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Optimization of the biochemical hydrolysis of cyanogenic glucosides, for the detoxification of bitter almond extracts, in a food context: the cas of rubber (*Hevea Brasiliensis*) kermel

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Abstract: Hevea kernels are rich in nutrients, but their toxicity due to the presence of hydrogen cyanide means that they are under-exploited. This is due to its hydrogen cyanide content (2712.3mg/kg), which is well above the recommended standard for food, cosmetics and pharmaceuticals (≤ 30mg/kg). As a result, cyanide detoxification is essential to enhance the value of extracts such as oil and cake. In fact, the oil is rich in essential fatty acids (59.3%) and the meal is rich in protein (41.2%). In this agricultural by-product, the toxin is locked in a heterosidic combination in glycosides such as amygdalin. Hydrolysis of these heterosides is therefore necessary to release the cyanides in the form of hydrocyanic acid. It was with this in mind that the biochemical hydrolysis of glycosides was optimised. The factorial design (2^3) carried out for this purpose during the cyanogenetic study of the almond made it possible to detoxify the oil and the cake. The Y90 model developed considerably reduced the level of cyanides in the almond to 0.38mg/kg HCN, giving a detoxification or amygdalin hydrolysis efficiency of 99.94%. Under optimal conditions, the model promises to produce consumable rubber tree oil.

Keywords: *Hevea kernel; hydrocyanic acid; cyanogenic glycosides; detoxification; biochemical hydrolysis; optimization*

1. Introduction

Hevea brasiliensis is a species of dicotyledonous plant in the Euphorbiaceae family, native to Amazonia. This plant is particularly cultivated for extracting latex to make natural rubber. In Côte d'Ivoire, rubber cultivation produces a large quantity of seeds, which are under-exploited. Hevea seeds are a source of fat (44% lipids) and protein (Okoma Muriel *et al.*, 2018). And only 10% of the production is used to make seedlings (Kouakou *et al.*, 2018), which means an annual production of around 75,000 to 100,000 tonnes of Hevea seeds for Côte d'Ivoire. This corresponds to a yield of 33,000 to 44,000 tonnes of Hevea oil, based on the lipid content of 44% of the plant material (Kone, 2019).

Detoxification or detoxication (detox for short) is the physiological or medicinal removal of toxic substances from a living organism, including the human body, which is mainly carried out by the liver

(Errami *et al.*, 2012; Genius et al., 2013; xxx). The detoxification of Hevea seeds is therefore essential if this oil is to be put to better use. These seeds present an opportunity in view of the growing global demand for vegetable oil due to galloping population growth (Houessionon, 2022).

This optimisation work was carried out with the aim of adding value to Hevea oil in the food sector. The biochemical hydrolysis of cyanogenic glycosides was introduced to detoxify the oil because of its interesting chemical properties, since this rubber tree oil is dry (iodine index greater than 150 I2/100g). This means it is rich in unsaturated fatty acids (Koffi *et al.*, 2022). It is also rich in essential fatty acids, particularly omega-3 and omega-6 (Kouassi *et al.*, 2020, Berriozabalgoitia *et al.*, 2022). This dryness is thought to be linked to the high unsaturation of some of its fatty acids, with up to 6 ethylenic double bonds (Alain, *et al.*, 2016). This is one of the reasons why rubber tree oil is most often used as a resinification aid in anti-corrosive coatings and adhesive products (Louise Ocho *et al.*, 2001).

Almond extracts are generally rich in polysterols (34mg per 30g). Polysterols are plant compounds which, when consumed as part of a healthy diet, are thought to reduce LDL cholesterol (Fumeron *et al.*, 2015) by 5-15%. They therefore help to combat bad cholesterol, and thus contribute to fighting the risk of cardiovascular disease, including type II diabetes, gallstones, hypertension and colon cancer, to name but a few. Because they are rich in essential fatty acids, bitter almond extracts are also very useful for menopausal women, for example (Lecerf, 2021).

In addition, work carried out in 2019 by Koné et al on the use of Hevea seed cake in guinea fowl feed highlighted the nutritional value of the extracts (Kone, 2019). However, despite all its potential, the oil has not yet been used in human food because it contains a high level of HCN (>50mg/kg), a level that would be dangerous according to standards if consumed as food (Scicom, 2016), and therefore harmful to human health. The cyanide ion, with its high affinity for haem groups, prevents haemoglobin from transporting oxygen. This ion therefore takes the place of oxygen by attaching itself to cytochrome oxidase, thus blocking the respiratory chain. This leads to nervous disorders (Puskarczyk, 2006).

Hydrocyanic acid is therefore a gangrene for the consumption of rubber oil and cake from rubber seeds. For humans, the acute lethal dose of HCN is estimated at between 0.5 and 3.5mg/kg body weight (CEAEQ, 2016). However, detoxification of Hevea seed extracts (oil, oilcake) remains complex, as most of the cyanides in this material are blocked in the heterosidic combination state by glycosides, such as amygdalin, for example. The biochemical hydrolysis of this material carried out in this work made a major contribution to detoxifying the Hevea oil. It made it possible to avoid using exogenous enzymes. Using β-glucosidase, amygdalin, the main glycoside in bitter almonds, is degraded to release this toxin in the form of hydrocyanic acid. The extracts from these almonds are then detoxified from the cyanides by entrainment of hydrocyanic acid vapour, using a steam generator adapted to the reactor.

This work is being carried out with the aim of adding value to the Hevea seed through its oil, in order to provide it with a high added value. To this end, we have undertaken to optimise the biochemical hydrolysis of cyanogenic glycosides, in order to detoxify Hevea kernel extracts (oil and cake) from this hydrocyanic acid toxin. Randomisation of biochemical hydrolysis was used to develop the Y_{90} isothermal model. This model provides optimal conditions for the detoxification of bitter almond extracts in a food context, given that industrial processes that use fewer chemicals (enzymes, acids, etc.) are increasingly in demand.

The present study sheds light on the factors influencing the hydrolysis of cyanogenic glycosides by almond emulsin and presents the optimum conditions for detoxifying bitter almond extracts in the food sector. It has contributed to the development of a new process for detoxifying Hevea almond extracts, in particular the oil and the cake.

2. Materials and methods

2.1. Equipment

2.1.1 Plant material

The plant material consisted of rubber tree (Hevea brasiliensis) seeds of clone *PB 260 collected from the various plantation sites of the CNRA (Centre National de Recherches Agronomiques) de Man in Côte d'Ivoire (**Figure 1-b**). These seeds were sun-dried on racks for about 2 weeks. They were then dehulled, oven-dried at 105°C for 24 hours, and ground. The moisture content was considerably reduced (H < 6%), to ensure that the samples were well preserved. This pre-treatment work on the almonds (**Figure 1-a**) was carried out at the LAPISEN laboratory of the institute National Polytechnique Félix HOUPHOUËT-BOIGNY in Yamoussoukro, in order to protect the samples from the degradation effects of mould and other undesirable enzymatic activities (**Figure 1-c**).

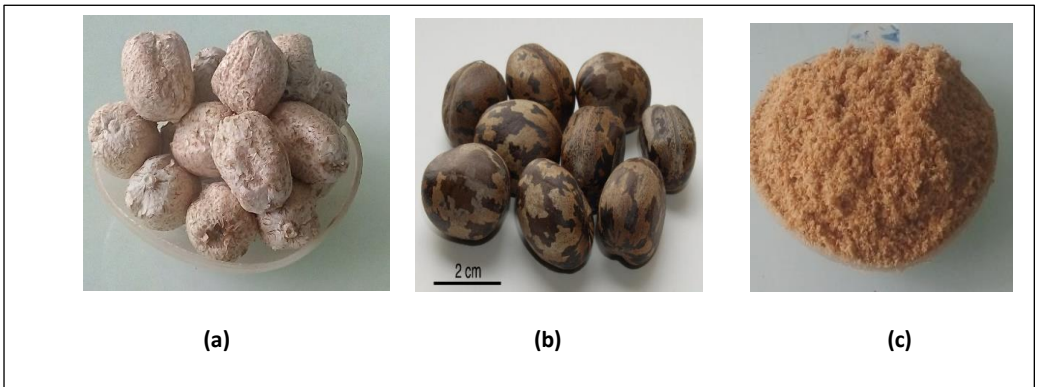


Figure 1: Some images of the pre-treatment of the almond: Hevea almond (**b**); Almond mash (**a**); Detoxified almond mash (**c**)

2.1.2. Chemical and technical equipment

2.1.2.1. Chemical materials

The chemicals used in this work were: picric acid (Sigma-ALDRICH ®), sodium carbonate (ACROS Organics), distilled water, Grosseron "Watchman" 3MM CHR paper (1cm x 5 cm) 0.34mn thick and with an absorption speed of 130mm/30min, and Sanitex allure paper. Glassware and other standard laboratory equipment such as a magnetic stirrer and PH meter were also used.

2.1.2.2. Technical equipment

The technical equipment consisted of a spectrophotometer (JASCO V-530, Japan) for dosing the samples, a camera (HUAWEI), a magnetic stirrer (IKAMAG, RCT), a PH meter (HANNA, HI5221) and a laptop computer (HP CORE i5, vPro). The latter was used for statistical processing of the data collected after the assay.

2.2 Methodology

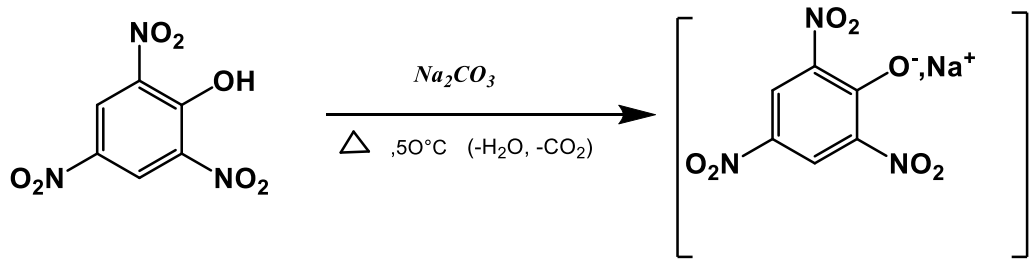
The various samples were prepared using pre-prepared picro-sodium paper. This paper was used as a sensor. It was used to detect the hydrogen cyanide vapour released during the biochemical hydrolysis of cyanogenic glycosides during emulsification. This method is based on that described by Rezaul Haque et al, 2012 (CAC/RCP 73-2013). It is a colorimetric determination of the cyanide ions contained in the coloured solutions, which obey Beer-Lambert's law. The hydrolysis yield was assessed after quantifying the hydrocyanic acid content released using the following expression from equation (**Eqn.1**):

$$(HCN(mg/kg) = \frac{420*A*100}{Pe} \qquad \textbf{Eqn. 1}$$

Pe: *poids du broyat*, **A**: absorbance

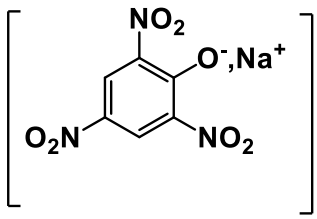
2.2.1. Paper preparation

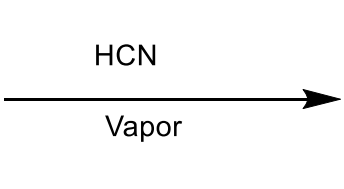
Watchman" paper (1cm x 5cm) is impregnated with a 1% picric acid solution, then removed and soaked in a 5% sodium carbonate solution, then dried in an oven at 50°C for approximately 20 to 30 minutes. The production of picro-sodium paper is made possible by the reactivity of the hydroxyl group on the aromatic nucleus of picric acid. The position of this group (-OH) group between the two nitrile groups makes the H^+ proton more labile. This leads to the substitution of the proton by the sodium ion. The mechanism of this chemical reaction involves a structural modification of picric acid (T.N.P). The reaction diagram is shown in equation (**Eqn.2**).

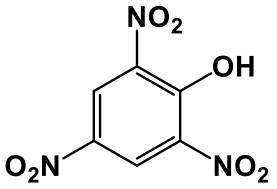


(Eqn.2)

picric acid solution *picro-soda paper*







+ (Na+, CN-) Eqn.3

picro-soda paper *coloured solution*

2.2.2. Preparation of samples for the assay

The various sampling stages for the spectrophotometric quantification of hydrogen cyanide content are shown in Figure 1. The detailed stages are as follows: capture of the gas, elution of the picro-sodinated paper in distilled water. During HCN vapour detection, once the picro-sodinated paper, initially yellow in colour, comes into contact with the hydrogen cyanide gas, it takes on a blood-red colour. Elution of the paper in distilled water (5ml) regenerates picric acid accompanied by sodium cyanide solution. This makes it possible to quantify the HCN released during the hydrolysis of cyanogenic glycosides by maceration. Samples of coloured cyanide solutions were thus obtained. The mechanism for obtaining them is based on the reaction scheme given by equation (**Eqn.3**).

2.2.3. Choice of factors influencing hydrolysis yield

Almonds are generally rich in mini-nutrients, so are mainly made up of: proteins, water, lipids, carbohydrates, vitamins and mineral salts. These different chemical components are complex molecules that take part in a number of reactions, the influencing factors of which are: temperature, maceration, mass, stirring speed and stirring time. We therefore chose these 4 influential factors to carry out the experimental design. The food context meant that we worked under isothermal

conditions, because of the nutritional quality of the product we are interested in, particularly the oil. Three isothermal models were developed for this purpose. The factors chosen were: the mass of the grind, the speed of agitation, the duration of agitation, and the volume of distilled water. It should be noted that the lesioning of plant tissue and hot maceration is a method of eliminating hydrocyanic acid from cyanogenic foods. This weak acid is highly volatile and easily lost during transport (Leibig-Denigès., 1971). For this reason, during the volumetric determination of total cyanides, because cyanide ions are highly reactive, they are usually trapped in an ammonia solution or in a sodium hydroxide solution (Han. W *et al.*, 2017). The various models were developed using a two-level factorial design, and the level of hydrogen cyanide HCN (mg/Kg) released was chosen as the experimental response.

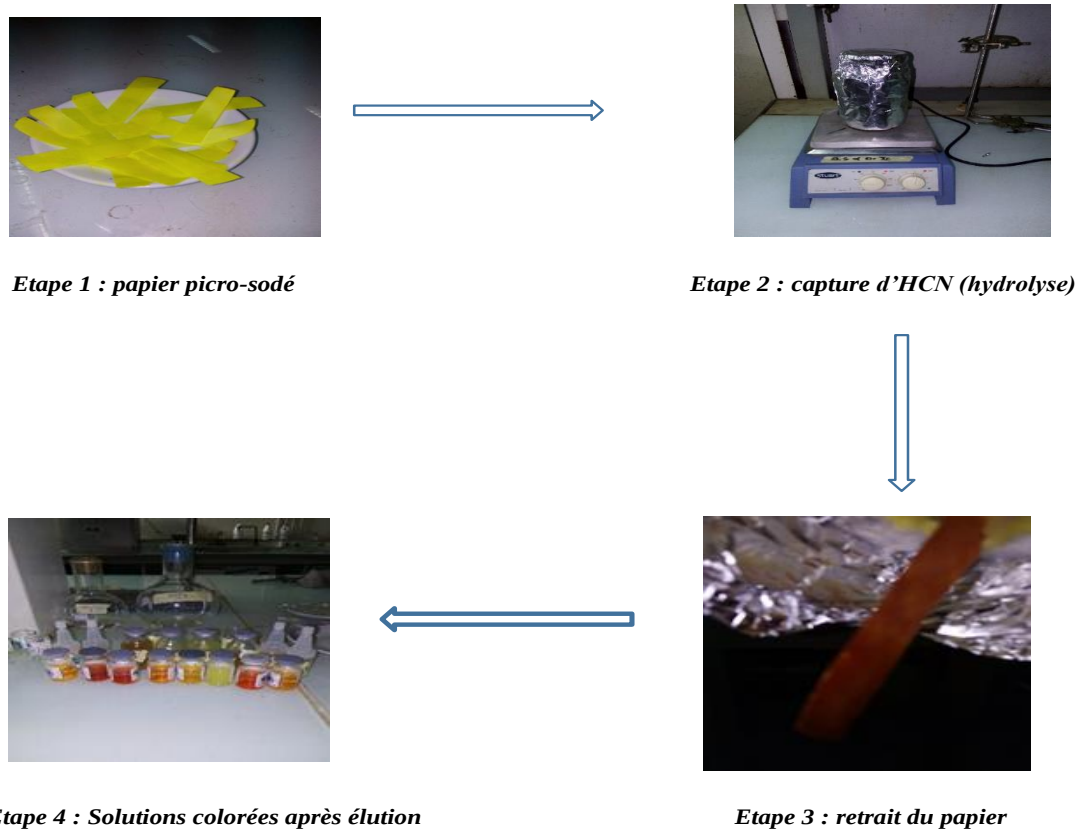


Figure 2: The different stages in sampling coloured cyanide solutions

2.2.4. Areas of experimentation

The different areas of experimentation were determined according to the equipment available in the laboratory. In terms of granulometry, the choice was also made in relation to the sieves available in our laboratory. Tables **I, II, III** and **IV** show the experimental areas according to the model and for each of the set temperatures.

2.2.5. General test procedure

The hydrogen cyanide gas was captured in the dark (**Figure 2**) and by maceration at various temperatures. A volume of distilled water was added to a precise mass of crushed material, the ratio of which was X1. The mixture is then allowed to macerate at a temperature based on the isothermal model and a stirring speed of X2. The maceration time is X3. During maceration, the picro-soda paper is suspended to detect cyanide ions (HCN, vapour). In the presence of cyanide ions, the paper takes on

a blood-red colour. The tests were carried out in triplicate under the different conditions in each area. The coloured cyanide solutions obtained were measured spectrophotometrically at 540nm.

Tableau I : *Domain of the Y_{50} model used to randomise hydrolysis (2^4)*

N°1(T=50°C)	**Experimental domain of factorial design 2^4**	
Level	**-1**	**+1**
X_1(A) : mass of crushed materiel (g)	**3**	**8**
X_2(B) : volume of distilled water (ml)	**50**	**200**
X_3 (C) : Stirring time (min)	**30**	**60**
X_4 (D) : Shaking speed (rpm)	**40**	**60**

Tableau II : *Domain of the Y_{50} to 50°C, model used to optimisation hydrolysis (2^3)*

N°2 (T=50°C)	**Experimental domain of factorial design 2^3**	
Level	**-1**	**+1**
X_1 (A) : Volume Weight (g/ml)	**2/50**	**5/50**
X_2 (B) : Shaking speed (rpm)	**60**	**90**
X_3 (C) : Stirring time (min)	**30**	**60**

Tableau III : *Domain of the Y_{70} to 70°C model used to optimisation hydrolysis (2^3)*

N°3 (70°C)	**Experimental domain of factorial design 2^3**	
Niveau	**-1**	**+1**
X_1 (A) : Volume weight (g/ml)	**2/30**	**5/50**
X_2 (B) : Shaking speed (rpm)	**60**	**90**
X_3 (C) : Stirring time (min)	**45**	**80**

Tableau IV : *Domain of the Y_{90} to 90°C, model used to optimisation hydrolysis (2^3)*

N°4 (90°C)	**Experimental domain of factorial design 2^3**	
Niveau	**-1**	**+1**
X_1 (A) : volume Weight (g/ml)	**2/30**	**5/50**
X_2 (B) : Shaking speed (rpm)	**60**	**90**
X_3 (C) : Stirring time (min)	**30**	**90**

2.2.6. Optimisation of isothermal models

The optimisation of each of the Y_{50}, Y_{70} and Y_{90} models obtained following the statistical processing of the experimental data was carried out using Microsoft Office Excel software with the solver add-in that allows optimisation using the Simplex method (Ribeiro-Santos R., 2017, Roslyn. M *et al.*, 2003). The influences of the various factors were ranked according to the Pareto principle.

2.2.7. Selection of the Y90 model developed at 90°C

Of the three models developed, the Y90 model was selected on the basis of its glycoside hydrolysis yield, in line with our aspirations in the food context. This choice was made following a comparative study carried out over a period of 80 minutes in optimal situations for each of the models. The samples were assayed using the silver method of Liebig-Denigès, 1971. The principle of this assay method is based on the property of silver nitrate to provide, with alkali cyanides, soluble double cyanides which are decomposed by an excess of silver nitrate to give a silver cyanide precipitate. The process is carried out in an ammoniacal medium, which ensures that the cyanide ions remain dissolved in the silver cyanide state. Under these optimum conditions for each of the models, the HCN released is vapour-drained and then measured using a silver nitrate solution (0.02N), in the presence of a KI solution (5%) and an ammonia solution (10%). Potassium iodide is used as an indicator at the end of the reaction, which is marked by a yellowish-white precipitate of silver iodide (a persistent opalescent cloud), due to the reaction of excess silver nitrate with potassium iodide, when all the cyanide is converted to the double salt.

3. Results and Discussion

3.1 Biochemical hydrolysis of cyanogenic glycosides

The results of the statistical processing of the experimental responses used to develop the various models are presented in the following figures: Figure 3; 4; 5; 6; 7 and 8 for the Y_{50} model carried out at 50°C, Figure 9; 10 and 11 for the Y_{70} model carried out at 70°C and Figure 12; 13 and 14 for the Y_{90} model carried out at 90°C. The tests carried out at 50°C for the two-level factorial design with the 4 factors (2^4) on the domain of Table I, contributed to the development of these models, as these tests made it possible to screen the influential factors according to their significant effects on the experimental response and their interaction effects with the other factors.

3.1.1. Results of the factorial design 2^4

The results of 16 randomisation trials of hydrolysis carried out at 50°C using almond emulsin provided a more or less clear understanding of cyanogenesis in rubber kernels. The ability of the kernel to supply hydrocyanic acid reflects the rate of glycoside degradation during hydrolysis. Data processing using Anova statistical software, based on p-values ($p<0.05$), revealed that factor (C), duration of agitation, did not influence the experimental response (Figure 3). However, its interactions with the other factors (A, B and D) were not negligible. So the factors likely to have the greatest influence on hydrolysis in the area mentioned above in Table I are still the agitation speed, the mass of the grind and the volume of water. Analysis of the results of factorial design 24 led to screening of the influential factors according to the Pareto principle (**Figures 4, 5 and 7**). This led to a reduction to 3 factors by combining the mass of the grind and the volume of distilled water.

By processing the data using the Pareto method, we were able to rank these factors in order of importance. Using this method, the causes are ranked in order of priority according to the effects of the different factors, as well as those of their interaction with the other factors. On each of the Pareto diagrams, the curve in red corresponds. These experimental data confirm the results of the work carried out by Gleadow et al in 2003 on amygdalin β-glycosidase in the toxicological study of cyanogenic plants of the genus 'Eucalyptus nobilis' in Australia. This experimental data also confirms the work carried out by Younes Zebbiche in 2013 on the determination of cyanides in bitter almonds and apricot kernels. According to these authors, almond emulsin activates the almond's own enzyme by bringing

amygdalin into contact with glucuronidase, thus optimising the degradation of amygdalin to release hydrocyanic acid (Zuzana *et al.*, 2021; Trincone, 2015; Codex Alimentaruis, 2013). Furthermore, this study shows that the biochemical hydrolysis of amygdalin by this pathway is optimised by controlling the above-mentioned factors, since the predominant factors for the release and quantification of cyanides remain pH and temperature (Silitonga *et al.*, 2016). Cyanogenesis in food can therefore be provoked or induced by taking these factors into account. Exogenous enzymes can therefore be avoided in order to reduce treatment costs. Many of the studies on β-glycosidase from sweet almonds (amygdalus comminus L.) are too old, so it remains difficult, if not impossible in most cases, to use these results for comparative purposes. Nevertheless, they agree that almond emulsin hydrolyses its glycosides (Yuan Si *et al.*, 2022), to remove hydrocyanic acid. This biochemical hydrolysis promotes the synergistic action of all the endogenous enzymes that hydrolyse almonds.

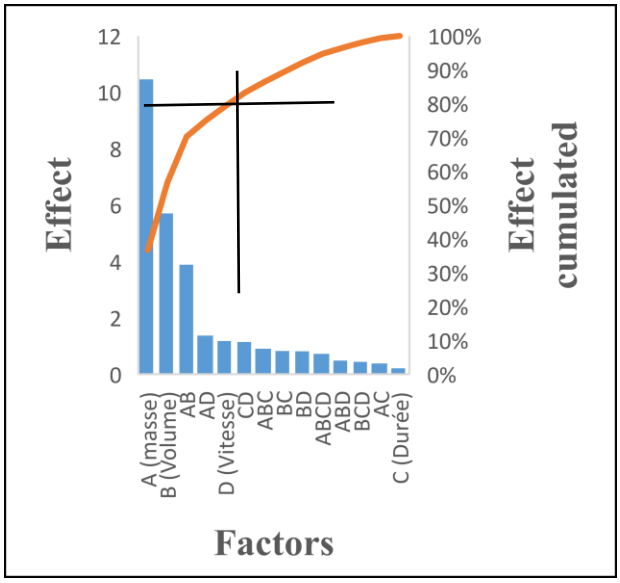


Figure 3 : Diagram of Pareto to Y_{50} (2^4)

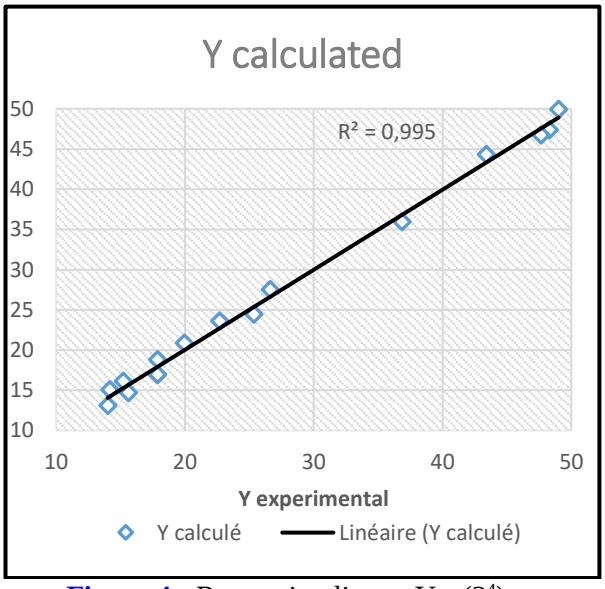


Figure 4 : Regression line to Y_{50} (2^4)

3.1.2. Modelling at 50°C (Y_{50})

The diagram reveals that the factors C (duration), A (mass/volume), interaction AC (density-duration) and interaction AB (mass-volume and stirring speed) are those that influence the experimental response. Their contributions are 33.54%, 20.27%, 12.18% and 10.51% respectively. Statistical analysis of the data led to the Y50 model, the mathematical expression of which is given by equation (**Eqn.4**).

$$Y_{50}= 3.008925-0.8331*XA +1.3748*XC- 0.4322XA*XC- 0.500575 \quad \textbf{(Eqn. 4)}$$

The tests at the centre of the domain shown in Table II were carried out. These were used to validate the Y_{50} model, as well as the other two models, following the method described by Faul in 2009 (Faul *et al.*, 2009). **Figures 4** and **8** respectively show the correlation between the values predicted by the model and the experimental values during the tests at the centre for factorial designs 2^4 and 2^3 carried out at 50°C. The correlation coefficients are respectively (R2=0.995) for factorial design 2^4 and (R2=0.9178). for factorial 2^3. The effects of the factors and their interactions with the other factors are shown in **Figure 6** and **Figure 7** respectively.

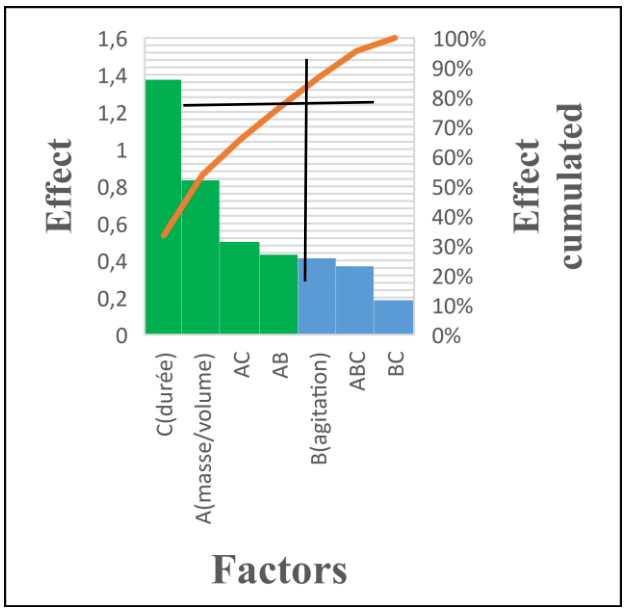


Figure 5 : Diagram of Pareto to Y_{50} (2^3)

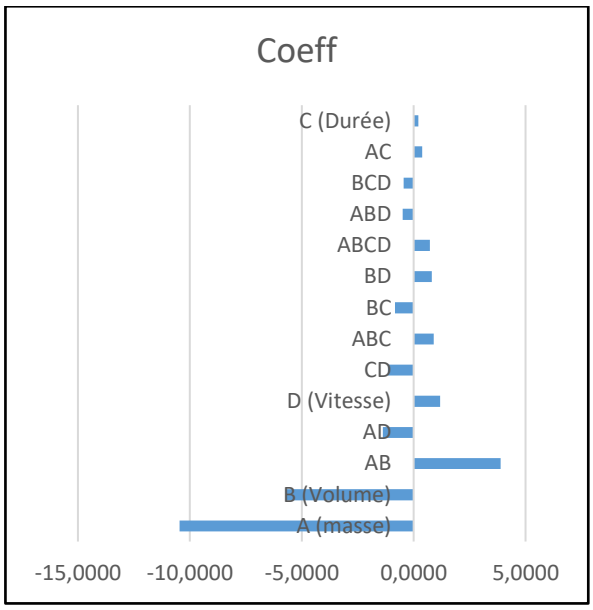


Figure 6: Effect coefficients for Y_{50} (2^4)

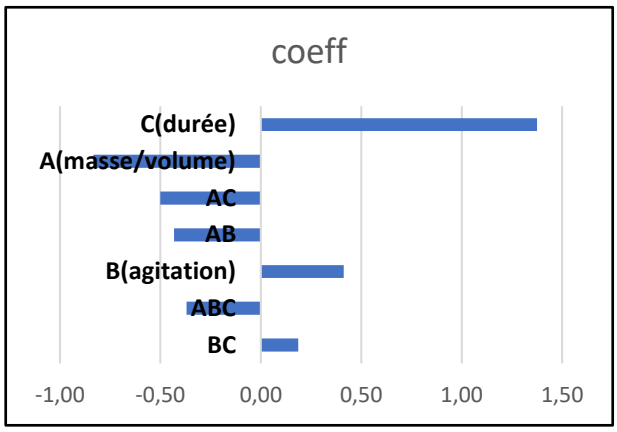


Figure 7 : Effect coefficients to Y_{50} (2^3)

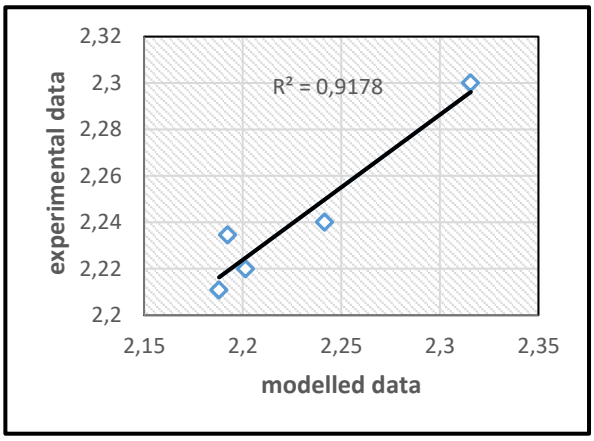


Figure 8 : Rgression line to Y_{50} (2^3)

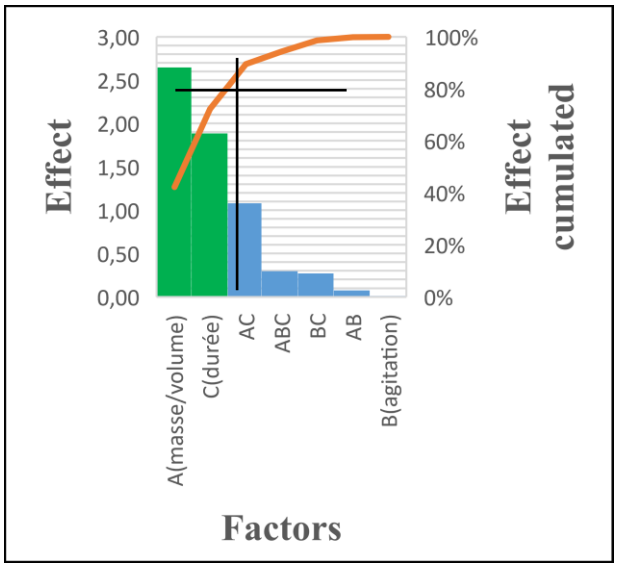


Figure 9 : Diagramme of Pareto to Y_{70} (2^3)

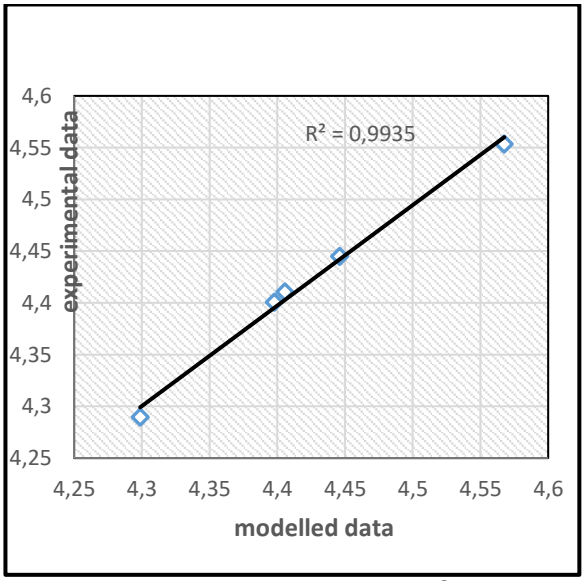


Figure 10 : Rgression line to Y_{70} (2^3)

3.1.3 Modelling at 70°C (Y_{70})

The previous diagram in Figure 9 shows that only factors A (mass/volume) and C (duration) should be considered when the temperature is around 70°C. As the temperature increases, the interaction effects and the effect of factor B (stirring speed) are not significant.

These factors contributed 42.18% for factor A and 30.07% for factor C respectively to the hydrolysis of almond glycosides by emulsin (Figures 9 and 11). This model was validated by performing the same tests in the case of the previous 50°C model. The resulting mathematical model is shown in equation (5). The correlation coefficient between the predicted values and the experimental values for the Y_{70} model is (R=0.9935), and is given in **Figure 10**. The effects of the factors and their interactions are shown in **Figure 11**:

$$\mathbf{Y_{70} = 6.28725-2.645625*XA+1.886175*XC} \qquad \mathbf{(Eqn.\ 5)}$$

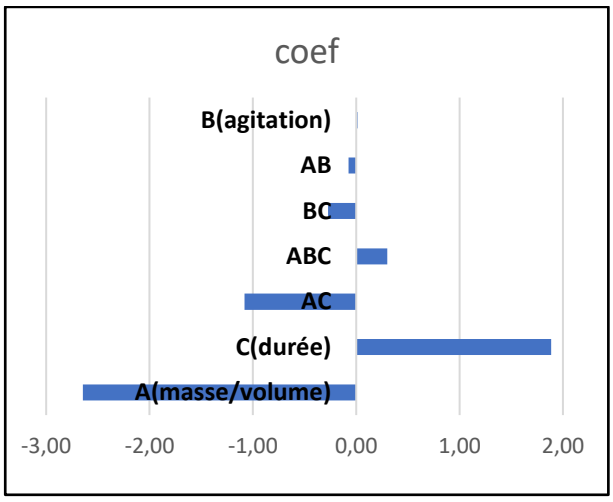


Figure 11 : The effect coefficients of Y_{70} (2^3)

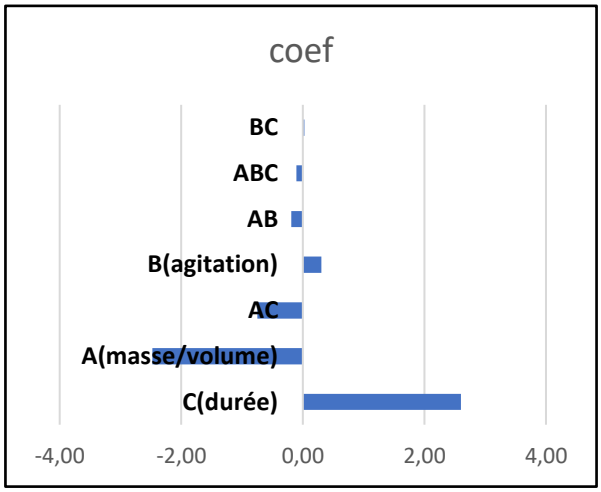


Figure 12: The effect Coefficients of Y_{90} (2^3)

3.1.4. Modelling at 90°C (Y_{90})

As confirmed previously, Figure 13 effectively shows that the two factors A (mass/volume) and C (stirring time) remain the only ones to be controlled in order to optimise our detoxification of rubber kernel extracts. At this temperature of 90°C, factor A comes second with a statistical weight of 38.37% after the 40.32% of factor C. The third resulting model is the Y_{90} model in equation (6). The correlation coefficient between the values predicted by the Y_{90} model and the experimental values carried out at the centre of the domain in the case of this Y_{90} model is given in **Figure 14** (R= 0.9779). The coefficients for the effects of the factors and their interactions are shown in **Figure 11**:

$$\mathbf{Y_{90} = 6.8132875- 2.4734375*XA+ 2.5989125*XC} \qquad \mathbf{(Eqn.\ 6)}$$

The models were subjected to a comparative study in order to select the ideal model for our detoxification work. The evaluation of the levels of HCN entrained by the steam over time, under the optimum conditions of each model over a period of 80 minutes, at 20-minute intervals. The analysis of the results ranked the Y_{90} model above the other two, as it presented the best yield, and was therefore well placed to optimise the biochemical hydrolysis of cyanogenic glycosides from rubber kernels. The model is therefore favourable in involving all the enzymes of its own, synergistically activating all the endogenous enzymes hydrolysing the kernel during hydrolysis of the cyanoglycosides. After 80min,

this model showed the highest hydrolysis yield of cyanogenic glycosides (94.37%). As for the other two models, after 80min, their cyanoglycoside hydrolysis yields were 76.43% for Y_{70} and 46.82% for Y_{50} respectively.

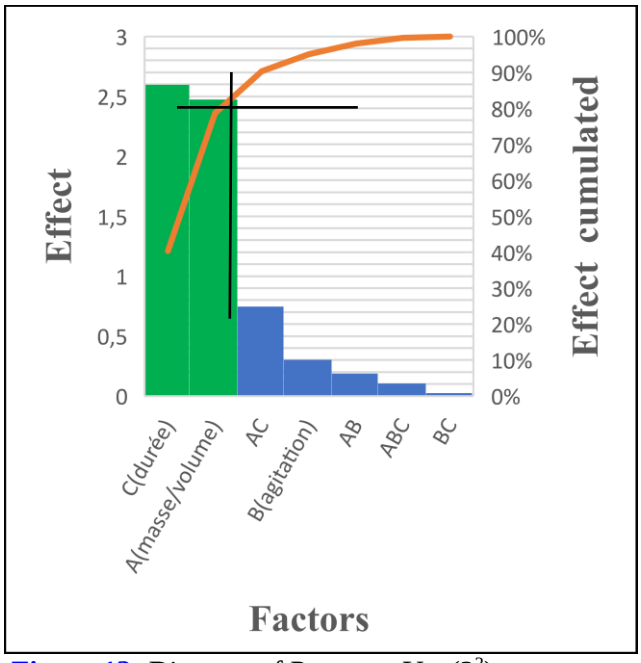


Figure 13: Diagram of Pareto to Y_{90} (2^3)

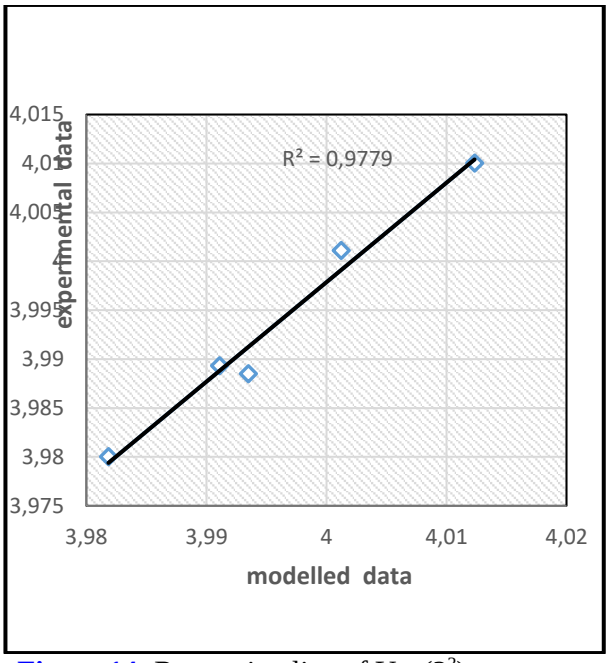


Figure 14: Regression line of Y_{90} (2^3)

After 60min of steam training, these same models Y_{70} and Y_{50} show approximately the same HCN content released, with a hydrolysis yield relatively equal to (42%). Unfortunately, the complex structure of some glycosides, such as amygdalin, also makes their hydrolysis complicated, compared with the hydrolysis of starch or sugar (FAO/WHO, 2012). For this reason, it seemed important to optimise the hydrolysis of these glycosides biochemically. The two-level factorial design was effective for this purpose. This method revealed that the Y_{90} model, under these optimal conditions, would be favourable, as it provided an estimated hydrolysis yield of 94.37% of the heterosides, the most abundant of which in bitter almonds is amygdalin, bearing in mind that complete degradation of 1 g of amygdalin releases around 59 mg of HCN (Dirk Selmar., 2016). This toxin prevents the consumption of bitter almond extracts, as the acute lethal dose of HCN is estimated at between 0.5 and 3.5 mg/Kg of body weight (Thi Thanh *et al.*, 2019).

3.1.6. Contribution and advantages of the model in food production

Detoxification methods for removing toxic compounds (cyanides) from almonds or oleaginous fruits are generally based on the physical properties of cyanogenic glycosides, including their solubility in water, their volatility and other factors that interfere with the hydrolysis reaction of these compounds, such as pH, water activity, temperature and physical state. The hydrolysis of cyanogenic glycosides is therefore the only way to remove cyanides in the hydrogen cyanide state from milled products for food use. Biochemical hydrolysis is an enzymatic reaction in which the catalytic enzymes used are specific to the almond (endogenous enzyme). It is the most frequently used process in food chemistry, as it does not use too many chemicals. Of the technologies available to detoxify cyanogenic foods, those based on enzymatic hydrolysis are the most promising, due to their good potential yields (Codex Alimentaruis., 2013). Although enzymatic hydrolysis processes are relatively recent, the last decade has seen good results in this field. However, there is still room for innovation to optimise yield and

productivity and reduce energy costs. In any case, the enzymatic hydrolysis of glycosides with an endogenous or exogenous enzyme gives the same experimental response, except that the catalytic route using an exogenous enzyme reduces the reaction time (Pan. R *et al.,* 2019). The Y90 model, a total detoxification of bitter almond extracts over a period of more than 80 minutes. Because all the factors listed are involved, all the cyanides are unblocked from their heterosidic combination states. These remarks are supported by the work of Pan et al 2019, and confirmed by the results of the work of KaLakuko et al in 2021, on the hydrolysis of linamarin to detoxify cyanogenic cassava (Kalakuko. E *et al.,* 2021). According to this work, despite the hydrolysis time of 70h, there was a residual HCN content of about 2.09±0.00mg/kg

So, with these data, we can say that the model in question is effective, because over the long term (long duration of experimentation), the extracts will be devoid of this toxin. The introduction of this Y_{90} model into the detoxification process that we have developed as a result of our work has made it possible to produce a detoxified, and therefore consumable, rubber tree oil. This was an important step in the process. The successive stages in the process developed to detoxify the Hevea oil are: solar drying of the seeds (2 weeks); drying of the kernel in an oven at 105°C (24h); grinding of the dried kernel; emulsification of the grind in water under the optimum conditions of the Y_{90} model; drying of the filtrate at room temperature (6h); steam roasting of the grind in 1h (0.1g/ml), then cooling and extraction of the oil using an organic solvent.

Conclusion

At the end of this work of randomisation of the biochemical hydrolysis of cyanogenic glycosides in the context of the detoxification of Hevea kernel extracts, we can affirm that the Y_{90} model optimises the detoxification of the crushed kernel with a hydrolysis yield of around 94.37%, including bitter kernels. This model makes it possible to rid almond extracts of most of the cyanides in the form of HCN hydrocyanic acid during the biochemical hydrolysis of rubber kernel glycosides. In addition, steam stripping is considered to be an essential step in the detoxification process of these extracts in the food context, as it does not use chemical inputs. The β-glycosidase of cyanoglycosides, such as amygdalin, is randomised under the following conditions of the model: duration 80min; temperature 90°C; mass/volume ratio of water 2g/30ml; stirring speed 50rpm, and therefore no exogenous enzyme is necessarily required since the food situation obliges. We plan to develop an innovative process for detoxifying rubber kernel extracts such as oil and cake for food purposes. A well-established flow chart setting out all the stages in the process will be developed, following the method for making atiéké from cyanogenic cassava. In this way, we would create added value for our agricultural by-product (rubber seeds) and improve the income from our rubber plantations.

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