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Impact of traditional transformation processes on the level of aflatoxin B1 in groundnut seeds sold in northern Togo

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Abstract: **The fight against food contamination by aflatoxins is essential to ensure the food security of populations. Among the control methods currently available, physical control methods such as sorting, washing, roasting have proven their effectiveness in controlling aflatoxins in foods. The objective of this work is to evaluate the influence of groundnut traditional transformation processes on aflatoxin B1 levels in order to propose an effective decontamination method to fight against aflatoxins in households. The samples were taken randomly from the Dapaong market. A total of five (05) samples of groundnut seeds of 10 kg each were taken. The traditional transformation processes such as sorting, roasting, groundnut paste making and groundnut oil extraction were applied on the samples. The results showed that only sorting and roasting are very effective in reducing aflatoxin levels in groundnut seeds. Manual sorting in this study reduced initial aflatoxin levels from 40.12 to 71.09%. Roasting groundnut seeds at a temperature of 160°C and 180°C for 15 minutes in a preheated roaster reduced aflatoxin levels respectively by 60.71 - 83.57% and 58.33 – 94.28%. Indeed, a strong positive correlation (R=0.99) is observed between the percentage reduction of aflatoxins, and the roasting time of groundnuts. The groundnut paste and oil extraction processes caused an increase in the aflatoxin B1 contents of the samples by 4.20% - 59.23% and 8.57% - 52.67% respectively.**

Keywords: **Groundnut; food processing; aflatoxin B1; roasting; manual sorting; Togo; ELISA.**

1. INTRODUCTION

Groundnut (Arachis hypogaea) is the thirteenth crop, the fourth source of edible oil and the third major source of vegetable protein in the world. It is grown on 26.4 million hectares worldwide for a total production of 36.1 million tons and an average productivity of 1.4 tons per hectare (FAOSTAT, 2011). In Africa, the sale of groundnuts is an income-generating activity for populations in both rural and urban areas (Diedhiou *et al.*, 2011). In Togo, groundnuts are one of the most consumed agricultural products; its production increased from 38,903 tons to 47,369 tons between 2014 and 2017 (FAO, 2019). Groundnuts can be eaten raw, boiled or roasted often outside of meals (Ndung'u *et al.*, 2013). It can also be transformed into groundnut paste to be incorporated into sauces, to extract oil or make patties (Perrin, 2015). Groundnut is a legume with high economic and nutritional importance (Eke-Ejiofor *et al*., 2012; Noba *et al*., 2014). Despite its economic and nutritional potential, groundnut is one of the oilseeds most contaminated by toxigenic fungi most often during cultivation, harvesting and during storage (Settaluri, *et al*., 2012; Krnjaja *et al*., 2019; Vabi *et al*., 2020). Indeed, these fungi (*Aspergillus*) secrete a toxin known as aflatoxin (Diakite *et al*., 2017). The contamination of food by aflatoxins can have several harmful consequences such as the alteration of organoleptic properties, the reduction of nutritional qualities, the appearance of diseases (allergies *et* mycoses), the production and accumulation of mycotoxins in food (Moghtet *et al*., 2012; Fofana-Diomande *et al*., 2019). Food contamination by mycotoxins (aflatoxin) affects both natural and processed products (Houmairi et Hicham, 2015). Indeed, a study carried out in Togo by Tedihou *et al*., (2019) on the prevalence of total aflatoxins in groundnut products had revealed the presence of total aflatoxins at levels greater than 900µg/kg in groundnuts, patties, groundnut oil etc. Other studies carried out in Nigeria have also revealed high aflatoxin levels of up to 338.33 µg/kg in roasted groundnut seeds and 53 to 2820 µg/kg in kuli-kuli. Groundnut seeds are likely to contain high doses of aflatoxins and very often these aflatoxin levels are found in foods after processing (Bol *et al*., 2016). The fight against food contamination by aflatoxins is essential to ensure the food security of populations.

Control methods currently available include chemical, biological and physical methods (Atehnkeng *et al*., 2014; Ba *et al*., 2015). Of all these methods, physical methods are the most numerous and are generally based on washing, drying, grinding, manual sorting, mechanical separation or heat treatment (Lizárraga-Paulín *et al*., 2013).

Some food transformation processes such as sorting or roasting have an influence on aflatoxin levels. Xu *et al*., (2017) in their study recorded a 96.7% reduction in aflatoxin B1 after manual sorting of groundnut seeds. According to (Martins *et al*., 2017) roasting groundnut seed at 180°C is also very effective in reducing aflatoxin levels by 83.6%. However, this reduction in aflatoxin levels during roasting depends on the food, time and type of temperature (Emadi *et al*., 2021). The need to find effective ways to prevent contamination or reduce aflatoxin levels in already contaminated groundnut seeds is essential.

The general objective of this study is to contribute to the food security of the Togolese population. More specifically, it was a question of (i) determining the effect of some conventional food processing processes on the levels of aflatoxin B1 contained in groundnut seeds and (ii) proposing effective decontamination methods against aflatoxins in foods made from groundnut seeds.

2. MATERIALS AND METHODS

2.1-Plant material

The plant material used for this study consists of shelled groundnut seeds.

2.2-Sampling

The samples to be analyzed were collected randomly in the Dapaong market. At each retailer, the total quantity of groundnut offered for sale was homogenized in a large basin before taking the sample. In total, five (05) mother samples (EM) of 10 kg each were taken from the dealers (Anyebuno *et al*., 2018). These samples were then wrapped in plastic bags, labeled and transported to the laboratory.

2.3- Determination of the effect of transformation processes on aflatoxin B1 content

The different groundnut processing methods most commonly used by processors in households were selected for this test. These are manual sorting (TM), roasting (TF), processing into groundnut paste (TPA) and groundnut oil extraction (THA). Thus, each mother sample (EM) of 10 kg initially taken from the retailers was homogenized manually in a large basin and divided into five samples of 2 kg each, for a total of twenty-five (25) sub-samples.

For each of the five (05) samples (ECH) from the same mother sample, the transformation processes were applied as follows (Figure 1). It should be noted that the transformation processes such as roasting, transformation into groundnut paste and extraction of groundnut oil were carried out entirely by the groundnut processors according to their usual method.

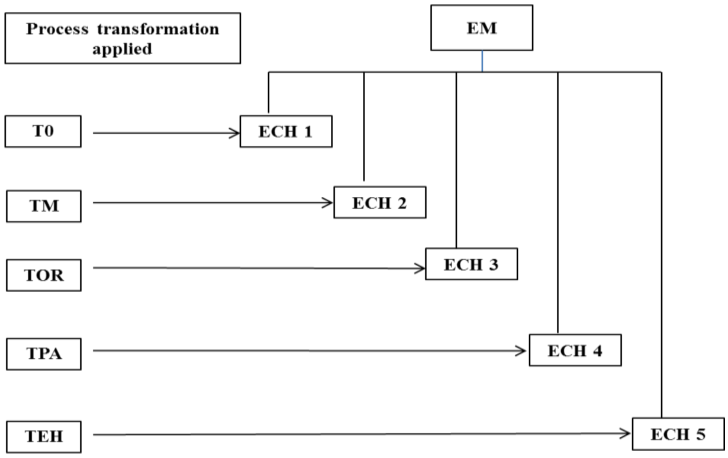

Figure 1: Representative diagram of sample preparation and the various applied transformation processes

T0 (Control): This consists of measuring the levels of aflatoxin B1 (AFB1) in the samples without any prior treatment.

TM (Manual sorting): This consisted of systematically identifying and eliminating all immature, moldy or damaged groundnut seeds before any analysis.

TOR (roasting): This step consisted in boiling the 2 kg of groundnut seeds in a liter of water containing 10 g of salt for 5 min. These seeds were then dried in the sun for eight (8) hours on a table covered with aluminum foil before being roasted for 15 minutes at a temperature of 172°C in a pot containing sea sand previously washed and dried for this purpose.

TPA (groundnut paste): Groundnut seeds were roasted in an aluminum pot at a temperature of 172°C for 15 minutes.

These seeds were left for 10 min at room temperature for cooling and then, the reddish seed coats were removed manually by friction by rubbing the seeds between the palms of the hand followed by winnowing. These seeds, thus cleaned, were ground in a blender for 10 min until a fine paste was obtained.

TEH (oil extraction): Groundnut seeds were roasted and ground into groundnut paste as before. To this paste, hot water was added (90-100°C) at the rate of 100 ml per 1 kg of groundnut paste. This paste was then kneaded on a board placed vertically in a container so as to collect the resulting oil.

2.4- Evaluation of the effect of temperature on the aflatoxins degradation during groundnut seeds roasting

In order to propose a method favoring a better reduction of aflatoxin levels during roasting, the roasting process usually used in households by processors has been modified. To this end, the temperatures of 160°C and 180°C which have been recognized as effective by Martins *et al*. (2017) in reducing aflatoxin levels to more than 80% have been retained for this test. The modification made to the method previously used by the processors consists in preheating the roasting device, so as to reach a sufficiently high temperature (160°C and 180°C) to cause aflatoxin degradation. A laboratory thermometer (0°C – 400°C) was used to measure the evolution of the temperature of the roasting device. A batch of 5 kg of groundnuts divided into two (02) samples of 2.5 kg each were roasted, one at 160°C and the other at 180°C. A sample was taken before the start of roasting to determine the crude aflatoxin (TO) content. During roasting and for each roasting temperature, samples (in duplicate) of 200 g were taken after 5 minutes, 10 minutes and 15 minutes of roasting in order to determine the aflatoxin levels and assess the effectiveness of the method of roasting offered.

2.5- Determination of aflatoxin B1 content

Aflatoxin B1 levels were determined by the ELISA method (Enzyme Linked Immuno Sorbent Assay) according to the protocol provided by the Bio-Shield B1 OP analysis kit, B3396 from Prognosis Biotech. The "Prognosis Data Reader" software was used for the determination of aflatoxin concentrations.

2.6- Statistical analyzes

Data for this study was entered into Excel and R version 4.0.5 software was used for data analysis. The analysis of variance (ANOVA) was performed according to Tukey's test at the 5% threshold.

3. RESULTS AND DISCUSSIONS

In order to highlight the effect of conventional transformation processes on aflatoxin levels, aflatoxin B1 levels were determined. These are initially shelled peanut seeds sold in the markets, then products resulting from the processing of these peanut seeds and finally peanut seeds that have undergone a modification of the process roasting usually used by processors. The results (figure 2) showed that the samples collected directly from the sellers and having undergone no prior treatment (T0) all contain aflatoxin B1 at levels above the maximum limit of 8µg/kg authorized by the European Union. These levels vary from 12.24µg/kg to 17.02µg/kg. The presence of aflatoxins in the samples could be explained by the poor storage conditions which surely favored the growth of aflatoxigenic fungi and the production of aflatoxins in the peanut seeds.

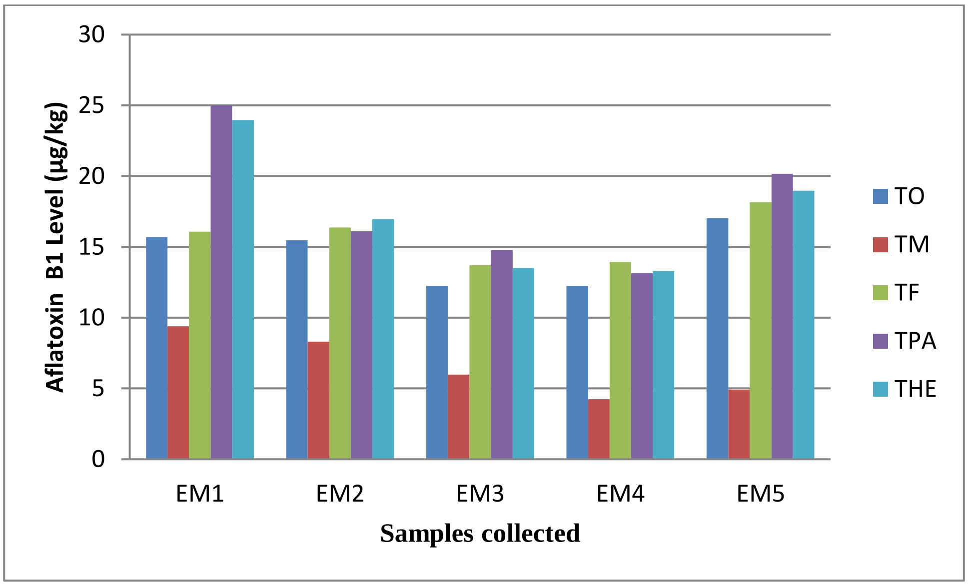

Figure 2: Influence of groundnut transformation processes on aflatoxin B1 levels

The application of transformation processes such as manual sorting (TM), roasting (TF), transformation of peanuts into peanut paste (TPA) and finally extraction of peanut oil (TEH) indicate that the various transformation processes had an influence on the AFB1 contents contained in the samples (Table 1). Depending on the type of transformation, either a decrease or an increase in AFB1 contents is observed. Indeed, a significant reduction (P=0.008) of AFB1 contents of 40.12 – 71.09% was observed after manual sorting of the samples. This reduction in AFB1 levels could be explained by the elimination during sorting of moldy, immature or damaged seeds which generally contain high doses of aflatoxins (Anyebuno *et al*., 2018; Tedihou *et al*., 2019). The similar results were obtained by Xu *et al*., (2017) who recorded a 96.7% reduction in aflatoxin B1 after manual sorting of groundnuts. This high reduction rate obtained by these authors can be explained by the ability of the grader to identify apparently healthy or moldy seeds. In fact, to be effective, sorting must be rigorous and allow the complete elimination of moldy, damaged, shriveled or discolored groundnut seeds which most often contain high doses of aflatoxins (Njeru, 2014).

Table 1: Effect of processing techniques on AFB1 levels

Food process	Aflatoxin level B1 (µg/kg)					Effect of transformation process (%)	
	EM 1	EM 2	EM 3	EM 4	EM 5	Reduction	Increase
T0	15,70±0,42^b	15,46±0,16^b	12,40±0,42^b	12,24±0,04^b	17,02±0,62^b	-	-
TM	9,40±0,14^a	8,30±0,28^a	5,98±0,35^a	4,24±0,08^a	4,92±0,02^a	40,12 – 71,09	-
TF	16,09±0,14^b	16,37±0,02^b	13,7±0^b	13,94±0,02^b	18,16±0,05^b	-	2,48 – 13,88
TPA	25,00±0,01^b	16,11±0,01^b	14,77±0,09^b	13,15±1,20^b	20,16±0,45^b	-	4,20 – 59,23
TEA	23,97±0,24^b	16,97±0,73^b	13,50±0,24^b	13,29±0,11^b	18,96±0,42^b	-	8,57 – 52,67

EM: original sample; **TO:** control sample; **TM:** manual sorting; **TF:** roasting; **TPA:** transformation into groundnut paste; **TEH:** groundnut oil extraction

The results also indicated that roasting peanut seeds for 10 min according to the process used by processors resulted in a slight increase in AFB1 contents of 2.48 – 13.88% compared to raw peanut seeds (T0). This small increase would be due not only to the low heating temperature but also to the too short cooking time, which was insufficient to cause degradation of the aflatoxin molecules. These results are different from those of Martins *et al*., (2017) who showed in their work that roasting peanut seeds in an oven at a temperature of 160°C for 20 min led to a reduction in AFB1 levels of 60.23%.

In fact, according to the method used by the processors, the cooking of the peanut seeds begins as soon as the seeds are put on the fire at a very low temperature and this temperature gradually changes with the roasting to reach 170°C after 10 min roasting. When the temperature necessary to cause aflatoxin degradation is reached, the sheaths are already cooked and must be removed; which prevents the seeds from being subjected not only to a high degradation temperature but also to a cooking time long enough to induce aflatoxin degradation. However, apart from temperature and duration of roasting, other factors such as the initial aflatoxin content of the contaminated food can also influence the reduction of aflatoxin levels during roasting (Arzandeh *et* Jinap, 2011; Emadi *et al*., 2021). According to (Martins *et al*., 2017) the higher the roasting temperature, the shorter the time required to cause degradation of aflatoxin molecules. Roasting peanut seeds at a high temperature (150°C – 200°C) and for a fairly long time is essential to reduce aflatoxins in food (Sipos *et al*., 2021). Roasting is known to influence aflatoxin reduction (Pankaj, Hu *et* Kevin, 2018; Peng *et al*., 2018). For this, the groundnut seed roasting method used by the processors was modified by inserting a preheating stage of the roasting device. The results of the assay of AFB1 levels after this new roasting method showed that roasting the peanuts led to a reduction in AFB1 levels (Table 2). In fact, a strong correlation (R2=0.99) is observed between temperature and aflatoxin levels. A gradual decrease in AFB1 levels is observed as a function of time. This decrease in AFB1 contents varies from 60.71 to 83.57% for samples roasted at 160°C and from 58.33 to 94.28% for samples roasted at 180°C (Figure 3).

Table 2: Reduction rate of aflatoxin levels after roasting

Temperature (°c)	Time (min)	Mean aflatoxin B1 content (µg/kg)	Percent reduction of aflatoxins (%)
0	0	8,4±0,63	0,00
160	5	4,3±0,41	60,71
	10	2,7±0,74	67,85
	15	1,38±0,16	83,57
180	5	3,5±0,41	58,33
	10	0,72±0,014	91,42
	15	0,48±0,12	94,28

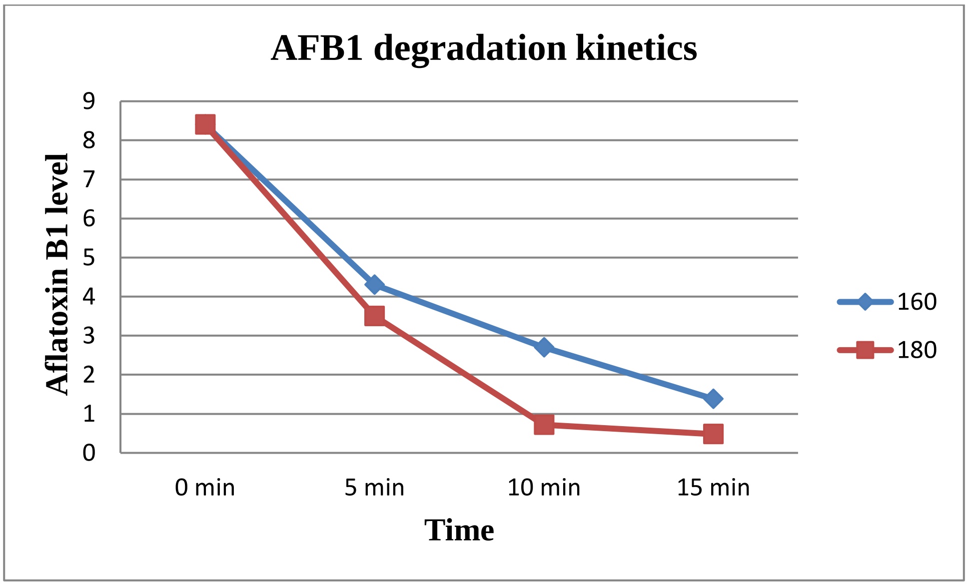

Figure 3: Influence of temperature on the degradation of aflatoxin B1

This decrease in levels could be explained by a degradation of aflatoxin molecules during roasting. These results are similar to those of Aygün, (2015); Karlovsky *et al*., (2016); Martins *et al*.,(2017) who showed that roasting peanut seeds at a temperature of 180°C for 20 minutes led to a reduction aflatoxin B1 levels of 80%. However, it should be noted that the amount of peanut seeds roasted and the heat source used are important factors to consider. Indeed, a larger quantity of peanuts to be roasted will require more cooking time than a smaller quantity; similarly, a gas roast could provide more heat than the charcoal used in this experiment and therefore impact the roasting time. Roasting must therefore be monitored because temperature has a limited effect on aflatoxins. In fact, Figure 4 shows that the high temperature necessary for the denaturation of aflatoxins can cause a modification of the organoleptic properties such as the carbonization of the peanut seeds, the degradation of certain nutrients or give a color and taste unacceptable to the consumer (Martins *et al*., 2017; Prusak *et al*., 2014). Indeed, Aygün (2015) observed a reduction in protein content after roasting peanuts at 200°C.

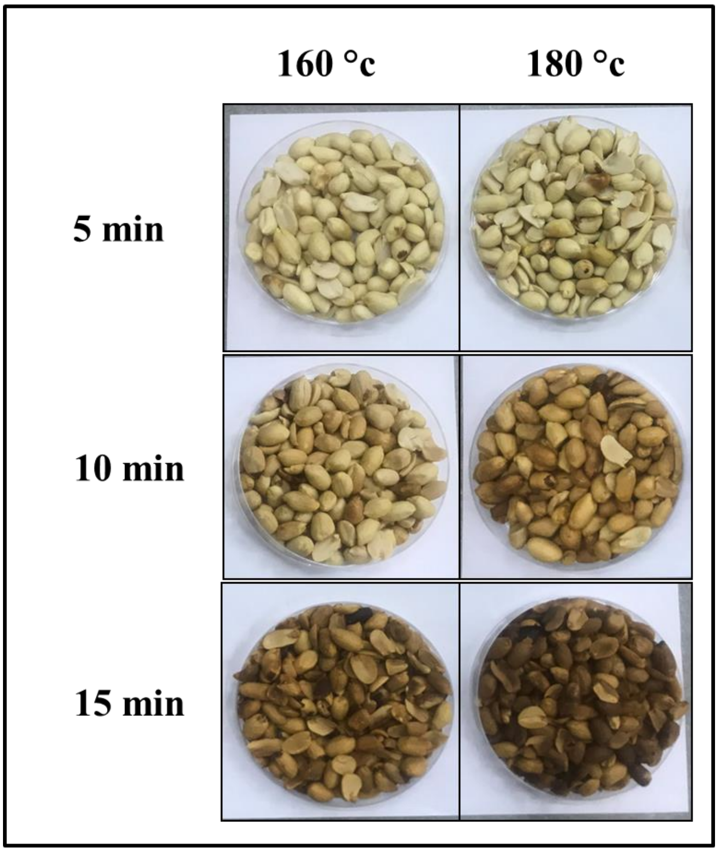

Figure 4: Effect of roasting time on groundnut seeds during roasting

The roasting and oil extraction processes caused an increase in aflatoxin contents of 4.20% - 59.23% and 8.57% - 52.67% respectively. This increase in aflatoxin levels could be explained by the total or partial absence of sorting of damaged seeds during the transformation process. Similar results with very high aflatoxin contents ranging from 0.0 to 2377.1µg/kg were obtained by (Ndung'u *et al*., 2013) in peanut paste. The traditional peanut oil extraction process does not promote decontamination of aflatoxins. Indeed, the results of this study showed that the aflatoxin levels observed in peanut paste are practically transferred to peanut oil. This transfer of aflatoxins could be explained by the extraction method used (Mahoney *et* Molyneux, 2010) which can in some cases favor the presence of fine particles of peanut paste in the oil. These results are similar to those obtained by (Schwartzbord *et* Brown, 2015) who observed an aflatoxin transfer of 185 µg/kg in the oil extracted from peanut seeds containing an aflatoxin content of 18200 µg/kg.

Indeed, some treatments such as alkaline refining, washing and effective bleaching of the oil removes aflatoxin from the oil can significantly reduce the aflatoxin levels and could explain the low aflatoxin transfer levels in the peanut oil. Nevertheless, based on pure solubility considerations, peanut oil should not contain aflatoxins. However, in practice there is a transfer of aflatoxins from contaminated seeds into the crude oil in the order of 15-35% of the initial level in the contaminated groundnut (Shephard, 2017).

4. CONCLUSIONS

This work has shown that sorting and the roasting process, when properly carried out, promote a reduction in aflatoxin B1 levels in foods. Manual sorting was effective in reducing aflatoxin B1 levels in groundnut seeds by up to 71.09%. Roasting, on the other hand, when carried out at a temperature of 180°C or 160°C for 15 minutes in a preheated roasting device can induce a reduction in aflatoxin levels of around 90%. Thus, in households, roasting to be effective must always be preceded by preheating for at least 8 or 15 minutes so as to start roasting at approximately a temperature of 160°C or 180°C. The groundnut paste and oil extraction process does not promote a reduction in aflatoxin levels in groundnut seeds. These two processes to be effective must always be preceded by rigorous sorting in order to eliminate all moldy or damaged seeds containing high doses of aflatoxin. Food contamination by aflatoxins is currently a real public health problem that must be resolved. The physical control methods tested in this study have proven their effectiveness in reducing aflatoxin levels in groundnut. Rigorous sorting of food for consumption combined with a preheating step of the groundnut roaster can be an effective decontamination method against aflatoxins in groundnut.

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