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Degradation of Rotenone in Yam Bean Seeds (*Pachyrhizus* sp.) through Food Processing

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ABSTRACT: The purpose of this research is to screen different processes that could potentially decrease or even eliminate rotenone, a toxic isoflavonoid, from *Pachyrhizus* seeds. Yam bean seeds have very interesting nutritional characteristics, especially their high protein and lipid contents, and could potentially increase food security in under-nourished populations. However, they contain rotenone, a natural molecule previously used as an insecticide inhibiting the respiratory mitochondrial chain. It was also proven to be toxic to mammals as chronic exposure leads to the development of Parkinson-like symptoms in rats. As the thermosensitivity of rotenone had been reported, this study tested different processes (drying, roasting, boiling, frying, alcohol extraction), tegument removal, and traditional Beninese culinary recipes. Rotenone was then quantified in end-products by a validated method, associating microwave extraction, solid phase extraction (SPE), and HPLC-UV. With these processes a rotenone removal of up to 80% was obtained. The most effective methods were the drying and roasting of the seeds and the maceration of their flour in local alcohol. Rotenone degradation and elimination were confirmed by cytotoxic assays, effectively inducing a decrease in sample toxicity.

KEYWORDS: *yam bean, rotenone, degradation, extraction, food processing*

INTRODUCTION

Pachyrhizus sp. or yam bean is a leguminous plant from Latin America, which is also cultivated in certain parts of South Asia. This plant has interesting agronomic advantages, such as low nitrogen fertilizer needs[1,2] and resistance to both drought and excessive rains.[2] This crop is easily cultivated, resulting in high yields, and the roots, which is the part that is usually consumed, have good nutritional characteristics.[1−3] Therefore, several research groups from different areas of the world studied the possibilities of using this plant as an alternative legume crop (AHIPA project).

Yam bean seeds have high protein (28.3%) and lipid (26.3%) contents,[4−6] so it would also be interesting to valorize these parts of the plant. However, the presence of rotenone in seeds restrains their use as a food crop.[7,8]

Rotenone is an odorless toxic isoflavonoid used as a broad-spectrum insecticide, piscicide, and pesticide. It was extracted from the roots of several members of the Fabaceae belonging, for example, to the *Derris*, *Lonchocarpus*, *Milettia*, or *Tephrosia* genera.[9]

This molecule is classified by the World Health Organization as moderately dangerous.[10] It is considered as mildly toxic to humans and other mammals but extremely toxic to insects and fishes. Its main mechanism of action is the inhibition of the NADH ubiquinone oxidoreductase, the first complex of the mitochondrial respiratory chain. The acute toxicity of rotenone in insects is attributed to this mechanism.[11] The main toxic consequence of this inhibition is the generation of oxidative stress,[12] which can cause DNA fragmentation, lipid peroxidation, and abnormal protein formation.[13] Furthermore, this oxidative stress seems to be related to a Parkinson-like disease and was proven in rats to cause a loss of dopaminergic neurons.[14]

Two cases of poisoning by yam bean seeds were previously reported. The first case was described in Thailand, where a man died after the ingestion of 100 g of *Pachyrhizus erosus* seeds,[15] and the second in Taiwan, where a group of five people were intoxicated after eating a soup prepared with 60 g of yam bean seeds.[16] The poisoning was characterized by nausea, vomiting, stomach- and headache, respiratory distress syndrome, and cardiac arrest.[15]

The elimination or reduction of the rotenone contents in yam bean seeds is thus essential to allow their human consumption.

In the literature, different methods (autoclaving, soaking, fermentation, roasting, etc.) have already been used to reduce the content of antinutrient or toxic compounds in various food crops. For example, some authors tried to eliminate antinutritional factors such as trypsin inhibitors, phytates, and tannins in lima bean[17] and other legume seeds[18] by soaking, boiling, autoclaving, or roasting seeds. Similar processes were also used to eliminate toxic compounds such as cyanogenic glycosides in cassava, for example.[18,19]

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To reduce or eliminate rotenone, different approaches are possible. First of all, rotenone is a light-sensitive compound. Its photosensitivity was widely studied for its use as insecticide[20] because it limited the duration of its efficiency. Cheng et al. identified that rotenone was degraded in at least 20 compounds, most of which were rotenoids. They reported that only one product, 6aβ,12$\alpha\beta$-rotenolone, was toxic.[20] Second, rotenone was also sensitive to degradation by microorganisms. Species such as *Ascomycetes*, *Basidiomyces*, or *Fungi imperfecti* were reported to possess an enzyme called laccase, responsible for the oxidation of rotenone. The main product formed is 12aβ-hydroxyrotenone.[21] Finally, rotenone has been described to be very sensitive to temperature: for example, Cavoski et al.[22] showed a degradation of rotenone in soils of up to 50% within 8 days at 10 °C and within 5 days at 20 °C. Heat being the most relevant factor used to prepare food, it was thus interesting to explore how temperature can be used to degrade rotenone in yam bean seeds, including use of culinary processes to potentially prepare a food.

Nevertheless, as far as we know, only one process has been described to reduce rotenone content in yam bean seeds. In 1995, Santos and his collaborators macerated the seeds into distillated water for 18 h and dehulled them manually. They were then boiled in water for 2 h and dried in an air-circulating oven for 48 h at 45 °C. The authors showed that this process induced a significant reduction of rotenoid contents in yam bean.[6]

The aim of the work presented here was thus to screen different cooking processes adaptable to the traditional scale to decrease or even eliminate rotenone from yam bean seeds of *P. erosus* and to test the feasibility of local Beninese recipes.

Furthermore it was tested if a decrease in rotenone would result in a decrease in the toxicity of the seeds, at least in an in vitro model, as our aim was to use seeds as food. Different transformation of the detoxified seeds could be imagined (bread, sauce, fritters, etc.), so that local populations could benefit from the interesting nutritional advantages of yam bean seeds, mainly their high contents of proteins and lipids.

MATERIALS AND METHODS

Plant Material. The seeds of two accessions of *P. erosus*, which have the collection numbers EC-533 (209018) and EC-KEW (209019), were grown and harvested in Benin at the IITA station (International Institute of Tropical Agriculture). Those seeds were prepared, milled, and sent to Belgium in August 2011. (The numbers in parentheses are the accession numbers registered in the gene bank at CIP-Lima.)

Chemicals. HPLC standard rotenone (≥98%) was purchased from Enzo Life Science (Zandhoven, Belgium). Methanol of HPLC grade was from Prolabo, VWR (Leuven, Belgium), acetonitrile of HPLC grade from Fisher Scientific (Tournai, Belgium), and dichloromethane of reagent grade from Sigma-Aldrich (Belgium).

Cooking Processes. Whole or dehulled seeds were dried, roasted, boiled, or fried. These processes are the most prevalent cooking practices of Beninese populations. Cooking processes were performed on an electric heating plate (roasted, boiled, or fried) or in an oven (dried). To easily remove the teguments of seeds, these were soaked in a large volume of water (10 g of seeds in 500 mL of water) overnight.

To estimate the effect of drying, seeds were dried overnight in the oven at 105 °C. Roasted seeds were cooked on a heating plate at 90 ± 5 °C during 1 h. Boiled seeds were warmed at 100 °C during 3 h in a large volume of water (10 g in 500 mL). Finally, to estimate the effect of frying, the seeds were fried in 10 mL of peanut oil in a stainless steel pan during 1 min and 30 s at 185−190 °C. Every process was repeated three times on 10 g of whole seeds and on 10 g of dehulled seeds of both accessions.

Beninese Culinary Recipes. Three recipes (atta, fried cowpea dumplings;[23] adowe, boiled cowpea dumplings; and fermented porridge) traditionally prepared in Benin with cowpea seeds (*Vigna unguiculata* L. Walp.) were prepared with yam bean seeds. After processing, samples of products made with all recipes were stored at −18 °C.

Atta. Fifty grams of yam bean seeds was steeped in 300 mL of water during 1 h, and then the teguments were removed. Dehulled seeds were then soaked in 50 mL of water during 30 min. Moistened seeds were crushed with a grindstone to obtain a dough. Then 2 g of cornstarch was added to improve the consistency of the dough; fritters were formed and then fried for 2 min in 125 mL of peanut oil, heated on a wood fire.

Adowe. Fifty grams of dry seeds was dehulled with a grindstone to obtain slightly crushed seeds. Dehulled seeds were then placed in 200 mL of water, covered with a lid, and cooked on an electric plate. The cooking was watched to avoid burning, and some water was gradually added. The quantity of added water varied from 600 to 900 mL. When beans were considered as sufficiently cooked, they were crushed in the remaining water into a dough. The duration of cooking varied from 2 h to 2 h and 20 min.

Fermented Porridge. Twenty-five grams of yam bean seeds was crushed finely to obtain flour. This flour was then mixed with 30 mL of water to obtain consistent dough, which was left overnight in a covered bowl at room temperature protected from light. The next day the dough was mixed with 50 mL of water to obtain a premix that was added to 100 mL of boiling water. The preparation was then cooked for 30 min.

Extraction Processes. To extract rotenone by alcohol, 10 g of whole, dehulled seeds or flour from whole seeds was soaked in 100 mL of Sodabi (traditional palm alcohol from Benin) during one night in triplicates. The residues of the extraction were milled and stored at −18 °C.

Determination of the Conversion Rate of the Process. To express the quantity of rotenone in milligrams per gram of untreated whole seeds, it was necessary to determine the conversion rate of each process.

For that purpose, the average mass of seeds after treatment was divided by the average mass of seeds prior to treatment.

For dehulled seeds, we proceeded in the same way, taking into account the conversion rate of the dehulling calculated as 124.34%. A yield >100% is understandable because dehulled seeds are filled with water and have a much higher weight than whole seeds when initially weighed.

Extraction of Rotenone and Cleanup Procedure. The microwave-assisted extraction (MAE) conditions were optimized in Lautie et al.[24] MAE were performed in a closed-vessel system (MARS, CEM Corp., USA), and 20.0 mL of a 50:50 mixture of DCM/MeOH was placed in the glass extraction vessels with two stirring bars. The different vessels were placed into the extractor, which was programmed to make a cycle of 11 min at 55 °C, with the potency of the extractor set to 400 W. At the end of each extraction, the vessels were allowed to cool to 38 °C before opening. The vessels were then centrifuged for 5 min at 3000*g*. Four milliliters from every vessel was then taken and dried in a RapidVap system (Labconco).

The resulting dry extract was reconstituted with 5.0 mL of 100% DCM, and $^1/_{10}$ was purified by SPE as described previously by Lautie et al.[25] The fraction eluted with DCM/MeOH (98:2, v/v) was collected, dried, stored at −18 °C, and then solubilized with 5.0 mL of methanol on the day of the HPLC analysis.

UV-HPLC Analyses. Analyses were performed according to the validated HPLC conditions described in Lautie et al.[25] on a LaChrom Elite HPLC integrated system (Merck Hitachi, VWR, Leuven, Belgium) equipped with an L-2300 oven, an L-2130 autosampler, and an L-2130 pump all piloted by EZChrom software. The chromatographic separation was performed on an RP-18e 250 mm × 4 mm LiChroCART column (5 μm) equipped with a guard column. It was eluted at a constant flow rate of 1 mL/min by a 45 min gradient

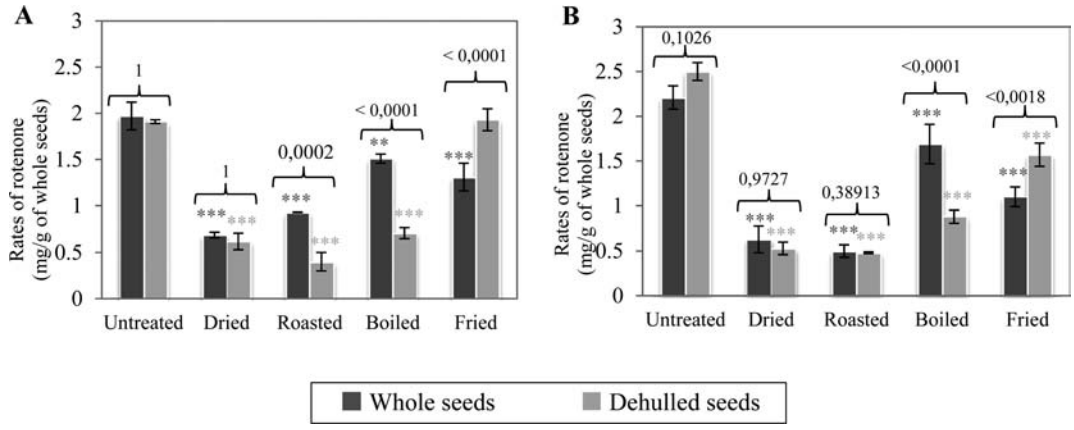

Figure 1. Effect of various cooking processes on the contents of rotenone in whole and dehulled yam bean seeds of two accessions: EC-533 (A) and EC-KEW (B). Bar data are expressed as the mean ± SD (n = 3). Asterisks above the same color bars compare the rotenone contents after the process with those in untreated seeds (p < 0.01, **; p < 0.001, ***). The values above bars represent the p value of the test comparing the concentration of rotenone in processed whole seeds and processed dehulled seeds.

of acetonitrile/water with the following steps: initial mobile phase, 48:52 (v/v), during 26 min, then a 1 min gradient to 68:32 (v/v) stabilized during 9 min, and finally back to the initial conditions stabilized for another 9 min. Analyses were carried out at room temperature, and UV detection used a wavelength of 295 nm. The quantification was performed using the external calibration method.

Cells and Media. The human normal fibroblast cell line, WI38 (ATCC CCL-75 from LGC Standards), was cultivated in vitro in DMEM (Gibco) containing 4 mM L-glutamine, 1 mM sodium pyruvate supplemented with 10% heat-inactivated fetal bovine serum (Gibco), and penicillin–streptomycin (100 UI/mL to 100 μg/mL).

The Chinese hamster ovary cell line, CHO (ATCC CCL61 batch 476-52-75), was cultivated in vitro in Ham-F12 medium supplemented with FBS (10%), penicillin–streptomycin (100 UI/mL to 100 μg/mL), and amphotericin B (2.6 μg/mL).

The cells were incubated in an atmosphere with 5% CO_2 at 37 °C.

Cytotoxicity Assay. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay was performed as previously described.[26] The colorimetric method is based on the cleavage of the tetrazolium salt reagent MTT (Sigma) by dehydrogenases in viable cells.[27] Rotenone (Enzo Life Science) was used as a positive cytotoxic reference compound. A stock solution of the purified extract obtained as described previously after the extraction and cleanup procedure was prepared for untreated whole seeds of EC-533 (UWS), in DMSO at 7.08 mg/mL, which corresponds, according to quantified rotenone content, to 1 mg of rotenone/mL. A stock solution of a purified extract of dehulled roasted seeds (EC-533) at 7.08 mg/mL corresponding to 0.31 mg of rotenone/mL was also prepared (RDS). The solutions were further diluted in medium with a final concentration ranging from 10 to 1 × 10^{-5} μg/mL. All experiments were repeated at least eight times.

Statistical Analysis. Statistical analysis was performed with JMP 5.0 software. To make multiple comparisons, the Tukey test was used.

RESULTS AND DISCUSSION

Effect of Thermal Processing on Total Rotenone Contents in Yam Bean Seeds. The concentrations of rotenone in untreated whole seeds were 1.97 ± 0.02 and 2.21 ± 0.13 mg/g, respectively, for EC-533 and EC-KEW. Results are thus higher than the residual quantity of rotenone found in other foodstuffs when rotenone is used as a pesticide. For example, contents of rotenone in raw honey were <0.2 mg/kg honey,[28] and for tea, contents of rotenone were even weaker, going from 0.012 to 0.016 mg/kg tea.[29] It was thus very important to find adequate processes decreasing the content of rotenone in yam bean seeds.

As rotenone may be considered as a phytoalexin protecting the seeds from predators, we chose to measure concentrations of rotenone in the integuments of the seeds. Indeed, numerous molecules from the defense system are localized in the integument such as flavonoids, polyphenols oxidases, and peroxidases.[30−32] Interestingly, these concentrations are quite low with 0.17 ± 0.03 (EC-KEW) and 0.21 ± 0.002 (EC-533) mg/g of dried integuments. The rotenone is thus mainly contained in the cotyledons of the seeds.

The concentrations of rotenone in whole and dehulled seeds from both batches (EC-KEW and EC-533) after the different cooking processes are shown in Figure 1.

First, it is obvious that the different processes cause reduction in levels of rotenone in yam bean seeds. This observation is in agreement with an earlier report that heating reduces rotenone contents.[22] We can also see on this histogram that for both batches, the same treatments entail similar results. The drying and roasting of seeds seem to be the most effective processes in decreasing rotenone content. After drying whole seeds, we detected concentrations of rotenone of 0.69 ± 0.09 and 0.63 ± 0.15 mg/g of untreated whole seeds for batches EC-533 and EC-KEW, respectively, corresponding to degradations of 64.97 and 71.49%. In the roasted seeds, concentrations of 0.93 ± 0.1 (52.79% degradation) and 0.50 ± 0.07 mg (77.38% degradation) of rotenone/g of whole untreated seeds for batches EC-533 and EC-KEW were measured. The fact that roasting leads to a higher level of degradation for EC-KEW than for EC-533 (p = 0.0079) could be attributed to experimental conditions or resistance of the integuments: in fact, we observed that the integuments of seeds were cracked, favoring direct contact between the heating plate and the cotyledons, allowing a better penetration of the heat and a higher rotenone degradation. In 2005, Ogunsanwo et al. studied the effect of roasting on the aflatoxin contents in Nigerian peanut seeds. Aflatoxins are, like rotenone, lipophilic ketonic compounds sensitive to heat. They showed in their paper that there are positive correlations between loss of aflatoxins in the products and roasting conditions: an increase in time or in temperature entails a higher aflatoxin loss. In their work a modification of 10 °C already induced a great loss difference.[33] Therefore, our range of temperature from 85 to 95 °C could maybe explain the difference observed for the different batches.

The protective coating of the seeds can be removed and the impact of this dehulling on the thermal degradation of the rotenone measured. As a matter of fact, processes of drying and roasting dehulled seeds seem to be a little more effective in reducing rotenone levels for both accessions than the same processing made on whole seeds, but these treatments do not produce significantly different results.

Boiling yam bean seeds was the least efficient process; only 23% degradation of rotenone was obtained in both accessions. This low degradation could be due to rotenone not being broken down or extracted by water due to its low polarity. Also, when seeds are boiled, the teguments prevent the penetration of the warm water in the various structures of the seed. This was confirmed with boiling dehulled seeds, which triples the efficiency of the boiling process with 62.83 and 64.80% degradations for EC-533 and EC-KEW, respectively. Making a soup with dehulled roasted or dried yam bean seeds would maybe allow an even more important degradation of rotenone.

By deep-frying seeds in peanut oil, we expected a thermal degradation of rotenone and its extraction by the oil. However, this process resulted in low degradation of rotenone compared to drying or roasting. During the frying of seeds in oil warmed at 185−190 °C, we observed that seeds quickly burned. It was thus necessary to limit the time of cooking, but 1 min and 30 s does not seem to be enough for a good penetration of oil in the seed structures. The combination of time and temperature of frying needs to be optimized in further tests to degrade a higher percentage of rotenone. Frying was the only process that seemed to work better on whole seeds than on dehulled seeds; the dehulling process itself may explain this fact. Indeed, dipping the seeds into water to remove the tegument entails inevitably a high degree of hydration of the seeds that limits the penetration of warm oil and thus the degradation.

In these tests it was shown that drying and roasting of the seeds would allow up to 80% degradation of rotenone. We can also conclude that for these two treatments it is not necessary to dehull the seeds, a process that would be more time- and labor-consuming. Moreover, we could see that rates of rotenone degradation measured after treatments are comparable for both accessions. Our processes are thus repeatable and easy to use by local populations to potentially reduce rates of rotenone in yam bean seeds.

The thermosensitivity of rotenone was mostly studied in the case of its use as insecticide and piscicide. It was shown in particular that when it was used as piscicide, the half-life of rotenone contained in the pond was shorter in summer compared with that in spring or autumn. A correlation between the water's temperature and rotenone half-time was demonstrated.[34] Zubairi et al. studied the influence of the temperature on the yield of rotenone extracted from *Derris elliptica* root. They concluded that rotenone is strongly affected by a temperature above 40 °C during the extraction process.[35] However, according to Pagan et al., the rotenone content of the fresh *Derris* root after all treatments (effect of direct sunlight and oven-drying at 80 °C) does not have any significant effect on the reduction of rotenone content.[36] It seems that the thermosensitivity of rotenone greatly depends on the matrix because we showed here that rotenone content in yam bean seeds was affected by oven-drying at 120 °C during one night. It is thus difficult to predict the behavior of rotenone content on the basis of the literature because it is the first time that someone attempted to decrease rotenone contents in yam bean seeds.

Contents of Rotenone in Beninese Culinary Recipes. In this case the content of rotenone was expressed as the mass of rotenone in 1 g of culinary preparation. The results are shown in Table 1. For the atta preparation,[23] which consists of the preparation of fried fritters, 0.62 ± 0.05 and 0.57 ± 0.03 mg of rotenone/g of recipe were detected for EC-533 and EC-KEW, respectively, whereas in adowe preparations (porridge of dehulled seeds) amounts of 0.71 ± 0.01 and 0.61 ± 0.07 mg of rotenone/g of recipe were quantified for EC-533 and EC-KEW. No significant difference was detected between both accessions in these two recipes. Finally, for the fermented porridge, rotenone contents of 0.30 ± 0.02 and of 0.51 ± 0.01 mg of rotenone/g of recipe were obtained for EC-533 and EC-KEW, respectively.

Table 1. Contents of Rotenone in Beninese Culinary Recipes[a]

	atta	adowe	fermented porridge
EC-533	0.62 ± 0.05	0.71 ± 0.01	0.30 ± 0.02
EC-KEW	0.57 ± 0.03	0.61 ± 0.07	0.51 ± 0.01

[a]Values are from duplicate recipes and expressed as the mean ± SD (mg of rotenone/g of recipe).

To determine if yam bean seed preparations could be used for food, we compared these results to the dose of rotenone that a man can ingest without adverse effect (NOAEL).

To determine the human NOAEL, the U.S. Environmental Protection Agency (EPA) applied to the mouse NOAEL (15 mg/kg/day)[37] an uncertainty factor of 1000. This corresponds to the multiplication of three risk factors of 10: the neurotoxicity uncertainty factor and the interspecies and intraspecies uncertainty factors. This information suggests that some animals are less sensitive to rotenone; for example, the oral LD_{50} for birds (1600−2500 mg/kg) is much higher than for rodents (40−100 mg/kg).[38]

Then, the dose of rotenone that can be ingested by a man of 70 kg without producing effects on health is 1.05 mg a day in acute diet.[37] This means that a man could eat, without risks, <2 g of these preparations.

Although an important degradation of rotenone content in yam bean seeds was observed after making these three Beninese culinary recipes, the residual amount is still too high; therefore, it is not safe for humans to eat those seeds after using these processing techniques. However, our processed seeds could be used as food for less sensitive animals, but this option needs further studies to determine processes and maximum levels of exposure.

Contents of Rotenone after an Extraction Process. The soaking in alcohol of the whole seeds, the dehulled seeds, and the flour of whole seeds showed interesting results, which are shown in Figure 2 for EC-KEW; the results obtained for batch EC-533 showed a similar profile.

Soaking the whole seeds in alcohol does not entail an important decrease of rotenone (5−6%), whereas for dehulled seeds, a decrease of 55% is observed. The maceration of the flour of whole seeds gives better results with a decrease of about 73.3% of the quantity of rotenone.

To verify if the decrease of the quantity of rotenone in the seeds was caused by the extraction of the compound or by its degradation at room temperature, levels of rotenone in the alcohol of maceration were determined. Figure 2 shows clearly that as the quantity of rotenone of the residue decreases, the

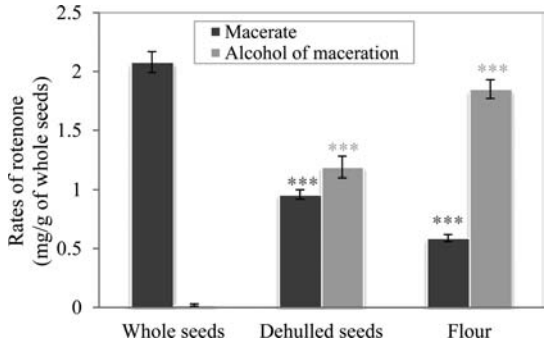

Figure 2. Effect of alcohol maceration on rotenone concentration in seeds (mean ± SD). Asterisks above the same color bars compare the contents of rotenone between UWS and processed seeds.

quantity of rotenone detected in the maceration alcohol increases and that the total amounts correspond to initial quantity. It shows that rotenone is extracted by alcohol; the more alcohol that enters in contact with the different parts of the seeds, the more effective the extraction is. This is in agreement with the findings of Sae-Yun et al., who showed that rotenone could be extracted from *Derris* roots by maceration in ethanol even if chloroform was considered as a better alternative for its extraction.[39]

Furthermore, it was also demonstrated that an ethanol Soxhlet extraction at 70 °C gave a better yield in rotenone than a stirring maceration at room temperature.[40] These results indicate that the temperature has a great influence on the rotenone solubility in ethanol. Thus, it would maybe be interesting to test the extraction of rotenone contained in yam bean seeds by maceration in hot alcohol to improve our results.

Cytotoxicity Assay. To determine if the degradation of the rotenone contained in the yam bean seeds decreased the toxicity of these seeds, preliminary results were obtained with cytotoxicity tests realized on human fibroblasts (WI38) and Chinese hamster ovary cell line (CHO) with two purified extracts of yam bean seeds.

First, we observed (Figure 3) that rotenone is around 150 times more toxic to the human fibroblasts (IC_{50} = 0.003 μg/mL) than to the hamster cells (IC_{50} = 0.49 μg/mL). On human cells, rotenone seems to be approximately 10 times more toxic

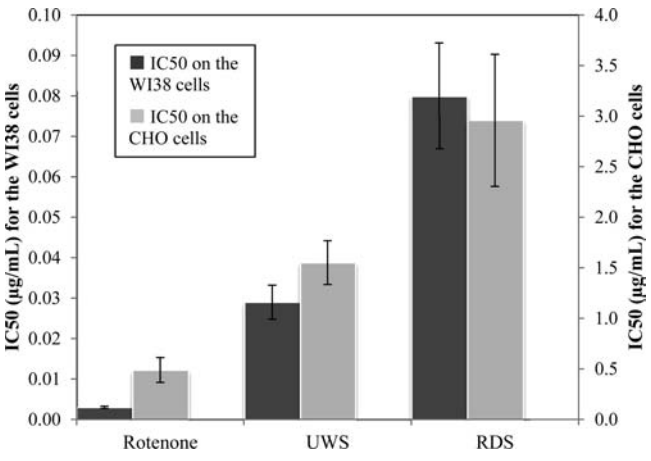

Figure 3. Cytotoxicity of the different extracts on the CHO and WI38 cells (mean ± SEM; SEM = standard error of the mean): $x = SD/\sqrt{n}$. UWS, untreated whole seeds extract; RDS, roasted dehulled seeds extract.

than the untreated whole seeds extract (UWS) (IC_{50} = 0.029 μg/mL). The same tendency is observed for animal cells with a slightly lower ratio.

The dehulled roasted seed extract containing 3 times less rotenone than the UWS extract is also less toxic, with an IC_{50} for human cells of 0.08 μg/mL, which is approximately 3 times higher than the UWS extract IC_{50} (0.029 μg/mL). For the animal cells, the tendency is the same but the ratio between IC_{50} values is around 2. These results confirm that a decrease of rotenone content entails a decrease of the cytotoxicity of the corresponding purified extract.

As a conclusion, this screening of various technological processes allowed us to select the most interesting processes: drying, roasting, and maceration of the seed flour in alcohol, which showed a significant reduction of the quantity of rotenone in the seeds. We did not observe any significant difference in the efficiency of processes for the two accessions used here (EC533 and EC-KEW), except for roasting, which shows that the efficiency does not depend on genotype.

The whole seeds of these two batches had an initial content of rotenone of more or less 2 mg/g that could be decreased, through the most effective processes (roasting and drying), by about 80%. The different technological processes are adaptable to the traditional scale and, for most of them, they are as effective on dehulled seeds as on whole seeds. In the future, it would also be interesting to evaluate the influence of those different treatments on the yam bean seed nutritional values, especially the high protein and lipid contents,[4,5] and micronutrient levels. Thermal treatments could degrade these compounds, but they may also improve the digestibility of proteins by various factors such as disruption of protein structures and cell-wall encapsulated starch and physical disintegration of the legume seeds.[41] The extraction of rotenone by alcohol was also shown to be very effective, with up to 73% rotenone extracted when seeds were pulverized into flour, irrespective of the accession used. The main objective of this project was to select processes that would reduce the rotenone content in yam bean seeds to allow local populations to eat these seeds and benefit from their high protein and lipid contents. However, despite the important rate of degradation obtained, the potential for consumption of yam bean seeds remains very limited, because levels are not yet below safe levels. It would thus be interesting to combine some of these processes to increase the degradation of rotenone. For example, maceration of flour in alcohol two times instead of one could be tested to further decrease the residual amount of rotenone. Then this flour could be used to make bread, fritters, or other recipes that would expose the products to high temperature, also degrading the residual amount of rotenone after extraction. Another strategy would be to look for accessions or genotypes of seeds containing initially less rotenone to reach the objectives of making yam bean seeds safe for human consumption. In the screening realized by our group,[42] we found one accession containing less rotenone than the quantification limit. We estimate the content of rotenone at 0.29 mg/g of seeds. If we consider an 80% degradation, we may reduce the quantity of rotenone to 0.06 mg/g of seeds. According to the NOAEL acute reference dose, a man of 70 kg could then eat 17.5 g of yam bean seeds. If we consider this accession, our degradation processes allow well the human consumption of those seeds.

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Notes

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