *Escherichia coli* and *Salmonella* sp have been used to assess microbiological safety, sanitization condition during processing and keeping quality of ready-to-eat food (Owhe-Ureghe et al., 2003). The isolation of these organisms in peanut butter is in line with the findings of Adesiyun and Balbrishing (1996); these workers reported that *Escherichia coli* and *Salmonella* sp are among the most important food borne bacteria pathogens in ready to eat food. In the pathogenesis of salmonella infection, it is known that the main route of entry into the host is the mouth (Sokari, 1991). *Escherichia coli* cause dysentery (Nester et al., 1995; Adebayo-Tayo et al., 2012c).

The presence of the most frequently isolated index of food and water quality and indicators of faecal contamination such as *E. coli* reported in this study is an indication of faecal contamination of the peanuts butter samples used for this study and unhygienic handling of the products right from the source or contamination of the fishes itself during harvesting, handling and storage and this might have adverse effect on the health of the consumers (Okonko et al., 2008a,b,c, 2009a,b; Adebayo-Tayo et al., 2012a). Selected strains can cause a wide variety of infections in hospitals and community setting (Donnenberg, 2005; Adebayo-Tayo et al., 2012a). *Escherichia coli* is commonly used as surrogate indicator, its presence in food generally indicate direct and indirect fecal contamination (Clarence et al., 2009; Adebayo-Tayo et al., 2012a). Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programs (Ternhag et al., 2008; Adebayo-Tayo et al., 2012a).

*Bacillus* which is known to be one of the highest occurring bacterial isolate causes toxin-mediated disease rather than an infection (Adebayo-Tayo et al., 2012b). *Bacillus* sp, is a normal inhabitant of the soil and a poisoning organism associated with animals. This is however, because of the survival advantage which the spores have in air and in other harsh condition. Contamination could be from the water and materials used in processing the peanut butter traditionally. The enterotoxins produced by this organism are stable at pH 8-10. Sokari (1991) reported the isolation of *Bacillus* sp, in ready-eat-food in Nigeria. This organism is capable of surviving very harsh conditions including roasting temperature to which the peanut were subjected during processing. Spore-forming bacteria are usually associated with spoilage of heat-treated foods because their spores can survive high processing temperatures (Doyle, 2007; Adebayo-Tayo et al., 2012b). These Gram-positive bacteria may be strict anaerobes or facultative (capable of growth with or without oxygen) (Doyle, 2007). Other thermophiles (*Bacillus* and *Geobacillus* spp.) cause a flat sour spoilage of high or low pH canned foods with little or no gas production, and one species causes ropiness in bread held at high ambient temperatures (Pepe et al., 2003; Doyle, 2007; Adebayo-Tayo et al., 2012b). Mesophilic anaerobes (*Bacillus* spp.), growing at ambient temperatures, cause several types of spoilage of vegetables (Chang and Kang, 2004; Doyle, 2007). Psychrotolerant spore-formers produce gas and

<table>
<thead>
<tr>
<th><strong>Serratia sp.</strong></th>
<th>2(11.1)</th>
<th>1(50.0)</th>
<th>1(50.0)</th>
<th>0(0.0)</th>
<th>0(0.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salmonella sp.</strong></td>
<td>3(16.7)</td>
<td>1(33.3)</td>
<td>2(66.7)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>4(22.2)</td>
<td>1(25.0)</td>
<td>1(25.0)</td>
<td>2(50.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>18(100.0)</td>
<td>4(22.2)</td>
<td>8(44.4)</td>
<td>3(16.7)</td>
<td>3(16.7)</td>
</tr>
</tbody>
</table>

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### 4. DISCUSSION

The assessment of the quality and safety of food is important in human health. A high bacteria colony alone dose not make food unsafe but it dose suggest non-hygienic handing, poor storage, inadequate general hygiene during processing and/or poor quality raw materials (Newsome, 1988). The present study evaluated the bacteriological quality of traditionally processed peanut butter at retail points in two local markets (Mile 1 and mile 3). The results revealed that *Staphylococcus* sp, *Salmonella* sp, *Serratia* sp, *Escherichia coli* and *Micrococcus* sp, were isolated from mile 1 peanut butter samples and *Micrococcus* sp, *Staphylococcus* sp, *Escherichia coli* and *Bacillus* sp, were also isolated from mile 3 peanut butter samples. The prevalence and degree of occurrence of one or two of these organisms over others are dependent on the environment (Okonko et al., 2009a,b). The presence of these organisms was also reported in a previous study on ready-to-eat seafood and palms of food handlers by Okonko et al. (2008a,b,c, 2009a,b) and Adebayo-Tayo et al. (2012a).

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sickly odors in chilled meats and brine-cured hams (Clostridium spp.) while others produce off-odors and gas in vacuum-packed, chilled foods and milk (Bacillus spp.) (Doyle, 2007; Adebayo-Tayo et al., 2012b).

The occurrence of Staphylococcus aureus in peanut butter samples may be a reflection of repeated hand contact with these foods at the point of sale. In addition, temperatures of 26-38°C are often encountered in the retail points from which the samples were purchased, hence, growth of this organism may have been favoured. Outbreaks of staphylococcal food poisoning have been reported to occur as the result of contamination of precooked food, often through unsanitary holding and food at temperatures that allow the growth and toxin production (Newsome, 1988, Neihart et al., 1988; Synder and Poland, 1991). The observed high percentage of occurrence of Staphylococcus sp. in the peanut butter samples may be attributed partly to post-processing contamination from the variety of customers who patronize these retailers (Noble et al., 1987). Additionally, this may result from the washing of cookery, grinding machines and other utensils. The sanitary conditions of the environment of these markets may also lead to contamination of food and food products. The presence of these microbes is an indication of possible contamination resulting from the use of well water, which is mostly used in local food processing industries are not free from microbial contamination (Potter, 1983; Adebayo-Tayo et al., 2009, 2012c). S. aureus known for production of heat stable enterotoxin (Stewart, 1974) and potentials for multiple antibiotic resistances when they get into the living tissue (Foster, 1996; Allen and Cowan, 1997; Okuma et al., 2002; Scott, 2002; Klein et al., 2007; Adebayo-Tayo et al., 2012c) makes the product of immense epidemiological danger (Adebayo-Tayo et al., 2009, 2012c).

The microbial contamination of ready-eat foods could be closely related to the method of preparation and handing. As would be expected, heat-stressed microorganisms that survived roasting was probably capable of growing if sample were not conserved under appropriate temperature conditions (Sokari, 1991; Devriese et al., 1986). International microbiological standards recommend limits of bacterial contamination in the range of 10-10^5 cfu/g for total aerobic plate count (ICMSF, 1986). Since large numbers, typically > 10^6 cfu/g, are required for the production of enough toxins to cause illness, contamination is necessary but is not alone sufficient for an out break to occur (Adams and Moss, 1999). Hood et al. (1983) found that fecal coliform levels were above the recommended wholesale level suggested by the National Shellfish Sanitation Program (less than or equal to 230/100 g). This is in agreement with earlier report by Agbu et al. (1998) in Kastina in terms of high viable counts of coliform density in the water ecosystem. In particular, holding the product for sale at temperature and time that allow the organism to grow to hazardous levels could be risky. It is an acknowledged fact that unsold samples are usually presented for sale the next day perhaps with gentle heat treatment. Such unwholesome practices could result in higher levels of toxins due to build-up on subsequent days. Staphylococcus aureus strains demonstrate elevated thermal resistance, which precludes inactivation by current culinary heating techniques (Synder and Poland, 1991: Acco et al., 2003).

The isolation of Salmonella spp. and S. aureus in this study is of practical impact. It shows that most of the seafood products might have been contaminated from source (Adebayo-Tayo et al., 2012a). It is an evidence of poor sanitary conditions. E. coli and S. aureus are normal flora in human and animals, their presence in foods are indications of excessive human handling (Clarence et al., 2009; Adebayo-Tayo et al., 2012a). Escherichia coli is implicated in newborn meningitis and infantile diarrhea, Salmonella paratyphi is the causative agent of paratyphoid fever in humans, who are the only reservoir of this organism (Nester et al., 1995; Adebayo-Tayo et al., 2006, 2012c). Enterococcus sp. has been implicated in human infections like pharyngitis, scarlet fever and pneumonia (Adebayo-Tayo et al., 2009, 2012c). Whatever the cause of contamination, appropriate control measures should be applied from the raw materials through processing to packaging and/ or storage, this will certainly reduce contamination to a very safe level. Attractive packaging and hygienic display of processed peanut butter in protected cabinets in the markets will also minimize potential hazards.

5. CONCLUSION

This study result that the bacteriological quality of traditionally processed peanut butter creates a potential danger with regard to public health. Therefore, there is need for systematic and universally applicable approach to food safety control. The isolation of organisms like Staphylococcus sp, Escherichia coli, Bacillus sp, and Salmonella sp, which are of public health significance in traditionally processed peanut butter sample do not only pose health hazards to indigenous consumers but also to visitors exposed to consumption of such enforcing proper sanitation and monitoring of products by relevant regulatory bodies. Education of the local producers by regulatory bodies such as NAFDAC on the use of the hazard analysis critical point (HACCP) concept and quantities risk assessment (QRA) from the raw
material through processing stages to storage and/or retailing is advocated in view of possible microbial hazards in traditionally processed peanut butter.

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