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Research Article

Production and characterization of exopolysaccharides from loss cooked sweet potatoes (*Ipomoea batatas*) by *Lactobacillus plantarum*

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Abstract

The aim of the work was to produce and characterize the exopolysaccharides from loss cooked sweet potato (*Ipomoea batatas*) using fermentation with *Lactobacillus*. The cooked sweet potato was fermented according to the factorial design with the following factors: the time (21.51h-38.48h) and the amount of *Lactobacillus* ($1.58*10^6$-$5*10^6$UFC). Responses were represented by production yield, pH, and titrable acidity. Characterization of the exopolysaccharides was then done by determination of total sugars, solubility index and viscosity. The results show that the pH of unfermented cooked potatoes was between 6.23 to 6.63. The pH of fermented potatoes varies from 3.96 to 4.06. The lactic acid content was found from 7.75 to 9.9% for the fermented samples. The production yields are 1.90% for the samples fermented for 21.5 hours with $3.5*10^6$UFC of bacteria and 5.62% for those fermented for 30 hours with the same volume of inoculum. The average viscosity of the products was 4mPas regardless of the fermentation time. Chemical characterization indicates glucose contents of 68.21 and 94.01% in fermented potato for 24h and 21h respectively. The solubility index gives values of 70.3 ± 0.16 and 88.11 ± 0.23 for fermentation times of 21h and 24h respectively. Results of this work indicated that cooked sweet potatoes ferment for 21h was a promising substrate for production of exopolysaccharides.

Keywords: expolysaccharides; fermentation; *Lactobacillus*; yield; titrable acidity

1. Introduction

Sweet potato (*Ipomoea batatas* L.) belongs to the Convolvulaceae family. It is the seventh major worldwide food crop after wheat, rice, corn, apple land, barley and cassava [1].It is the second foodstuff after cassava in tropical countries [2] and the third in Sub-Saharan Africa after cassava and yam [3] Cameroon had a production of 348,618 tonnes in 2013 and this production increases over time [4].

After cooking, sweet potato is the subject of losses, which could be an alternative to produce exopolysaccharides useful in food industries, health and agronomy. Indeed, when the cooked potato is left at room temperature for 24 hours, it gets slimy on the surface layer, indicating the presence of exopolysaccharides produced by bacterial during ambient fermentation. Exopolysaccharides (EPS) are also called capsular polysaccharides (CPS), or exo cellular polysaccharides [5].

They are of great interest in health, agro-food, and cosmetics. In food, they can improve the rheological and sensory properties of fermented products such as cheese and yogurt [6]. In bread making, they improve the viscoelastic properties and volume of the dough, and increase the self-live of bread, while reducing the hardness of the bread crust [7-8].

Most exopolysaccharides used in the food industry are obtained from plant substrates. Starch and its derivatives (gums, corn starch, tapioca, and cellulose) are the most used polymers to produce exopolysaccharides [9]. Exopolysaccharides can be produced from *Tibetkefir* with the use of *Lactobacillus kefiranofaciens* [10]. Lactic acid bacteria during growing have the capacity to generate exopolysaccharides. However, several factors can influence the production of exopolysaccharides such as: temperature, pH, number of microorganisms, time of fermentation, the fermentation substrate [11].

To bring added value to sweet potato cooked and to valorize the loses after cooking in Cameroon, the general objective of this work is to produce exopolysaccharides by lactic fermentation using the loses sweet potato after cooking. Specifically, the cooked sweet potatoes were ferment as a function of time and ratio microorganisms. The characterization of the exopolysaccharides was also performed.

2. Materials and methods

2.1. Microorganisms and root tubers

The root tuber used in this work is sweet potato (*Ipomoea batatas*) the yellow variety which was bought at the Mokolo market inYaoundé in the Centre Region of Cameroon in July 2019. The *Lactobacillus plantarum* was kindly supplied by Professor Sado (Department of microbiology, University of Yaounde I, Cameroon). The microorganism was maintained at 4 °C in lactose (1g milk+20g of 1L of water and 1mL of Tween 80) and subcultured every 2 weeks. Figure 1 shows the general diagram of the work carried out.

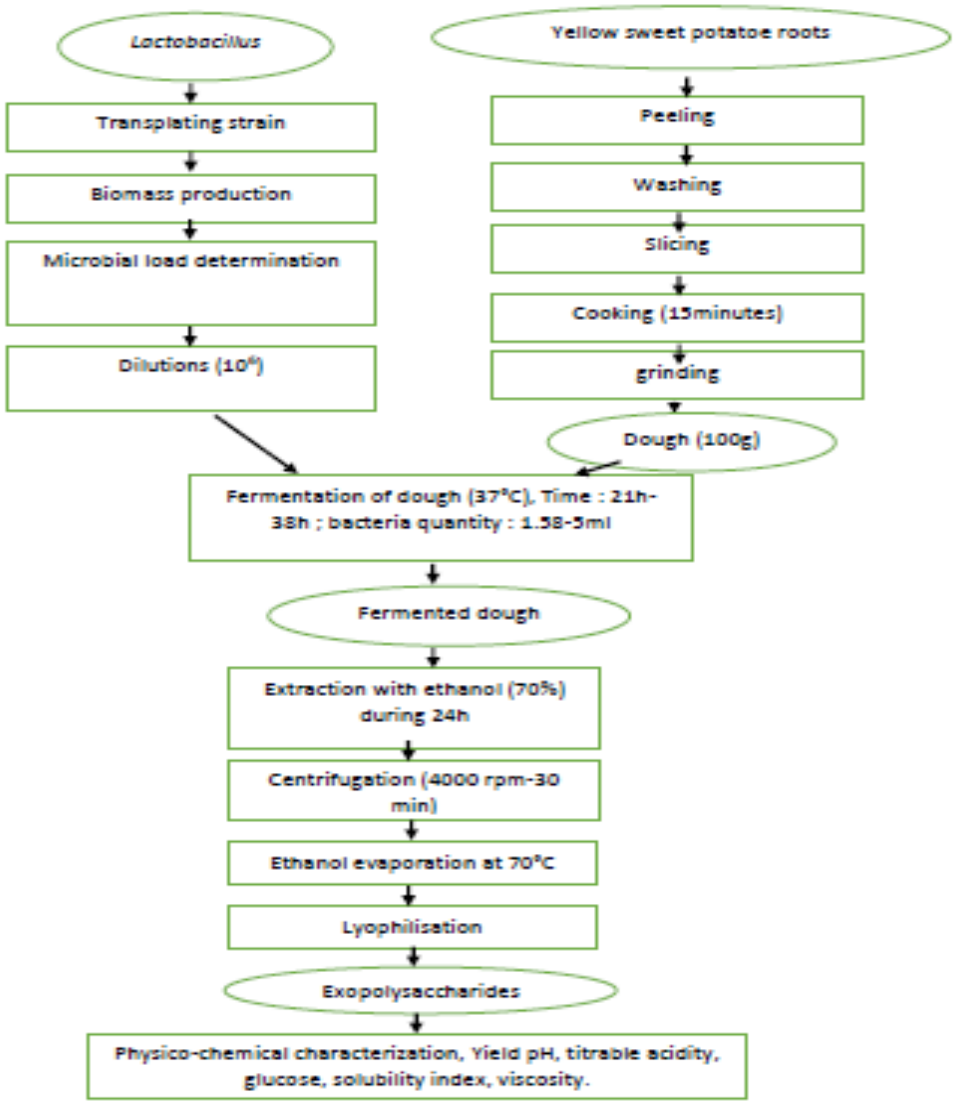

Figure 1. Synoptic diagram of the work carried out.

2.2. Transplanting the strains

Transplanting of the strains (figure 2) was done to readjust the microorganisms to their environment. The microorganisms stored at -18 °C are stressed. They were put in optimal conditions so to renew young cells.

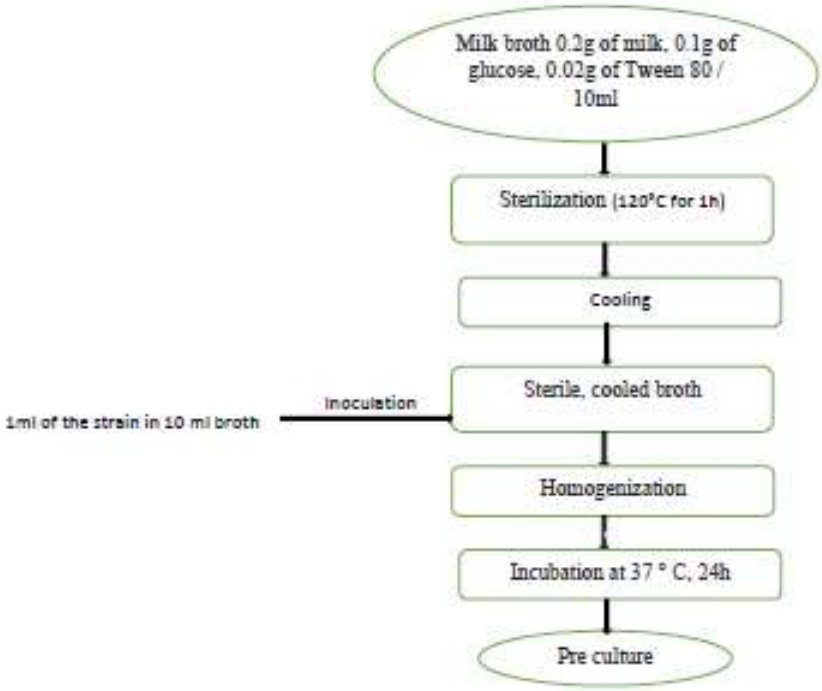

Figure 2. Method for obtaining a preculture of the *Lactobacillus sp strains.*

2.3. Production of one liter biomass

Biomass production was carried out to obtain a number of bacteria important for analyzes. It was done by the culture of bacteria in a nutritious medium at 37 ° C for 24h as shown on figure 3.

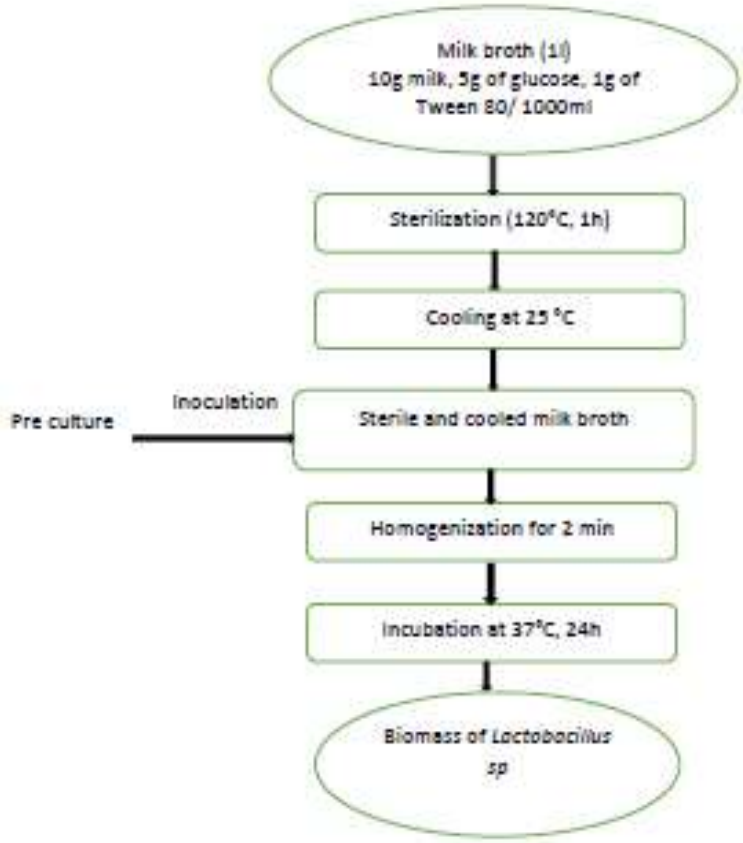

Figure 1. Production of *Lactobacillus sp biomass*

2.4. Determination of the microbial load of the obtained biomass.

The microbial load is determined in order to ferment the cooked sweet potato roots with fixed charges. For this, the biomass previously obtained is centrifuged at 4000 rpm for 30 min. The pellet is suspended in physiological water (1.7 g / 200 ml) and transferred to a sterile conical flask 0.1 ml of this mixture is sown in Petri dishes containing the medium culture Plate Count Agar (PCA: 6.9g / 300ml) previously sterilized and cooled. The whole was incubated at 37 °C. The counting is carried out after 24 hours.

2.5. Fermentation of cooked sweet potato roots.

The fermentation was conducted in 250 mL Erlenmeyer flask using 50 mL modified EPS production medium. The inoculated flask was incubated at 37 °C for different times (21h-38h) of fermentation under static condition. This fermentation process is illustrated in figure 4.

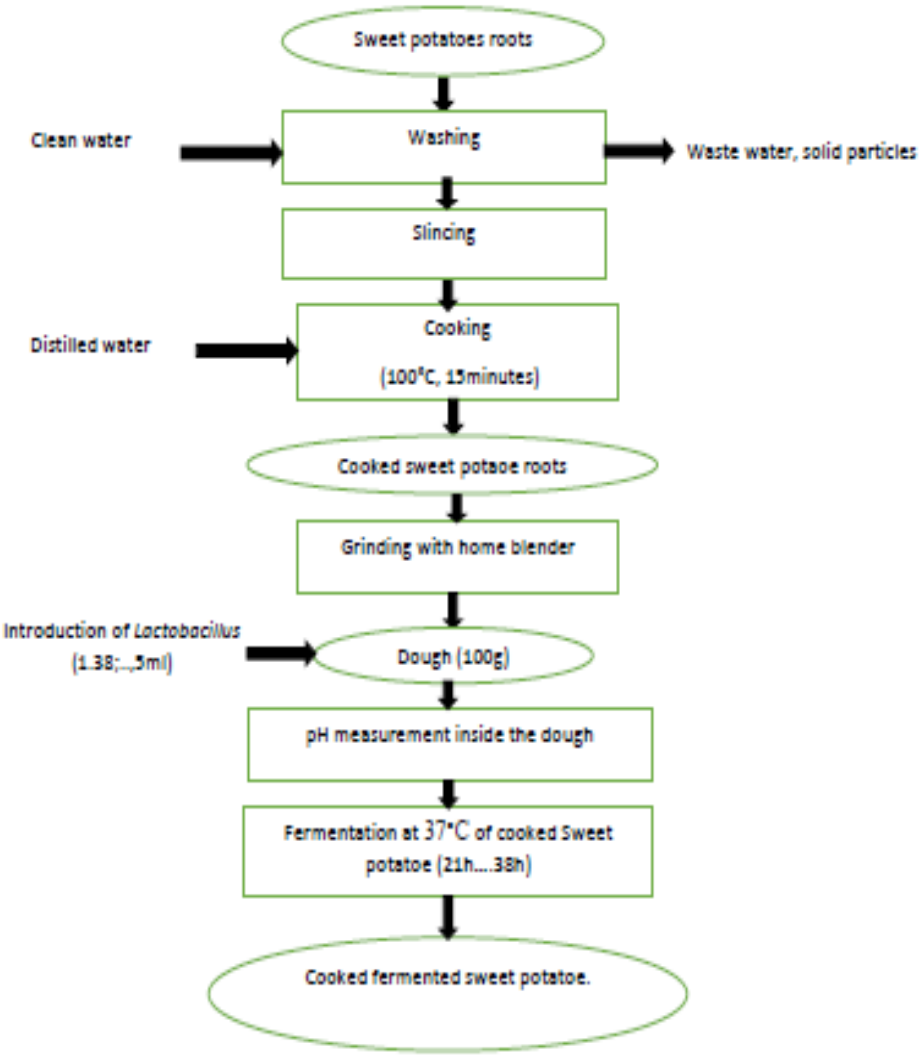

Figure 2. Fermentation process of cooked sweet potato roots.

2.6. Extraction of exopolysaccharides.

Exopolysaccharides were extracted according to the method of [12] after boiling the culture at 100°C for 10 min to inactivate the EPS-degrading enzymes. The extraction of exopolysaccharides was done as shown in figure 5.

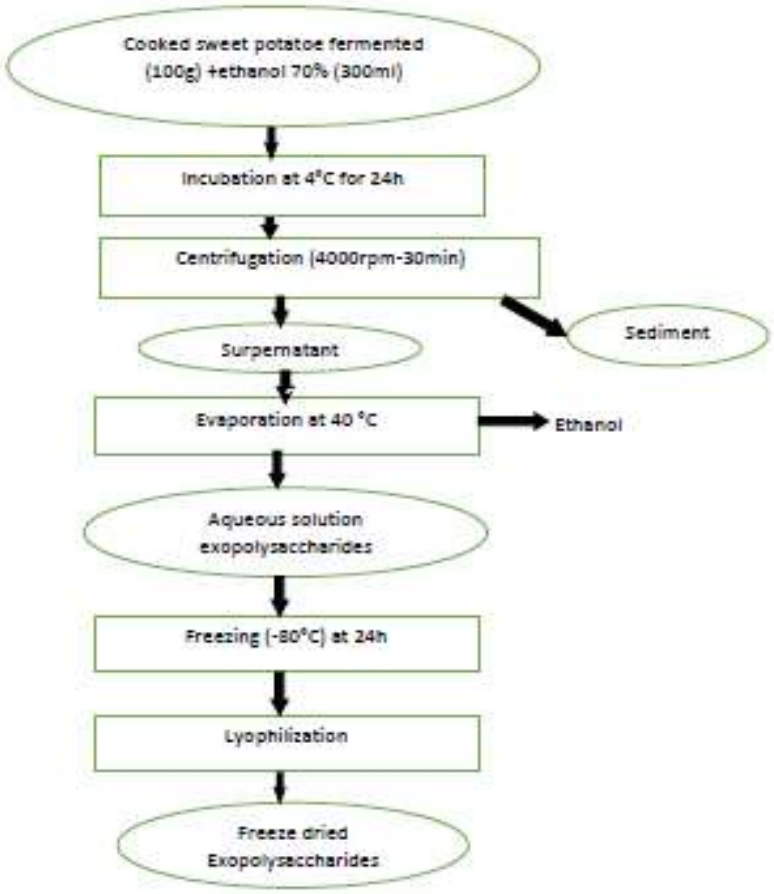

Figure 5. Process for extracting exopolysaccharides from cooked and sweet potato roots fermented.

2.7. Characterization of exopolysaccharides.

To characterize the obtained exopolysaccharides, the analyses carried out were: titrable acidity, viscosity measurement, the solubility index, determination of total sugars. The yield was also calculated.

2.7. 1.Titrable acidity.

The titrable acidity of the fermented sweet potato was performed as follow: In 10g of fermented potatoe, a few drops of phenolphthalein were added. Then the mixture was titrated with 0.1N NaOH until the appearance of a pink color.

2.7. 2.Viscosity

The viscosity of exopolysaccharides was measured using a viscometer (Brookfield). Solutions (10% W/V) of exopolysaccharides are prepared. The mixture was stirred. The rotational viscometer was used to measures the viscosity. The stem is driven in rotation by a motor passing through a calibrated spring. Resistance to the flow increases as a function of the size of the rod and or the speed of rotation. Viscosity was expressed in mPas.

2.7. 3.Solubility index.

Solubility in water was performed according to the procedure of [13]. 10% exopolysaccharide (W / V) solutions were prepared. The mixture is stirred for 30 min then centrifuged at 3500 rpm for 15min. The solubility index is the percentage of exopolysaccharides dissolved in water after determination of the dry matter of the supernatant (24 h at 105° C). The solubility index is given by the equation:

$$Solubility\ index\ (\%) = \frac{Total\ carbohydrate\ concentration\ in\ supernatant}{Weight\ of\ sample\ (dry\ weight\ basis)} \times 100$$

2.7.4. Total sugars.

Total sugars in exopolysaccharides, was quantified using reducing sugar assay described by [14]. The results were expressed as glucose equivalent.

2.8. Statistical analyses.

A fractional factorial design, Box-Behnken model, was employed for the statistical optimization of the production conditions. The experimental design consisted of thirteen runs and the independent variables were studied at two different levels, a high level and a low level. The high level is commonly coded as +1 and the low level as -1. It is necessary to include center points as well (in which all factors are at their central values).

Two factors selected for optimization were the time of fermentation and the amount of microorganisms. A design was generated with these factors, having a low level and a high level. The low level for time of fermentation was 21h and the high level was 38h. The low level for amount of microorganism was $1.38*10^6$ ml with a high level of $5*10^6$ ml. The Minitab 18Software (v. 6.0, Stat-Ease, Inc., Minneapolis, USA) was used for experimental design. The pH, the titrable acidity of the media and the yield value of the produced EPS was taken as the response.

The statistical analysis of the model was performed in the form of analysis of variance (ANOVA). This analysis included the Fisher's *F*-test (overall model significance), its associated probability p(*F*), correlation coefficient R, and determination coefficient R^2 which measures the goodness of fit of the regression model. It also includes the Student's *t*-value for the estimated coefficients and associated probabilities p(*t*). The quadratic models were represented as response surface graphs. Validation of the experiment was also performed by selecting different combinations of the factors as recommended by the software. All the experiments were performed in triplicates.

3. Result and discussion

3.1. Microbial load of the biomass obtained

The microbial load required for the fermentation of sweet potato roots was 10^{-6}. Dilutions were done to obtain these microbial loads. After 24h of incubation at 37°C, the colonies for each petri dish were counted. Several dilutions were carried out to obtain the microbial load. These results are presented in table 1. The dilutions used in this work were those between 10^{-6} and 10^{-8}. In these cases, the dilution of 10^{-6} was used.

Dilution	Numbers of colonies	
	Trial 1	Trial 2
10^{-1}	Incountable	Incountable
10^{-2}	Incountable	Incountable
10^{-3}	Incountable	Incountable
10^{-4}	Incountable	Incountable
10^{-5}	Incountable	Incountable
10^{-6}	200	341
10^{-7}	Incountable	185
10^{-8}	291	203
10^{-9}	Incountable	85
10^{-10}	Incountable	Incountable
10^{-11}	Incountable	Incountable
10^{-12}	12	112
10^{-13}	3	5
10^{-14}	13	3

Table 1. Enumeration of colonies obtained after the culture of *Lactobacillus plantarum.*

3.2. Yield of exopolysaccharide

The low yields of exopolysaccharide production by most *Lactobacillus* species are the main reason of their noncommercial exploitation. Therefore, among factors which affect the yield production of exopolysaccharides are time of fermentation, amount of microorganisms, the fermentation substrate, the temperature of fermentation, the pH of the media and the production of lactic acid [15-16]. In this study, the fermentation time and the amount of microorganism does not affect the yield of exopolysaccharides (table 2). The quadratic effect of the quantity of microorganisms and the time*quantity (p=0.91) interaction does not have effect on the yield (table 2).

Source	Df*	Adj SS*	Adj MS*	F-value	P-value
Model	5	4.41	0.88	1.17	0.41
Linear	2	1.06	0.53	0.71	0.52
Time	1	0.93	0.93	1.24	0.30
Quantity	1	0.13	0.13	0.17	0.69
Square	2	3.33	1.66	2.21	0.18
Time*Time	1	3.08	3.08	4.09	0.08
Quantity*Quantity	1	0.52	0.52	0.69	0.43
2-Way interaction	1	0.00	0.00	0.01	0.91
Time*Quantity	1	0.00	0.00	0.01	0.91
Error	7	5.28	0.75	-	-
Lack-of-Fit	3	3.86	1.28	3.62	0.12
Pure Error	4	1.42	0.35	-	-
Total	12	9.70	-	-	-

DF*: Degree of Freedom; Adj SS*: Adjusted sums square; Adj MS*: Adjusted means square

Table 2. Anova table of the effect of time and quantity on the yield of exopolysaccharide.

The yield of cooked and fermented potato exopolysaccharides varies as a function of time and the amount of *Lactobacillus* (figure 6). At the minimum amount of $1.58*10^6$ UFC, the production was 3.268g/100g for 30h. When the amount of microorganisms was increased up to $3.5*10^6$

UFC, the yield was 5.682g/100g. Between $3.5*10^6$ and $5.62*10^6$ UFC a slight decrease of the production of exopolysaccharides is observed with yields of 5.682g/100g to 4.518g/100g respectively.

The production of exopolysaccharides increases with the time of fermentation. After 21.51h, the production of exopolysaccharides was 1.9g/100g. While after 38h 48min (the longest fermentation time), the production yield of exopolysaccharides was 4.321g/100 g. The maximum yield was obtained after 30 hours of fermentation and the corresponding value was 5.6g/100g. The time and quantity of microorganisms at which a maximun yield is obtained are 30h and 3.5ml of *Lactobacillus* respectively. Sethuraman et *al.* [17] had 9.3 g/l of exopolysaccharides after 100 hours of fermentation. They used the cooked sweet potato water as a growing medium. However, Yuliani et *al.* [18] found 3.8 g/l of exopolysaccharides after 48h 8min of fermentation. The yields observed by Sethuraman et *al.* [17] and Yuliani et *al.* [18] are lower than yields obtained with cooked sweet potato water. This can be explained by the fact that, after a long fermentation time, the amount of lactic acid in the medium was high. In fact, lactic acid stresses bacteria.

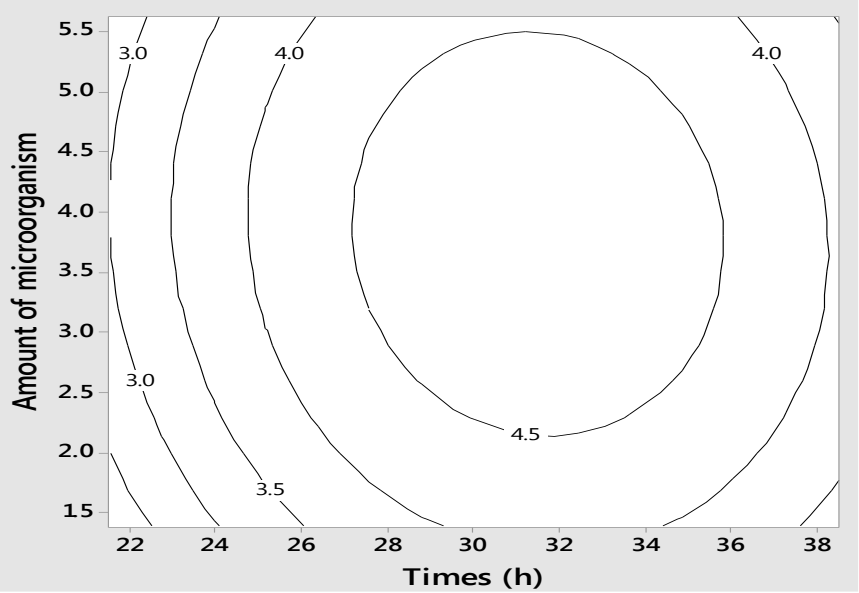

Figure 6. Contours plots of the effect of time and the amount of microorganisms on the yield of exopolysaccharides after fermentation.

3.3. pH of the media

It has been shown that the pH of the media have a great impact on the production of exopolysaccharides. The best pH to produce exopolysaccharides varies around pH 5 and 6 [19]. The pH was measured before and after fermentation. The pH of the various treatments before fermentation is between 6.2 and 6.6. There is no significant difference between these values.

The effect of fermentation time and the amount of *Lactobacillus* are presented in table 3. The P value (0.95) of time and the quantity of microorganisms show that these two factors have no effect on the pH. Quadratic effect of quantity and interaction does not affect the pH. Yuliani et *al.* [18] extracted the exopolysaccharides from the water distilled *soschu* made from sweet potato and obtained maximum yield at pH 6.2. Sivakumar et *al.* [20] obtained exopolysaccharides from *Frateuria aurentia* by varying the pH from 3 to 9, after 72h of fermentation at 30 ° C. The pH of 6 and 7 were those at which the yield is maximum. This shows that the high yield depends on the experiment. The study of these authors shows that, to have an optimal production exopolysaccharide, the pH should be between 6 and 7. For this work, at the start of fermentation the pH values were between 6.2 and 6.6. At the end of fermentation the pH value range between 3.9 and 4.6 as shown at Figure 7. The drop of pH in the medium was due to the production of lactic acid and hydrogen by bacteria. That is why the next result was to verify the amount of titrable acidity in the medium.

Sources	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	0.47	0.09	1.79	0.23
Linear	2	0.19	0.09	1.80	0.23
Time	1	0.19	0.19	3.60	0.09
Amount	1	0.00	0.00	0.00	0.97
Square	2	0.28	0.14	2.66	0.13
Time*Time	1	0.26	0.26	4.93	0.06
Amount*Amount	1	0.00	0.00	0.11	0.74
2-Way Interaction	1	0.00	0.00	0.00	0.95
Time*Amount	1	0.00	0.00	0.00	0.95
Error	7	0.37	0.05	-	-
Lack-of-Fit	3	0.37	0.12	1716.68	0.00
Pure Error	4	0.00	0.00	-	-
Total	12	0.85	-	-	-

DF*: Degree of Freedom; Adj SS*: Adjusted sums square; Adj MS*: Adjusted means square

Table 3. Analysis of variance of the effect of the fermentation time and the amount of *Lactobacillus* on the pH.

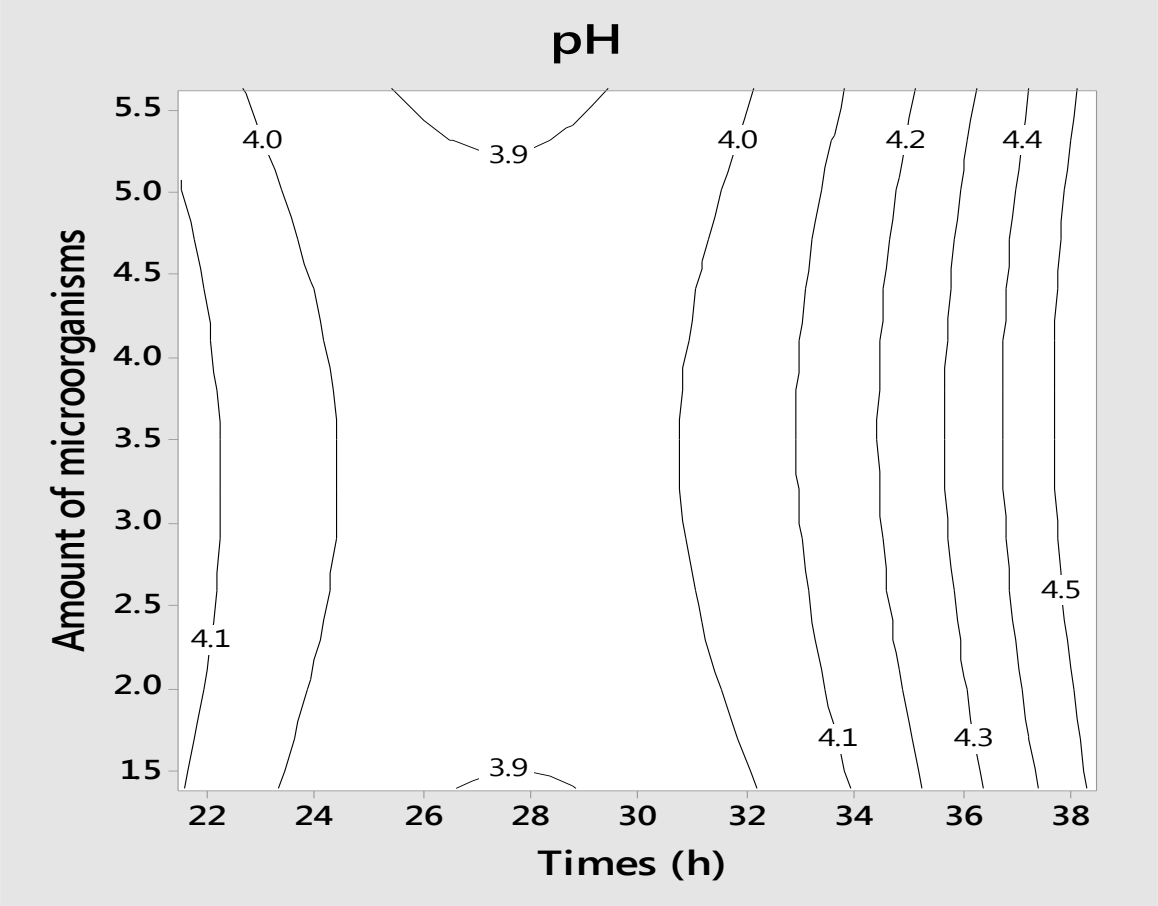

Figure 7. Effect of fermentation time and amount of Lactobacillus on pH.

3.4. Titrable acidity

Lactic acid bacteria as dominant organism in food fermentations will convert free sugars to lactic acid. The occurrence of lactic acid is based on the production of amylolytic enzymes by the bacteria that degrade starch granules and hydrolyze amylose and amylopectin [21].This enzyme production depends on the time of fermentation and the amount of microorganism in the media. Table 4 presents the analysis of variance of the effect of the fermentation time and the amount of lactic acid bacteria on the titrable acidity. The P-value ($P \geq 0.05$) of time and quantity shows that these two factors have no significant influence on titrable acidity. The effect of the time and the quantity of microorganisms does not also affect the titrable acidity at the secondary level and in interaction.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	2.42	0.48	1.58	0.27
Linear	2	0.62	0.31	1.02	0.40
Time	1	0.02	0.02	0.09	0.77
Amount	1	0.59	0.59	1.95	0.20
Square	2	1.54	0.77	2.52	0.15
Time*Time	1	0.07	0.07	0.26	0.62
Amount*Amount	1	1.52	1.52	5.00	0.06
2-Way Interaction	1	0.25	0.25	0.83	0.39
Time*Amount	1	0.25	0.25	0.83	0.39
Error	7	2.13	0.30	-	-
Lack-of-Fit	3	1.93	0.64	12.75	0.016
Pure Error	4	0.20	0.05	-	-
Total	12	4.56	-	-	-

DF*: Degree of Freedom; Adj SS*: Adjusted sums square; Adj MS*: Adjusted means square

Table 4. Analysis of variance of the influence of the fermentation time and the amount of *Lactobacillus* on the titrable acidity.

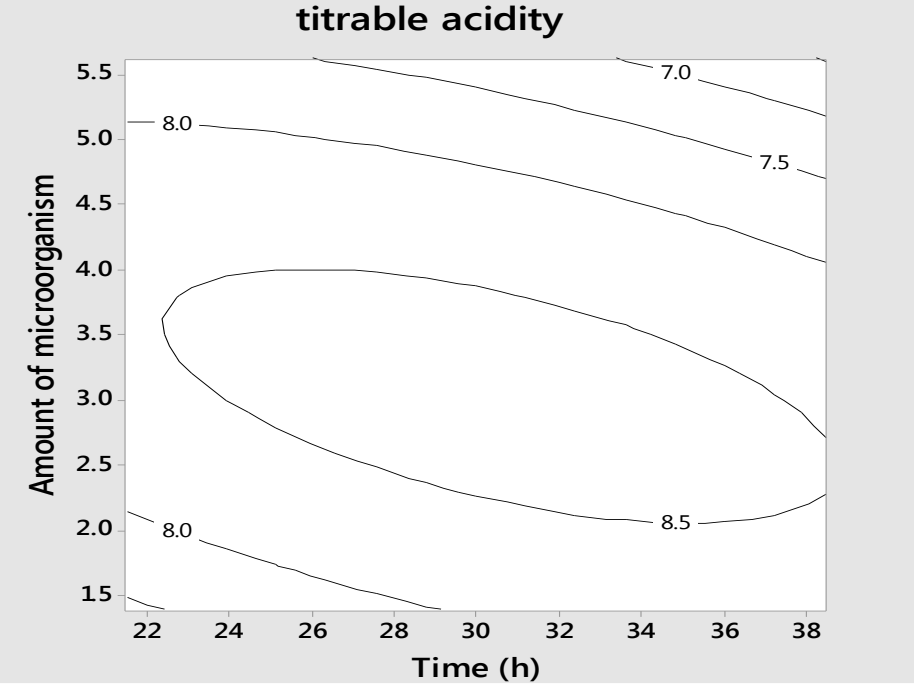

Figure 8. Effect of fermentation time and amount of Lactobacillus on titrable acidity.

The variation of titrable acidity of the fermented cooked sweet potato is shown on figure 8. At a minimum amount of *Lactobacillus* of1.58 * 10^6 CFU, there was a production of lactic acid at 9.2%. When the amount of microorganism increased to 3.5 * 10^6 UFC, the titrable acidity was 9.8%. From 3.5 * 10^6 UFC to 5.62 * 10^6 UFC the production of titrable acidity was observed in the environment with values drops from 9.8% to 7.75% respectively. After 21h51min of fermentation, the titrable acidity produced, was at 8.66%. At 38h48min, the value rate increases to 9.9%. Bergmaier et *al.* [22] obtained 0.90 g of lactic acid per g of lactose consumed in the environment during the production of exopolysaccharides from potatoes by *Lactobacillus rhamnosus*. These authors observed that the increase of the fermentation time is related to that of lactic acid in the medium. After a large production of lactic acid in the medium, the bacteria are stressed and they no longer optimally produce exopolysaccharides. When there is a large amount of lactic acid in the medium, the pH drops and becomes acidic. It will therefore be necessary to adjust the pH to promote the production of exopolysaccharides.

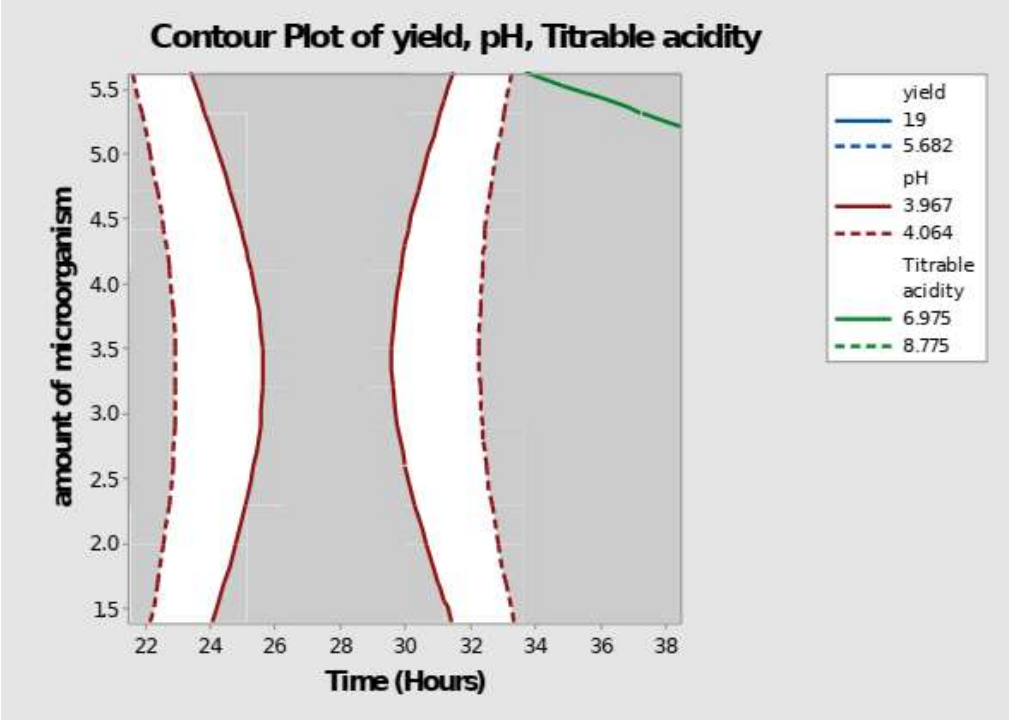

Figure 9. Optimal area for the production of exopolysaccharides.

Figure 9 shows the optimal area for the production of exopolysaccharides as a function of the fermentation time and the amount of microorganisms. The areas marked in white represent these optimal areas which are for the time being intervals ranging from 22h to 24h and 31h to 33h. The quantity of *Lactobacillus* used in this study has no influence on the production. Figure 9 confirms also that the lactic acid concentration and the pH are the parameters that affect the production of exopolysaccharides. This is why, Bergmaier et *al.* [22] found that a very high pH or a very low pH does not favor a high yield of exopolysaccharides production. In addition, the presence of a high amount of lactic acid in the environment stresses the bacteria and slows down their growth.

3.5. Physico-chemical characterization of exopolysaccharides.

In order to characterize the physico-chemical properties of the produced exopolysaccharides, the samples was analyses at the center and at the ends of the experimental design for their total sugars contents, solubility index and their viscosity.

3.5.1. Total sugars.

Exopolysaccharides are manly composed of sugars. Glucans are composed of glucose polymer backbone with various degrees of branching depending on producing strains. Exopolysaccharides were analysed for their sugar contents in term of glucose. Table 4 shows the amounts of sugar (glucose) in g present in 100gof exopolysaccharides from some samples of exopolysaccharides with respect to samples fermented for 21h, 24h, 30h and 38h. The glucose contents in the exopolysaccharide analysed samples vary from 68.21 ± 0.32g / 100g of exopolysaccharides and 94.01 ± 0.52g / 100g. These results show that glucose is the major sugar in these exopolysaccharides produced from sweet potatoes roots. Thus exopolysaccharides obtained would be glucans which are glucose polymers. They are composed of the monosaccharide monomer D-glucose, linked together by glycosidic bonds. Glucans can be divided into α-glucan and β-glucan.

3.6. Solubility index

Some glucans are both soluble and insoluble. That is why it was important to evaluate the solubility index of the exopolysaccharides. Solubility index is the maximum amount of substance that can dissolve in a certain volume of water. The solubility indices vary from 70.3 ± 0.16 to 88.11 ± 0.23%. These result shows that the exopolysaccharides obtained are very soluble in water. Zaheer et *al.* [10] extracted the exopolysaccharides from *Tibet kefir*. These authors obtained the solubility of 14.2%.The difference in solubility can be explained by the difference in the substrates. In addition to the substrate, the composition of the exopolysaccharides could bedifferent. In this investigation, after characterization, the exopolysaccharides contain more than 80% ofglucose. Glucose being a sugar very soluble in water, the solubility of exopolysaccharideswas very high.

3.7. Viscosity

The exopolysaccharides are visually slimy when they are produced. So, it is important to know the viscosity of the produced exopolysaccharide. The viscosity of the samples measured are all equal to 4mPas. Kanmani et *al.* [23] had viscosities equal to 208mPa at pH 6 and 226mPa at pH 3. Zaheer et *al*. [10] have obtained viscosity equal to 3.5mPa with a concentration of exopolysaccharides of2mg / ml and 4.5mPa with a concentration of exopolysaccharides of 4mg / ml. Kanmani et *al*. [23] observed that the viscosity of exopolysaccharides increases inacidic media. Zaheer et *al.* [10] demonstrate that the more the concentration increases, the more the viscosity increases. According to the concentrations (100mg/ml) used in this study, the viscosities (4mPas) obtained are lower than those noted by Zaheer *et al.* [10]. This can be explained by the fact that the viscosity values could vary with respect to the subtrates and the composition of exopolysaccharides.

4. Conclusion

This study opens new horizon for news sources of exopolysaccharides from local products. Loss cooked sweet potato root can be ferment and used to produce exopolysaccharides. These exopolysaccharides can be used in different field of food industry.

Acknowledgment

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Conflict of interest

The authors do not have any conflict of interest.

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