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Optimization of the Extraction of Sorghum's Polyphenols for Industrial Production by Membrane Processes

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Abstract

For a large-scale production of sorghum's polyphenols for food and medicine by membrane processes, different extraction parameters (temperature, duration and nature of solvent) were optimized. For a production in respect of environmental standards to an extrapolation of technology to the semi-industrial scale in the developing countries, 14 hours of extraction with magnetic or mechanical stirring at room temperature with a ratio of 1g of solids per 150 ml of water and a neutral pH were selected as optimum extraction conditions

Keywords: Sorghum caudatum, foliar sheath, temperature, solvent, polyphenols, extraction.

Introduction

Several studies revealed the sorghum richness in phenolic compounds[1-10] and their impact on human health[4,11-17]. But the extraction of plant polyphenols, especially from red sorghum is undoubtedly the most difficult technological operation in the production of these secondary metabolites. To overcome the instability of anthocyanin, polyphenols dual functions (natural dyes and molecules with biological activities), in neutral or alkaline solution, the extraction solutions are often acidified. Methanol[18], ethanol[19-21] and water[22], either alone or in mixture, acidified with various acids are commonly used as extraction solutions[23-26]. Acidified methanol seems to be the best extraction solvent and would be 20% more effective than ethanol and 73% more effective than water[27]. According to Metivier's studies in 1980 on the pulp of the wine[28] the most effective acids with methanol are citric acid 5% and hydrochloric acid 10%. Higher concentrations of HCl do not allow more important extraction, but rather decreases the rate of extraction, probably due to hydrolysis of anthocyanin into aglycones, which, much less stable, may be degraded into colorless compounds (chalcones) resulting in a loss of color of the medium[29-30].

Among the solvents commonly used for the extraction of polyphenols of sorghum we can mention the acidified methanol, the acetone/water (7/3)[31]. More recently in 2010, Khalil et *al*[32] used the butane-1,3-diol/ethanol to extract a new natural phenolic compound. In the food industry, the use of methanol as an extraction solvent is forbidden due to its toxicity. Aqueous solutions containing 300-1000 ppm of SO_2 are also used in these industries and provide crude extracts containing more than 80% anthocyanins[33].

In the present work, the objective of extracting the polyphenolic compounds in sorghum is to obtain extracts of polyphenols that can be used as ingredients in industrial circuits manufacturing of various bioproducts, as well health products (medicine, pharmaceuticals, and cosmetics) and nutritional products. Thus, the chemical nature of the extraction medium and conditions for implementation of the solid-liquid extraction must be compatibles with unitary technological operations that we plan to use for production testing at the pilot scale. The envisaged process must also be able to be operated in a least requiring technical environment and which is compatible with certain existing technological conditions in developing countries, producers of the vegetable (Africa) where this process and the associated know-how could be transferred to an local small and medium-size enterprises (SME). Total elimination of organic solvent in the extraction process would be an ideal condition for a production at low cost while meeting the requirements of the standard organic solvent. Moreover, in order to make the process as inexpensive as possible while respecting the enforcement objectives of the finished product, a compromise must be sought in energy expenditure, time of extraction, technology implement and the nature of acid or base used. The search for optimal conditions for extraction must be done under these constraints of technical objectives and market targeted.

Material and Methods

Plant Material: Samples of leaf sheath of Benin red sorghum (*Sorghum caudatum* L.) were harvested from "Kpakpassa", a village at 12 km from the city of Savalou in the center of Benin. A specimen was deposited at the National Herbarium at the Faculty of Science and Technic, Abomey-Calavi University. After collection, the samples were dried away from sunlight until stabilization of their mass and then cut and ground in powder to allow good penetration of the extraction solvent.

Methods: In the context of our studies, the conditions for polyphenol's extraction at laboratory scale, from the leaf sheath of *Sorghum caudatum* were defined in anticipation of use of membrane technologies conditions for extraction-concentration and physical characteristics of materials commonly used in these industrial production units. Thus, the chemical nature of extraction solvents and acidity were carefully selected in accordance with the usual industrial equipment (tubing, pumps, sensors) and production standards in the food industry.

An aliquot of 1 g of powder is put under slightly magnetic stirring (150 rpm) at cold with a volume V (ml) of extraction solution acidified or not for 2 h at room temperature (20 at 25°C). For choosing of extraction medium we compared the extraction at different pH environments in hydroalcoholic and aqueous medium. According to Extraction protocols published in the literature[37-39], methanol (M) and ethanol (E) were chosen as alcohols in proportions: 75% (M75, E75), 50% (M50, E50) and 25 % (M25, E25) in water. A mineral acid, phosphoric acid, was used at low concentrations, because it is no health risk and on the stability of the desired pigments (anthocyanins). This acid also has the advantage of being less aggressive for the equipment that we plan to use for large scale production. The extracts are then filtered and analyzed by UV/Visible spectrophotometry between 250 and 750 nm. The total phenolic content was estimated by Folin-Ciocalteu method[34], while aluminum trichloride method[35] was used to quantify the flavonoids. The anthocyanins determination was performed using the method of Ribereau et *al*[36] based on their property to be transformed in colorless derivates by the action of certain reagents such as bisulphite ions.

Results and Discussion

Choice of extraction medium: Extractions realized in aqueous methanol at pH =1 and pH =2 leads to similar UV/Visible spectra (Figure1). The two absorption bands at 280 nm and 470-502 nm are respectively attributed to total phenolic compounds[40] and to 3-desoxyanthocyanins. Indeed, according to the literature, the family of 3-desoxyanthocyanins is characterized by its wavelength of maximum absorption and a bathochromic shift with increasing pH[41]. The M75 UV/Visible spectrum differs from two previous by the appearance of a third absorption band around 360 nm corresponding to flavonols. We note here that the extraction rate is even higher than the proportion of methanol is important.

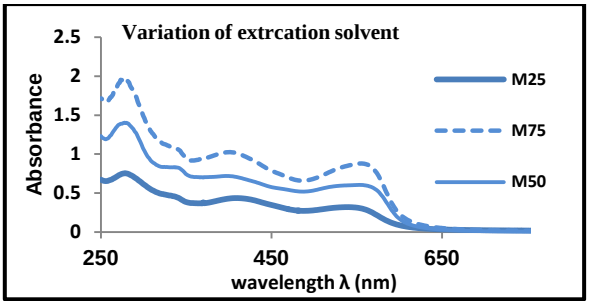


Figure- 1
UV/Visible spectra of methanolic extracts at different proportions of alcohol

For a substitution of methanol by ethanol, the efficiency of the two solvents was compared.

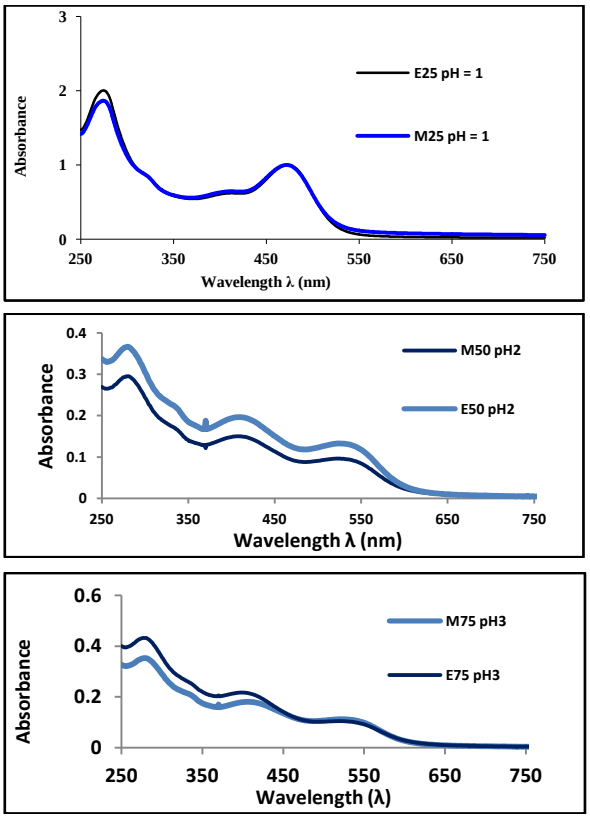


Figure-2
UV/Visible spectra of extracts obtained by magnetic stirring in $EtOH/H_2O/H^+$ and $MeOH/H_2O/H^+$ at different pH

Whatever the type of alcohol used, the results are nearly identical about the nature of the molecules extracted (overlapping curves) with an extraction yield slightly better with ethanol above 50% at pH 2 (figure 2). These results are consistent with the colors of the extracts obtained. But the use of organic solvents is limited to non-toxic solvents and a residual proportion of 25 to 33% by the europeenne regulations[42] for formulations in cosmetics and the production of food additives; we chose to compare the performance of the two alcohols staying within the limit of 25% and by varying the pH.

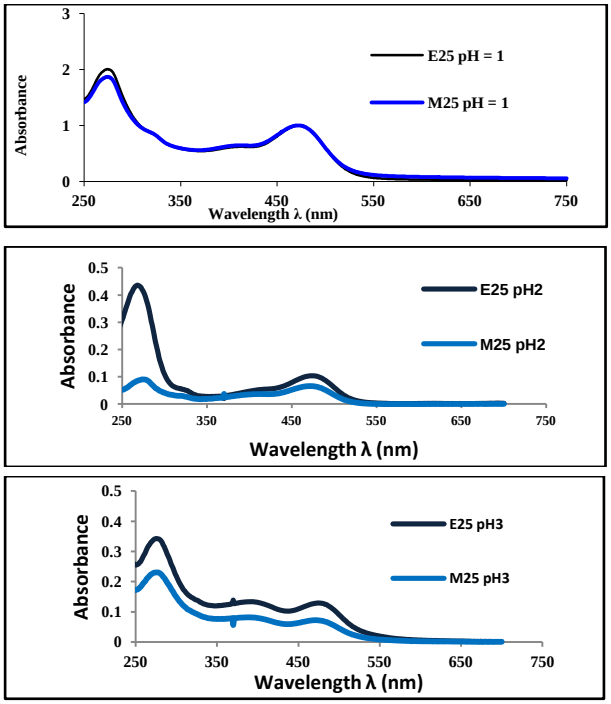


Figure-3
Effect of solvent extraction at different pH

The replacement of methanol by ethanol in water in the same proportions and at the same pH shows spectra with similar absorption bands in both ranges: UV and visible range and a perfect superposition of the spectra recorded at pH = 1 and pH = 3 (Figure3). In conclusion, the hydroalcoholic extract of the leaf sheath of sorghum in acid does not depend on pH and leads to the same families of phenolic compounds. In this case, ethanol is revealed slightly more efficient than methanol. This efficiency, coupled with its lower toxicity led us to choose ethanol for more detailed studies. Thus, we tested the effect of the proportion of ethanol and pH on the extraction of phenolic compounds.

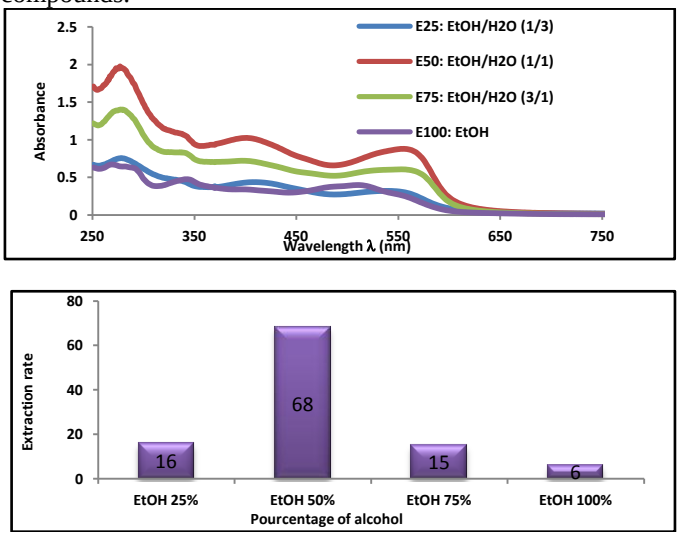


Figure-4
Effect of ethanol proportion on extraction at pH 7

The results show that 50% ethanol gave the best extraction rate (figure 4). This observation was confirmed by mass balance (figure 5). Pure ethanol gave the lowest extraction efficiency. The mixtures ethanol/water (50/50) and (25/75 and 75/25) are respectively 11 times and 4 times more efficient than pure alcohol.

For the effect of pH, aqueous ethanol 25% was used to compare three acid pH and neutral pH.

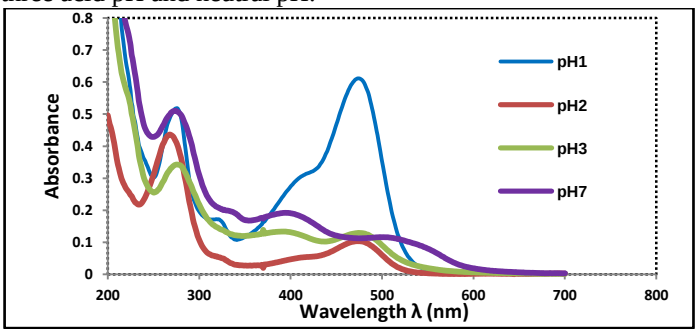


Figure 6
Effect of pH on polyphenols extraction by ethanol 25%

Table 1
Wavelengths of maximum absorption and families of compounds corresponding

pH	λ_{max} (nm)	Families of compound
neutre	280	Total Polyphenols
	310	Phenylpropanoids
	405	Flavones
	520	Anthocyanins
acide	280	Total Polyphenols
	310	Phenylpropanoids
	470	3-Deoxyanthocyanins

At neutral pH, there are four main absorption bands corresponding to four families of polyphenols as indicated in table 1. But at acidic pH there is the replacement of two absorptions in the visible (405 nm and 520 nm) with a main absorption at 470 nm characteristic of 3-deoxyanthocyanins with a shoulder at 405 nm. This observation is due to hydrolysis groups (possibly acyl and oside) related to the basic skeleton of a family of 3-desoxyanthocyanins. We suspect the presence of apigenidin which is the main anthocyanin identified in sorghum seed[30]. Otherwise, we notice a high value of absorbances recorded at pH=1 with regard to pH=2 and pH=3 (Figure6); this could be due to hydrolysis further connections when the acidity increases. It is necessary to note that the studies of Revilla and *al*. have shown that high concentrations lead to acid hydrolysis of anthocyanins, specifically acylated anthocyanins. These authors also indicate that some neutral solvents are as good as acidified solvents for the extraction of anthocyanins. Ethanol can be chosen between 25% and 50% in water for solvent extraction. The variation of pH would select the desired color of the pigment. But ethanol extract gives a very low solubility limiting the use of the finished product. This added to the basic

objectives led us to test the aqueous medium without organic solvent for extraction.

Aqueous extraction at different pH: The extraction is carried out in phosphoric acid solutions at different pH (pH = 1, 2 and 3) and in water at neutral pH (pH = 7). To test the conditions and traditional craft used by people at the base, three basic pH were compared to neutral pH. The base used here is potassium hydroxide.

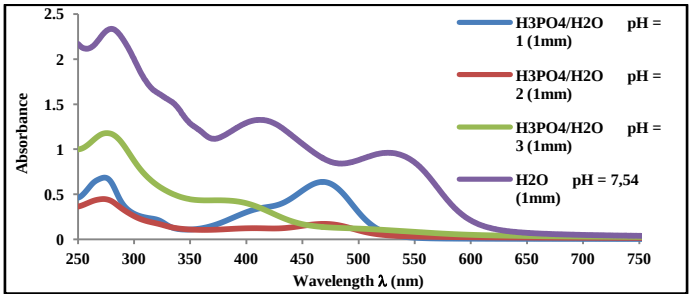


Figure-7
Effect of pH on the extraction in aqueous acidified solution

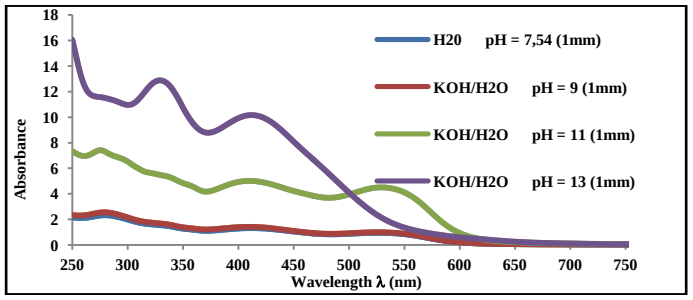
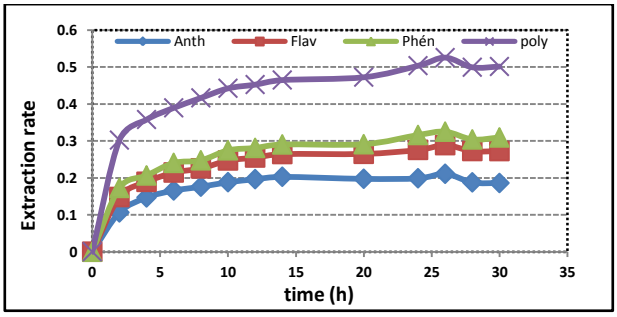


Figure-8
Effect of pH on the extraction in aqueous alkaline solution

At neutral pH, water extracts more anthocyanins and flavones in unhydrolyzed form (figure 7). These results are in agreement with those obtained previously using 25% ethanol. From neutral pH (pH 7) until pH= 11 the spectra are perfectly superimposable both in the UV than in the visible (figure 8). The same families of phenolic compounds are extracted. But in strongly basic pH (pH = 13) the spectral shape changes, suggesting the extraction of other families of compounds. But extraction in neutral medium followed by a modification of pH of the filtered extract gave similar results to those found when the extraction is performed directly to pH considered. The extracted compounds are similar regardless of the pH but undergo structural modifications when the pH changes. Then we retain in light of all these data that water at neutral pH would be the best solvent for extracting polyphenols from the leaf sheath of red sorghum for improved profitability of production in compliance with the requirements of the standards in the field application of these extracts.

Selection of extraction time (kinetics of extraction): In this section, the extraction rate was evaluated over time for 30 hours.



Anth = Anthocyanins; Flav = Flavonoids;
Phen = Phnylpropanoides; poly = Total Polyphenols

Figure-9
Kinetics of sorghum polyphenols extraction

We note that the extraction rate increases with time, regardless of the family of phenolic compounds considered, until 14h and then becomes almost constant (figure 9). After stirring for 24 h, the rate of extracted compounds fall at all the families of phenolic compounds extracted. So, we can retain 14h as stirring time enough for a good extraction yield at lower cost. However, this period may be extended to 24 hours without risk of degradation of compounds extracted.

Choice of extraction temperature: The extractions were performed in a thermostat bath at different temperatures and the results were presented in figures 10 and 11.

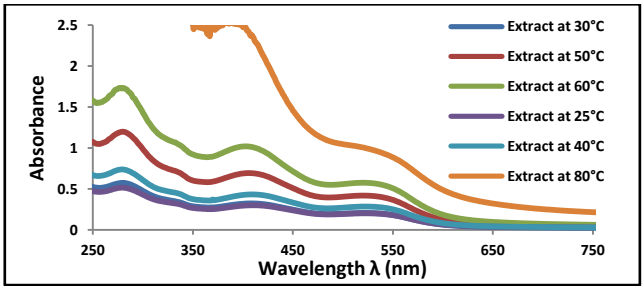


Figure-10
UV-visible spectrum of leaf sheath extracts at different temperatures

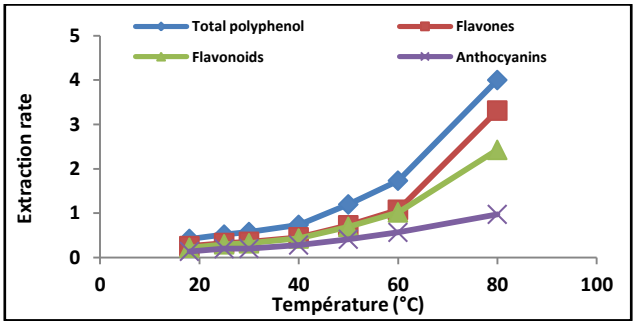


Figure-11
Extraction rate at different temperatures

These results show an increase in extraction rate according to temperature without degradation of the compounds in the temperature range tested. The sorghum polyphenols are stable up to 100°C and this is a huge advantage for the use of the

`finished product if we know that the thermosensitivity of polyphenols and more particularly that of anthocyanins is the bottleneck for industrials in the use of these natural pigments in food. This heat resistance offers a second advantage in technology and could minimize the use of the heat exchanger in the industrial process of extraction.

Evaluation of temperature effect upon reflux heating: The recorded results are identical, showing an increase in the rate of extraction of different families of compounds depending on the temperature and confirm the stability of compounds extracted (figures 12 and 13).

Parallel to the spectrometric analysis, a mass balance was performed to determine the mass yield of the extraction in function of temperature. The results are given in figure 14.

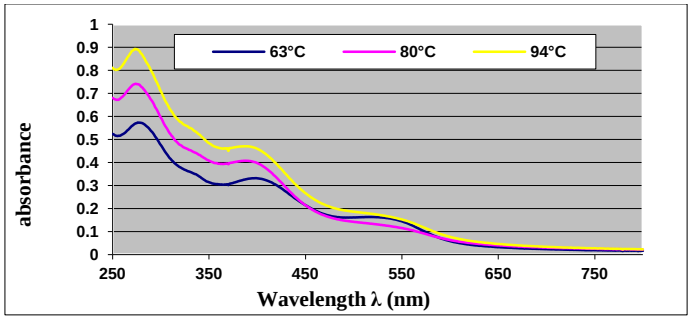


Figure 12
UV-visible spectrum of extracts obtained at different temperatures in refluxing

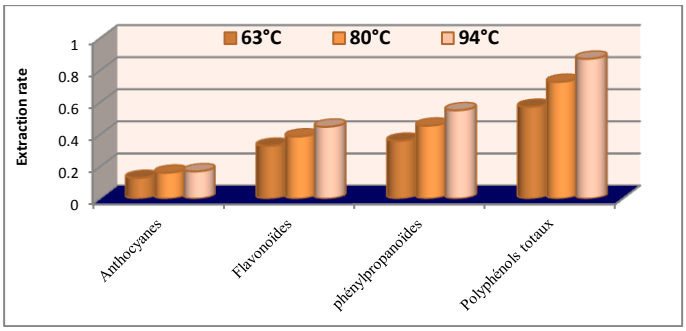


Figure 13
Extraction rate of different phenolic compounds families depending on the temperature

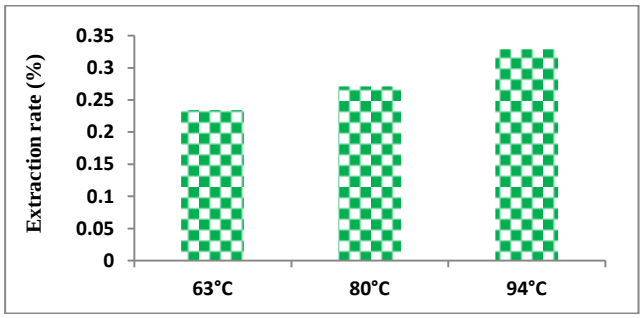


Figure 14
Extraction yield as a function of temperature

The yield increases with the extraction temperature showing a good correlation between the results of spectrometric analysis, metering and those given by the mass balance. The increase in temperature therefore exerts a positive effect on the extraction, but the implementation of these temperatures requires a financial cost. The final objective of optimization is to extrapolate the technology on an industrial or semi-industrial scale applicable in the southern countries already deficient in energy, so we recommend a longer extraction time to an increase in temperature.

Effect of agitation on the kinetics of extraction: Two sets of extraction were carried out: one under stirred and one without agitation. The kinetics was followed for 14 h which represents the time of maximum stirring extraction (Figure15).

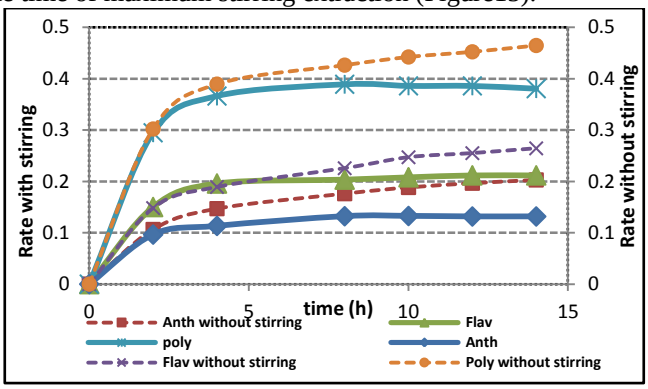


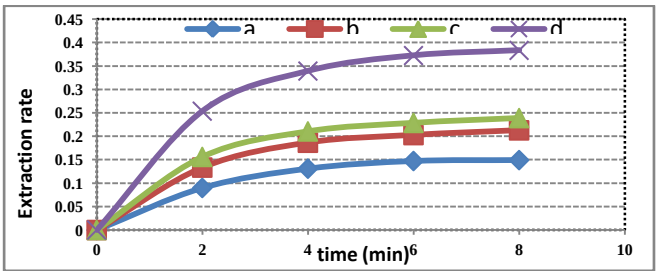
Figure 15: Effect of agitation on sorghum polyphenols extraction

Without agitation, the concentration of anthocyanin and flavones reached a maximum at 8h while the total polyphenol increases more after nearly 6 hours of extraction. From 8 to 24 hours of extraction, the anthocyanin and total polyphenols concentration is no longer increasing. When we realized the extraction with magnetic stirring, the concentrations of the three families of polyphenols reach their maximum after 14h. These rates are maintained up to 24 hours, and then falls after.

Influence of ultrasound: We used a unit with an output of 500 Watts and frequency 20KHz (SONICS Vibra Cell, Model VC 505) equipped with a probe 13 mm titanium alloy. The optimized conditions above have been applied; an ice bath was used to prevent the temperature rise during the extraction. The amplitude was set at 70% of the total power of the device. The kinetics of extraction was monitored for 16 minutes with 8 minutes of effective application of the pulsed ultrasound because the total irradiation time is double the effective sonication time.

The extraction times by sonication to get the same performance as 4h and 6h extraction by magnetic stirring are approximately 6 minutes for anthocyanins and 8 minutes for other families of phenolic compounds. This indicates a reduction of extraction time of over 70%. Ultrasound is therefore a complementary technology to conventional extraction techniques and improves

the penetration of the extraction solution in solid matrices to be extracted by generating an intense molecular agitation (cavitation) within it. But the implementation of this technology is not without drawbacks. It implies a financial cost and therefore requires technical knowledge which are difficult to have in developing countries, targets of the operation of the extraction technique optimized. In addition, the temperature control during the extraction involves an additional cost that may make the process expensive in the short term. The application of this technology for large-scale extraction is to study at financial point before its inclusion in the final process. At this stage of our study, extraction with magnetic stirring is used.



a = anthocyanins; b = Flavonoids;
c = Phnylpropanoides; d = Total Polyphenols

Figure 16
Kinetics of sorghum polyphenols extraction under ultrasound

The application of ultrasound does not change the nature of the phenolic extracts (Figure16 and table2). The same families of molecules were detected during extraction by magnetic stirring.

Choice of ratio: dry matter mass / extraction solvent volume: 1 g of dry ground was extracted in different volumes of distilled water (50ml, 100ml, 150ml and 200ml) with stirring for 2 hours. The results indicate that the quantity of phenolic compounds extracted increases with the ratio (dry matter mass/extraction solvent volume) until (1g/150ml) then becomes constant whatever is the family of phenolic compounds considered (figure17). We shall preserve this value of ratio (1/150) that these conditions are the most optimal for the maximal extraction of all the families of compounds polyphénoliques present in the sorghum.

Because the majority of solvent of extraction is destined to be eliminated after extraction, the use of large amounts of solvent would require an expenditure greater energy at the concentration step and would make the process more expensive.

Conclusion

As we can observed through this work devoted to the study of different parameters to optimize the production of polyphenols from the leaf sheath of red sorghum from Benin, the extraction operation is the most delicate and determines in large part, the financial cost of the production process. For a best extraction yield by an extraction process easy to implement in the Southern countries, while respecting the standards of quality and the environment, we found 14 hours of extraction with magnetic or mechanical stirring at room temperature with a ratio of 1 g of dry matter per 150 ml of water and a neutral pH. These parameters will be used for trying on a pilot scale for a reliable extrapolation of scaling for production of concentrated polyphenols of sorghum at a semi-industrial scale for SMEs and SMIs located in the southern countries.

Table-2
Kinetics of extraction of different phenolic compounds families compared to ultrasonic extraction with magnetic stirring

	Ultrasound		**Magnetic agitation**	
	Time (min)	**Extraction rate**	**Time (h)**	**Extraction rate**
Anthocyanins	2	0,09	2	0,11
	4	0,13	**4**	**0,15**
	6	**0,15**	6	0,16
	8	0,15	8	0,17
Flavones	2	0,13	2	0,15
	4	0,19	4	0,19
	6	0,20	**6**	**0,21**
	8	**0,21**	8	0,25
Phenylpropanoids	2	0,16	2	0,17
	4	0,21	4	0,21
	6	0,23	**6**	**0,24**
	8	**0,24**	8	0,25
Total Polyphenols	2	0,25	2	0,30
	4	0,34	4	0,36
	6	0,37	**6**	**0,39**
	8	**0,38**	8	0,44

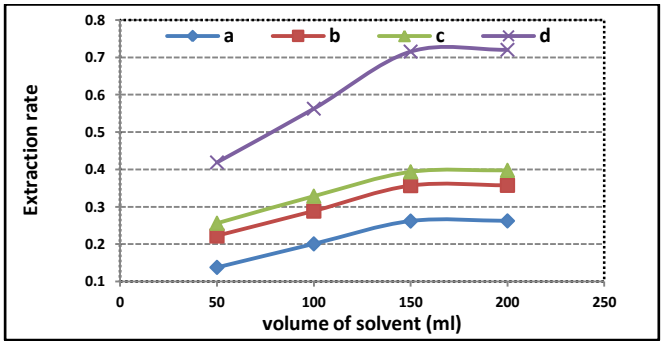


Figure-17
Effect of volume of solvent on quantity of phenolic compounds extracted

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