

Microflora and Processing method of *adjuevan*, an Ivorian fermented fish condiment

*Koffi-Nevry Rose*¹, *Alloue-Boraud W.A. Mireille*¹, *Koussemon Marina*¹, and *Pehuke Jean Gabin*¹⁻²

¹Biotechnology and Food microbiology laboratory, Department of Food Science and Technology,
University of Abobo-Adjamé, Abidjan, 02 BP 801, Abidjan 02, Côte d'Ivoire

²Faculty of Pharmaceutical Sciences,
University of Cocody, Abidjan, Côte d'Ivoire, BP V 34 Abidjan, Côte d'Ivoire

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ABSTRACT: This study was designed to describe the processing of a traditionally fermented fish named *Adjuevan* in Côte d'Ivoire. We studied the processing techniques, the percentage of salt used and the microflora composition of the *Adjuevan* prepared from *Chloroscombrus chrysurus*. The fermentation process is done in 4 or 7 steps respectively according to the size of fish: gutting, carving, washing, pre-fermentation, salting, fermentation and drying. *Adjuevan* is obtained with an average of 35.09% marine salt. A total of 72 samples of *adjuevan* were analysed. The Mean values of pH were 5.38 ± 0.27 . The microbial load of *adjuevan* samples revealed a range of $2.1 \cdot 10^5$ - $2.8 \cdot 10^5$ cfu/g for the total count. The predominant microorganisms were lactic acid bacteria ($1.3 \cdot 10^3$ - $1.2 \cdot 10^4$ cfu/g) followed by *Bacillus* ($4.7 \cdot 10^2$ - $3.3 \cdot 10^3$ cfu/g), with a frequency of isolation of 100%. *Pseudomonas* and *E. coli* were not detected in the *adjuevan* samples analysed but *Staphylococcus* was isolated with a prevalence of 60.65%. These contaminations can represent a risk for the consumers and also for the public health. It comes out that *adjuevan* is found not to be safe microbiologically.

KEYWORDS: Adjuevan, fish, fermentation process, salt, microorganisms.

1 INTRODUCTION

Fish is an important food in human diet. Artisanal fish processing remains the predominant and most important method of fish preservation in Africa. The principal methods are smoking, sun-drying, salting, fermentation, grilling and frying [1],[2]. These processes may either be used alone or combined in order to achieve the desired product. Salting and sun-drying are often combined to get a well preserved product. The final product is distinguished by peculiar qualities such as aroma, flavour and colour according to the consumer's preference [1], [3], [4].

The choice of fish processing method is greatly influenced by socio-economic factors. For instance, in Côte d'Ivoire, due to the availability of fuel wood and solar salt, a lot of fish is smoked, fermented or salted and dried. These cured fishery products are the most popular form in which fish is generally consumed in Côte d'Ivoire. Fish is usually consumed after being cooked in a sauce, braised, roasted, fried or cured [5]. Different types of cured fishery products are normally used to prepare the family meal in order to achieve a desirable flavour in the sauce which is eaten with the traditional starchy staples such as cassava, plantain, yam or rice [4]. Fermentation is one method of fish curing in which the development of a distinctive flavour in the final product is the principal objective [6], [7], [8]. Therefore, this product is mainly used as a condiment in the preparation of traditional sauces [9], [10].

In Southeast Asia the fermentation process often lasts several months and the final product is usually a paste, a sauce (liquid) [11], [12]. Fish sauce is called "nam pla" in Thailand, "nuoc mam" in Vietnam, "patis" in the Philippines, "shottsuru" in Japan, "ngan-pya-ye" in Burma, "tuk Trey" in Cambodia, "nam pa" in Laos, and "yeesui" in China. The name basically means "fish water" [1], [13], [14].

In the West African region, and particularly in Côte d'Ivoire however, fish fermentation lasts from a few hours to about two weeks. Fermented fish is called in Ghana "momoni", "lanhouin" in Bénin, etc. [9], [10]. Fermented fishery products in Africa may either be soft with a high moisture content, semi-dry or very dry. Some products are also heavily salted and dried whilst others are dried without any salting.

In Côte d'Ivoire, fermentation practices are also used and fermented fish conduct to a fermented condiment called "Adjuevan" [8],[4]. It is a fermented condiment used as flavouring agent in soup and sauces, as an exhauster of the taste, giving a particular flavour and authenticity. *Adjuevan* is produced in presence of high salt concentration. This product is popularly used and appreciated as condiment in many types of flavourings and cuisines for seasoning sauces for the consumption of *foutou* yam, *foutou* plantain, *attiéké*, *placali*, *bêdêcouman*, etc. [15], [3], [16], [17]. When used in sauce, *adjuevan* imparts a distinct aroma and flavour on its own. *Adjuevan* is inexpensive, available everywhere, convenient to all social classes and very popular despite its strong and unpleasant odour.

Despite the fact that *adjuevan* is well known and used by the Ivorian population, very few scientific study of this product was performed. Indeed, to the best of our knowledge, there is no report in the literature on *adjuevan* processing. Thus, the aim of this study is to determine the fermentation process, the percentage of salt used, and to evaluate the microbiological status of *adjuevan*.

2 MATERIAL AND METHODS

2.1 MATERIAL

The study was carried out on *adjuevan*, a fermented fish made in Côte d'Ivoire. The fermented fish were purchased the same day of each experiment from the unique collective fish fermentation processing site, located in Vridi Zimbabwé (Abidjan, Côte d'Ivoire). The salt was obtained from retail outlets in Abidjan.

2.2 METHODS

2.2.1 SAMPLING

Three processing posts (PP) coded PPA, PPB and PPC were randomly chosen among the 9 that were available at the unique collective fish fermentation processing site in Abidjan. Sampling was done every week over a period of 6 months. Samples were collected per processing site at the end of the fermentation process, from a basket containing the fermented fish, one day of a week and the same day of production. A processing site is defined as a space shared by 3 to 5 processors (women); it is equipped with a well, a dryer, and one or several round-shaped concrete tanks, barrels, buckets (for water drawing from the well), rattan baskets or made in bamboo bark. The capacity of a basket ranges from about 2000 to about 3000 fermented fishes according to the size of *adjuevan*. A sample consisted of 3 to 5 fermented fishes (200 to 300 g) from the same basket. A total of 72 samples of *adjuevan* were randomly and aseptically collected at a week interval. Samples were collected into sterile plastic bags (30x40 cm) and stored in icebox filled with ice and transported to the laboratory for analysis.

2.2.2 ADJUEVAN PROCESSING

The process of *Adjuevan* production and the environment of processing were observed using the 9 processing sites with 20 processors (women) at the unique collective production site in Abidjan, located in the periphery of the industrial zone of Vridi, township of Port-Bouet, a commune of the district of Abidjan, on the border line of the Ebrié lagoon. The site and the process observed were then described (fig.1).

2.2.3 DETERMINATION OF ADJUEVAN PH

The pH of the samples was measured with a pH-meter (Hanna Instrument HI 9318) on a mixture of 20g of blended *adjuevan* meat and 80 ml of distilled water.

2.2.4 DETERMINATION OF THE PERCENTAGE OF SALT USED

The percentage of salt used for *adjuevan* production was determined using the following technique: the total mass (M_p) of fish to ferment is determined as well as the initial mass (M_i) of the salt bag where the appropriate quantity of salt for fermentation will be withdrawn. After the withdrawal of the necessary amount of salt, the final mass (M_f) which is the quantity of the remaining salt in the bag is determined. The percentage of salt used during fermentation is obtained following this formula:

$$\% \text{ salt} = (M_i - M_f) \times 100 / M_p$$

M_i = initial salt bag mass (kg)

M_f = final salt bag mass after use (kg)

M_p = quantity of fish used for fermentation (kg)

2.2.5 MICROBIOLOGICAL CHARACTERIZATION OF ADJUEVAN SAMPLES

Each sample of *adjuevan* is cut into pieces and ground in a stomacher into a homogeneous mixture. Twenty five grams of the mixture were suspended in 225 mL of sterile peptone buffer and homogenized again for 30s at a normal speed. The homogenates were used for all the microbiological analyses. Counts were enumerated according to the methods stated in Compendium of Methods for the Examination of Foods [18] and Food and Drug Administration (FDA) [19]. Suitable decimal dilutions were pour-plated on Plate Count Agar (AES Laboratory) for total aerobic count.

Staphylococci spp. were determined on Baird Parker Agar (Oxoid CM 275) at 37°C for 24-48h. Typical black colonies with zones around and atypical black colonies were considered as *Staphylococcus* sp. *Escherichia coli* (*E. coli*) was determined on Violet Red Bile Lactose Agar (Oxoid CM 107) and incubated at 44°C for 24h. Pink-red colonies with precipitation were streaked on Endo Agar (Oxoid CM) and incubated at 37°C for 24-48 h. The isolates were characterized phenotypically using Portoir réduit De Leminor and a positive indole reaction in the Tryptone Water indicated the presence of *E. coli*. We investigated lactic acid bacteria on Man Rogosa and Sharpe Agar (MRS) (Biomérieux) with the pH adjusted to 5.5. *Pseudomonas* sp. were isolated on Pseudomonas Agar (Oxoid CM 559) aerobically at 30°C for 24-48 h. Oxidase (+) colonies were taken into consideration. Bacilli were investigated on Dextrose Tryptone Agar (DTA, Oxoid CM 75) and the plates were incubated for 48 h at 35°C. MRS plates were incubated anaerobically at 30°C for 48 h, while others were incubated at 30°C for 48 h. Selected distinct colonies were enumerated. Duplicate agar plates of between 30 and 300 colonies were counted, and mean counts calculated. The colonies appearing were counted as Colonies Forming Units / g wet weight of sample. All tests were carried out in duplicate.

3 RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 ADJUEVAN PROCESSING

The unique collective site for *adjuevan* production in Abidjan, is an open site of about 3000 m² surrounded by refuse with at least 40 brine barrels and 15 drying grids and leads used by about thirty processors. For the preparation of *adjuevan*, different types of fish can be used, but usually *Chloroscombrus chrysurus*, an atlantic bumper is frequently used. The fishes are gutted followed by carving (larger size) washing, pre-fermentation, salting, fermentation and drying. The plastic bags, barrels and baskets are never washed and the microorganisms inside these materials may serve as starter cultures for spontaneous fermentation.

Two processing techniques exist in Côte d'Ivoire depending on the size of the fish: smaller fish (weight < 600 g) technique and that of larger species of fish (weight > 600 g). Only fish of the same species are fermented together. The mixture of species is proscribed. The end product named *adjuevan* is golden light brown in colour with a semi-dry, firm texture and a characteristic strong smell (fig.1).

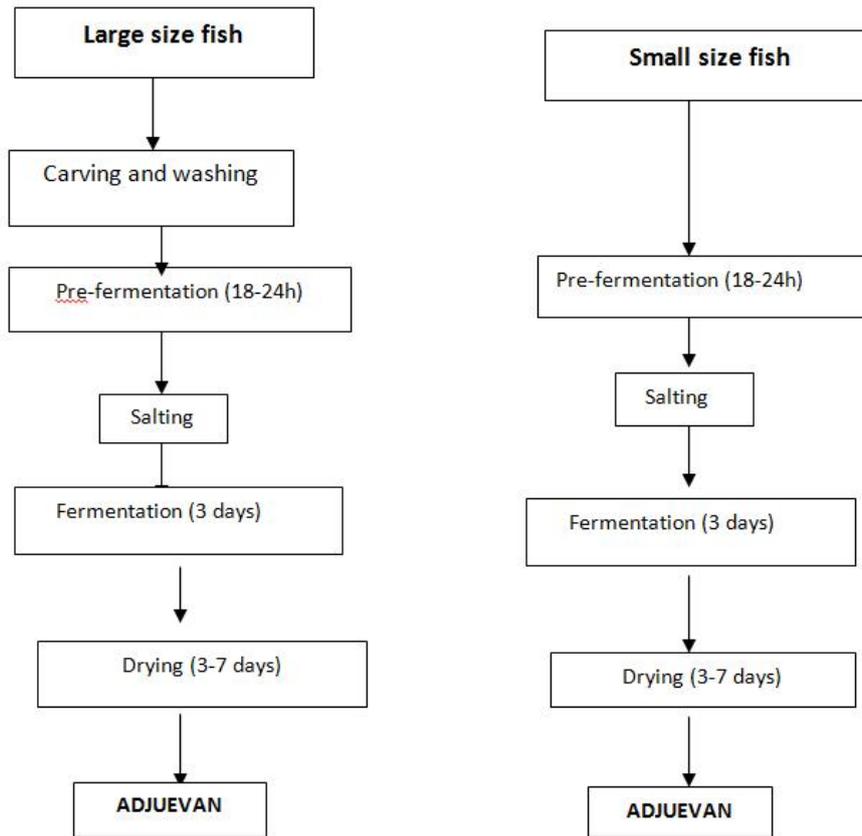


Figure 1: *Adjuevan* processing of large size and small size fish

3.1.2 PROCESS OF LARGE SIZE FISH FERMENTATION

When fish weight is at least 600 g (shark, captain, carp...), fermentation is made by immersion. Fish are first gutted, rinsed, cut into pieces of at least 400 g, rinsed again and then arranged into baskets. The baskets are covered with plastic or jute bags for about 18-24 h. This is the pre-fermentation stage. After the pre-fermentation, the fish are withdrawn from the baskets, placed in concrete fermentation tanks with alternate layers of salt. The tanks are topped with a layer of salt. Then a woven bamboo mat or plastic bags are placed over the fish and weighted down with heavy rocks to keep the fish from floating. After three days of fermentation, the fermented fish are withdrawn from the tank, sun-dried during an average of three days before being marketed as *adjuevan*. However, the fermented fish can remain on the driers until the delivery that takes place within a maximum of seven days. During that period, *adjuevan* is withdrawn from the driers every evening to be dried again the following day.

3.1.3 PROCESS OF SMALL SIZE FISH FERMENTATION

As opposed to the fermentation process of larger fish, the fermentation of the smaller fish (<600 g) as generally that of the Carangidae family (jacks, pompanos, jack mackerels, and scads, leatherjackets, etc.) is not made by immersion.

The whole small fresh fish are not gutted but wrapped directly in bags made of jute, and left to ferment for 12-24 h. that is the pre-fermentation step.

Then, the pre-fermented fish are placed in concrete fermentation tanks with alternate layers of salt as stated for larger size fish, then the following steps are the same as for the larger size fish.

3.1.4 PERCENTAGE OF SALT AND PH OF ADJUEVAN

Depending on the processing post, the percentage of salt used for *adjuevan* production ranged from 30.42 to 44%, while the pH of *adjuevan* ranged from 4.9 to 6.01 (Table I).

Table 1. Percentage of salt used during *adjuevan* production and mean value of pH in *adjuevan* according to processing post (PP)

	I	II	III	Mean values
Mass of salt used (kg)	25.23 ± 20	28.5 ± 11	22.72 ± 10	25.5 ± 14
Mass of fish produced (kg)	83 ± 12	72 ± 6	63 ± 9	72.06 ± 9
Percentage of salt (%)(w/w)	30.42	39.6	44	35.09
pH	6.01 ± 0.3	5.23 ± 0.21	4.9 ± 0,3	5.38 ± 0.27

3.1.5 MICROBIOLOGICAL CHARACTERISTICS OF ADJUEVAN

The microbial load of *adjuevan* samples revealed a range of 2.1×10^5 to 2.8×10^5 cfu/g for the total count, 4.7×10^2 to 3.3×10^3 cfu/g for *Bacillus*, 1.1×10^2 to 4.1×10^3 cfu/g for *Staphylococcus*, 1.3×10^3 to 1.2×10^4 cfu/g for lactic acid bacteria. *Pseudomonas* and *E. coli* were not detected in *adjuevan* (Table 2). Total aerobic count, *Bacillus* and lactic acid bacteria were found in all the samples of the three processing posts with a prevalence of 100%, while *Staphylococcus* spp. prevalence ranged from 58.14 to 62.5% depending on the processing post (Table 3).

Table 2. Mean values of bacterial load (cfu/g) in *adjuevan* according to the processing post (PP)

Microorganisms (cfu/g)	Processing posts			Mean values
	I	II	III	
TAC	$2.8 \times 10^5 \pm 7.1 \times 10^4$	$2.4 \times 10^5 \pm 7 \times 10^4$	$2.1 \times 10^5 \pm 6.8 \times 10^4$	$2.4 \times 10^5 \pm 7 \times 10^4$
<i>Escherichia coli</i>	<1	<1	<1	<1
<i>Staphylococcus</i> sp	$4.1 \times 10^3 \pm 7.1 \times 10^2$	$1.1 \times 10^2 \pm 84$	$2.2 \times 10^3 \pm 1.2 \times 10^3$	$2.1 \times 10^3 \pm 6.6 \times 10^2$
<i>Bacillus</i> sp	$2.8 \times 10^3 \pm 1.3 \times 10^3$	$4.7 \times 10^2 \pm 3.2 \times 10^2$	$3.3 \times 10^3 \pm 3.8 \times 10^2$	$2.2 \times 10^3 \pm 6.3 \times 10^2$
<i>Pseudomonas</i>	<1	<1	<1	<1
LAB	$1.2 \times 10^4 \pm 8.6 \times 10^3$	$1.2 \times 10^4 \pm 1.3 \times 10^3$	$1.3 \times 10^3 \pm 10^3$	$8.4 \times 10^3 \pm 3.6 \times 10^3$

TAC = Total aerobic count ; LAB = Lactic acid bacteria

Table 3. Prevalence (%) of bacteria isolated in *adjuevan* according to processing post

Microorganisms	Processing posts			Mean values
	I	II	III	
Total aerobic count	100	100	100	100
<i>E. coli</i>	00	00	00	00
<i>Staphylococcus</i> sp.	62.5	58.14	61.3	60.65
<i>Bacillus</i> sp.	100	100	100	100
<i>Pseudomonas</i>	00	00	00	00
Lactic acid bacteria	100	100	100	100

3.2 DISCUSSION

Adjuevan is made from small or large fish, but small fish, particularly the Atlantic bumper, *Chloroscombrus chrysurus*, an ecologically important species in the southern Gulf of Côte d'Ivoire, which is seasonally abundant, is used. This small fish (length and width ≤ 20.5 and 8 cm respectively) would otherwise have little value for consumption. Smaller fish are easily dehydrated by the salt and dry readily. Larger varieties of fish, such as captain and shark or carp also make good *adjuevan*, but because they are relatively more expensive due to their value as food fish, they are seldom used in the commercial production of fermented fish. This argument confirms that of [20] and also [4] who reported that fish used for fermentation are usually small, with low commercial value and seasonally abundant.

Larger and smaller fish fermentation processes are almost similar. *Adjuevan* processing is done in 4 or 7 steps according to the size of the fish used: gutting, carving, washing, prefermentation, salting, fermentation and drying. The results of this study are almost similar to those of [10] who indicated that "lanhouin", a fermented fish produced in Benin, is processed in 5 or 6 steps. Gutting reduces the microflora of fish gut; washing cleans residues of viscera and others and carving is done to favour brine spreading. Washing can reduce or eliminate some microflora from digestive tract. However, the development of microorganisms is essential for the final characteristic of product. Consequently, unwholesome or inferior quality small raw fish is often processed into fermented products which are, however, acceptable by traditional quality standards.

The objective of the prefermentation is to obtain the softening of the fish. This step is done in 18-24 h. During this step, the fish undergo a process of tissular degradation due to the activity of microorganisms which leads to a soft product because the fish lose their rigidity (*rigor mortis*) [21]. For larger fish, the prefermentation is realized after gutting, carving and washing while for smaller fish, the prefermentation is done directly after purchase. The *post mortem* degradation processes that take place in the fish muscle gradually modify the initial state of freshness. Tissue degradation is accompanied by drastic myofibrillar proteolysis produced as a consequence of the activation of proteolytic enzymes [22], [23].

Salting in fish processing may either be by dry salting (smaller fish) or wet salting (larger fish). In dry salting, the granular salt is applied directly to the fish either in the gills or on the surface. *Adjuevan* is obtained with an average of 35.09% salt (w/w). In Africa, the percentage of salt for fish fermentation process is different for each country depending on the cost of the salt [1]. In the coastal countries such as Côte d'Ivoire, Ghana and Senegal, marine salt is produced by the natural evaporation of sea water and is readily available and inexpensive (easily accessible). Therefore, fermented fish is heavily salted. This could explain the high usage of salt for *adjuevan* processing. The results obtained in this study are in accordance with those used for fermented fish in European and Asian countries [24] [25], [9], [20]. Our results are also similar to those of [10] who reported the percentage of salt used to process "lanhouin" to be between 20 and 35%. [20] stated that the high concentration of salt was used to maintain the product under adequate microbiological control. Salt and fermentation time are responsible and influence the final product quality. Salting inhibits the environmental microorganisms from the water used for washing or on the working material and the undesirable microorganisms not necessary for fermentation. Salting also dehydrates the fish partially through osmosis. The partial dehydration inhibits the photolytic endogenous enzyme activity responsible for histamine (a toxic compound for human) production [20].

Fermentation for *adjuevan* production lasts 2 to 3 days. Only the microorganisms that are able to develop under high salt concentration will participate to this step. *Adjuevan* is prepared through a spontaneous anaerobic fermentation, commonly initiated by repeated use of the same utensil and reuse of the brine at ambient temperature ($30\pm 2^\circ\text{C}$). The texture of *Adjuevan* was not significantly affected by the fermentation, compared to fermented fish such as Norwegian røkefisks, Swedish surchomings and Vietnam fermented fish [1], [26]. This means that the anaerobic degradation is not advanced and consequently, the fish can be used as a condiment but not eaten as food fish because of the strong smell.

The last step is sun-drying which reduces the water activity in *adjuevan*. This step is also observed during the fabrication of "lanhouin", but to the difference of *adjuevan* which is directly dried after fermentation, "lanhouin" is washed after fermentation before the drying step [10]. Drying is a delicate and hard operation that lasts from 3 to 7 days depending on the season. This step is partly responsible for the quality of *adjuevan*. An insufficient drying can favour not only a quicker microorganisms development during the preservation, but also enzymatic reactions which can negatively modify the organoleptic quality of the contaminated product. During the drying process, fermented fish are exposed to insect infestation and microorganisms contamination, and therefore requires periodic re-drying to maintain a good quality. *Adjuevan* is obtained after this last drying step.

The pH values of the *adjuevan* (below 7) are similar to those of *momoni* and *lanhouin*, fermented fish from Ghana and Bénin respectively [9],[5] and are also in accordance with pH values usually reported on other fermented products. These pH values are due to acid production during the fermentation.

The enumeration of the total aerobic count on samples showed a high microbial population in *adjuevan*. The predominant microorganisms were lactic acid bacteria followed by *Bacillus*. Various authors have reported similar microflora in fermented fish products [24], [27], [9], [8], *Pseudomonas* and *E. coli* were not detected in *adjuevan*. This result shows that *adjuevan* is weakly subjected to faecal contamination and *Pseudomonas* spoilage. This may have been the result of the salt concentration. These observations correlated with the work of [28] who demonstrated that some lactic acid bacteria strains had bactericidal activity on *E. coli*. Bacilli and lactic acid bacteria generally considered as a normal microflora of such products were present in all the samples analyzed with a prevalence of 100%. Lactic acid bacteria could be useful for fermentation.

The presence of *Staphylococcus* sp with a prevalence of 60.65% is significant and shows that there is a need for improved handling and processing procedures of *adjuevan*. These results are in agreement with those of [5] and [29] who indicated the presence of *Staphylococcus* in fermented fish. *Staphylococcus* may play a potential role in fermented fish with production of volatile fatty acids [30]. *S. aureus* are normal flora in human and animal, their presence in foods is an indication of excessive human handling [31]. The concentration of the salt used could not only inhibit the microflora of the fish surface, but also select microorganisms such as *Staphylococcus* and lactic acid bacteria responsible for the fermentation [5].

The reuse of the brine may be a potential source of bacterial contamination to fresh batches of fish. The bacterial load of the brine becomes extremely high. Contamination of fermented fish can come from the processing equipment (tanks, drying

racks, baskets...) which are reused [8]. These contaminations can represent a risk for the consumers and also for public health. However, fermented fish have not been reported to be responsible for infection [3].

4 CONCLUSION

Fermentation is an important method of preservation and helps to salvage fish which would otherwise have been thrown away. It comes out that *adjuevan* is found not to be safe microbiologically. Bacilli and lactic acid bacteria were found in all the samples of *adjuevan*. The sanitary conditions of fermented fish production were generally found to be poor and the processing methods were not standardized. In the light of the observations made, we suggested to the processors to respect hygienic conditions in order to improve the quality of *adjuevan* and enhance the intra-regional trade of fishery products.

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