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STUDY THE EXTRACTION OF SUGARS FROM CAROB PODS

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ABSTRACT

The study of the sugars extraction of the carob pods shows that biological material can be considered a renewable carbon source. By maceration in the water at room temperature, a large fraction of sugars extracted may be intended for the agro-alimentary sector .For the chemical industry, can be treated the fruit of the carob by sulfuric acid or oxalic acid to 60% of sugars extracted. Concerning the production of bioethanol, and the sugars have good fermentation, which after a period of three days, are totally converted.

Keywords: *Carob Pulp, Sugars; Maceration, Hydrolysis, Fermentation etc*

1. INTRODUCTION

Fossils sources, still occupying an important place in the socio-economic activities of nations. These resources are limited and their utilization is detrimental to the quality of life by generating of pollution in all forms, Furthermore, climate change because of the greenhouse effect resulting mainly from the accumulation of carbon dioxide in the high atmosphere [1-2] concerned about increasingly the scientific community. Sustainable development is a concept that imposes on the nations in quest of progress. Sustainable development is a concept that requires nations in quest of progress. The integration of renewable resources in the conception of industrial processes is in total agreement with this essential concept for a better future of human. Moreover the countries with neither of petroleum neither natural gas, are exposed to the vagaries of international markets of these fossils sources whose courses are often subject to speculation. A view to reducing the screw-in dependence on fossil sources and to face climate change, we considered various prospects to replace fossil fuels without restricting the supply of energies to cope with the growing demand foreseen for the future [3] Within that framework, renewable energy can play a significant role since their use of which generates relatively little harmful substances. Non-oil countries such as Morocco by developing that type of energy can reaching socio-economic objectives of prime importance the reduction of oil dependence and the creation of employment.

The biomass is a renewable source of primary energy; it can sustainably meet the demand for energy. Lignocellulosic raw materials such as organic waste, forest residues .they were the subject of several research projects; they concern mainly conversion of biomass into biofuel said of the second generation whose use inevitably contributes to the reduction of greenhouse gases [4-5].

Also the carob tree presents a good drought resistant; it can reach height ranging from 8 to 15 m and live up to 500 years. It is often used for controlling against deforestation and desertification by limiting erosion. The pulp from carob pods is very rich in sugars [6-7]. The latter have enormous development potential, in fact, after their extraction, sugars can be a significant source of biomaterials suitable for a variety of chemical or biochemical transformations to obtain new higher value-added materials. The fermentation of sugars produces bioethanol substituent used as gasoline. This substitution, at least partial, showed prove with the so called first generation bioethanol.

Besides that, sugars from the pulp of the carob, may constitute the basic material of the chemistry of sugars, for example, may be mentioned the production of the biodegradable plastic. , So this is a great opportunity that take advantage of The availability of the biomaterial derived from the carob whose national production can rapidly evolve as needed, in fact, the carob tree is a tree that easily adapts to the unexploited arid areas which Morocco has enormous potential. The present work is devoted to the extraction and identification of the sugars of the carob.

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The study was carried out on specimens from three different areas of northern Morocco. For the actual extraction we have achieved in the simplest conditions possible, in fact regardless of the end product desired, the complexity of the process of extracting sugars will a manner or other a repercussion no beneficial onto the approach adopted for valuing the carob pulp. Thus, we examined the maceration at room temperature previously dried carob powder. We have studied the effect of some parameters such as the nature of maceration solution, the pH, temperature and the maceration time [8-9].

Hydrolysis tests on the solid residue which persists in the maceration were performed to extracting the sugars advantage. The quantity of sugar obtained after each treatment of the carob pulp was estimated by spectrophotometry. The identification of the nature of the sugars extracted is carried out by high-performance liquid chromatography (HPLC).

2. MATERIALS AND METHODS

2.1. Aqueous extraction of carob pulp

The sugars extraction operation of carob pods is made after removing the seeds are then drying and grinding of the carob pods. The flour which obtained mixed with distilled water as the S / V ratio (g/ml) = 1/4, indeed the ratio of the mass of the solid flour (S) and volume of the water (V), it can affect the amount of sugars extracted, [10]. After 24 hours of maceration, the residue is separated from the liquid part by a centrifugal machine with 2000 revolutions/minute for 10 minutes, and subject to a second and third extraction with the same conditions. The three filtrates are mixed for measurement the quantity of sugars extracted. After the third extraction, the solid part is dried and weighed to estimate the mass of disbanded of the pulp, We have reviewed the effect of the temperature 4 ° C and room temperature as the S / V ratio = ¼, a qualitative study was conducted in according to the quantity of the sugars in function of time during the maceration in water at T = 4 ° C.

2.2. Hydrolysis of carob pulp

After the past maceration, the only distilled water not allows the dissolution of sugars, to get advantage, can undergo an hydrolysis treatment in the presence of acid or base to attack by mass the remaining lignocellulosic materials, to this effect we used different chemical agents for the study of the hydrolysis, such as acids (sulfuric, tartaric and oxalic) and bases (sodium hydroxide and potassium hydroxide) at the temperature 80 ° C for eight hours. In the case of sulfuric acid other temperatures were explored. Other tests, using sulfuric acid and sodium hydroxide as the hydrolyses agents, were relized at T = 100 ° C for ten hours on the powder of the pulp carob directly without maceration in water.

2.3. Fermentation

The filtrate containing sugars extracted is subject to an anaerobic fermentation process at room temperature for three days. The pH is adjusted from 5.2 to 6.5 by adding sodium hydroxide (4N).The fermentation is carried out using the two instant yeasts saf indonusa brand in Indonesia and brand Lesaffre Rafiaa in Morocco, dry yeasts are placed in water (10 g / L) the yeast inorganic nutrients were added: ammonium phosphate (3.2 g / L), potassium sulfate (1 g / L) and magnesium sulfate (1.8 g / L).

2.4. Analysis Techniques

2.4.1. Determination of moisture content in the powder of the carob pulp

About 5 g of powder of the carob pulp weight was measured and placed in beaker (previously heated and weighed). After drying at 105 ° C for 6 hours, cooled to room temperature in a desiccator. The moisture is considered as the loss of mass of the sample after drying. It is calculated by the following formula:-

$$\text{moisture}\% = \frac{m_0 - m_1}{m_0} \text{x}100$$

m0: mass of the sample before drying,
m1: mass of the sample after drying.

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2.4.2. Estimate the quantities sugars

2.4.2.1. Total sugars

For determining the quantity of total sugars (sucrose - fructose- glucose) in the aqueous solution of the carob pulp was used (phenol / sulfuric acid) method, the absorbance is measured at λ=489nm [11-12], the identification and quantification of sugars have been made in some cases, by high-performance liquid chromatography (HPLC).

HPLC separation of sugars was carried out on column Agilent Zorbax Carbohydrate Analysis aminopropyl dimension 4.6mm x 250mm 5um 100A, flow rate 1 ml / min of the mobile phase in isocratic acetonitrile / water 75:25, the temperature of the column of 30 ° C, volume of 20ul. Under the same conditions of procurement solutions of glucose, fructose, sucrose at a concentration of 10 mg / ml was injected to observe the order of elution of the sugars and their retention time (standard).

2.4.2.2. Reducing sugars

The reducing sugars assay was performed according to DNS (3, 5-di nitro salicylic acid) method ,the principle of this method consists of reacting salicylic acid 3, 5 di nitro with reducing sugars to form the compounds that absorbs strongly of light in the vicinity of 540 nm [13].

3. RESULTS AND DISCUSSION

3.1. Characterization of carob pod

According to the table (1) we have three samples from three different areas of north of Morocco do not present a notable difference; Relative moisture to three samples about 10% ,With regard to the amount of sugars is obtained about 60% that is shared into two almost equal fractions 30% reducing sugars and 30% non-reducing sugars.

Table 1 Carob pod characterization of samples studied

Characteristic factor	sample1 (Tetouan)	sample 2 (Al-Hoceima)	sample3 (Chefchouen)
Moisture (%)	11	8	9
Total sugars (%)	57	59	62
Reducing sugars (%)	30	30	30
Non-reducing sugars (%)	27	29	32

3.2. Aqueous extraction of sugars from carob pulp

Fig (1) shows the maceration in water as a function of time. The quantity of sugars extracted evolves in a regular manner, however we can notice that in the early hours of maceration mixture is extracted the majority of sugars while the fraction obtained during the rest of time (over 20 hours). This due to the effect of simple diffusion as it may be related by the bonds in the nature of sugars and the matrix of the pulp.

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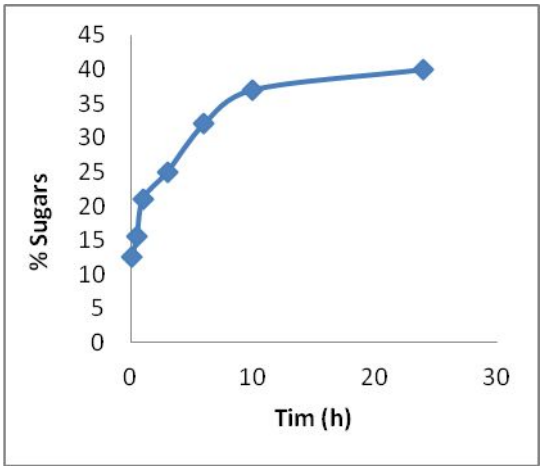

Fig.1. Aqueous extraction sugars of carob pulp at 4°C

Fig (2) shows the temperature of the maceration has an effect on the amount of sugars extracted. And Pass 4 ° C to room temperature, improvement for the extraction of 5%. Unfortunately we did not realize other maceration experiments in higher temperatures. However there may be a regular increase, because the temperature generally has a tendency to accelerate physicochemical transformations.

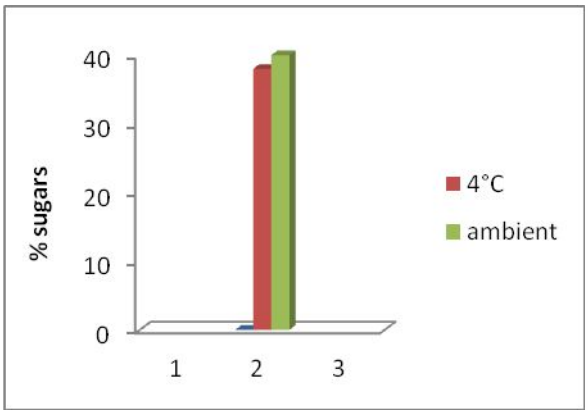

Fig.2. Aqueous extraction sugars of carob pulp at 4°C and room temperature

3.3. Hydrolysis of Carob pulp

Fig (3) represents the effect of the temperature in the presence of Sulfuric acid of the sludge remaining. For each temperature treatment duration of the order of 10 hours. With a temperature to each other, can be seen A marked increase in the percentage of sugars, however between 80 and 100 ° C the extraction appears of complet.

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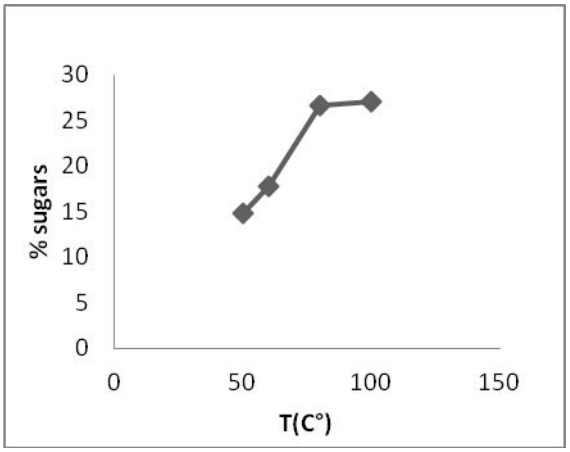

Fig.3. Hydrolysis of carob pulp using sulphuric acid and different temperature (50 °C -60 °C -80 °C – 100 °C).

Fig (4) shows the effect of different treating agents on the three samples which have been the subject of this study. It appears that the sample from the Chefchaouen area is more suitable for different treatments, above all in the case of tartaric acid and oxalic acid. Nevertheless the difference between the three samples is not spectacular. Furthermore, oxalic acid provides the best result as compared to other species.

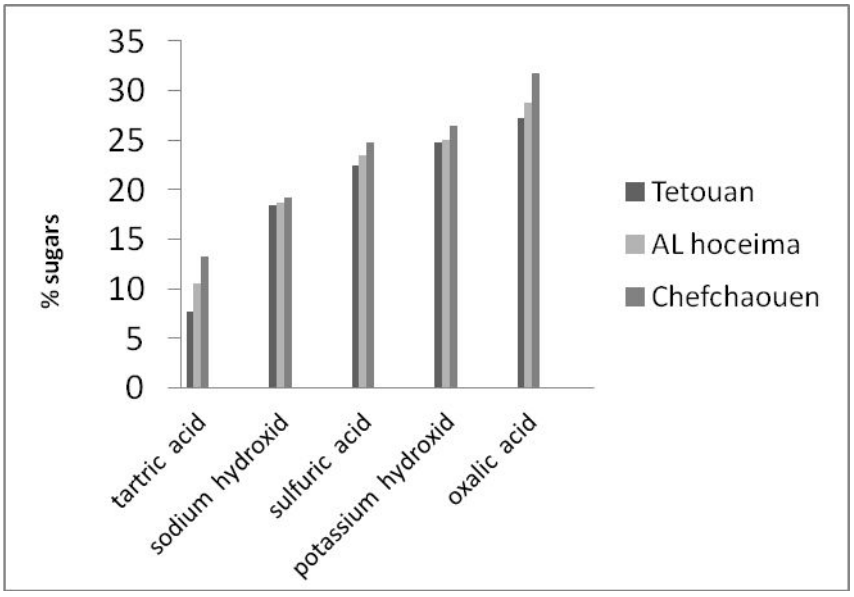

Fig.4. Hydrolysis of carob pulp using different treatment agents at temperature 80 ° C

Fig (5) represents the effect of sulfuric acid and sodium hydroxide about in powder pulp of carob. For each treatment the duration of the order of 10 hours and the temperature 100 ° C, the percentage of the quantity of sugars extracted is slightly higher the sulfuric acid.

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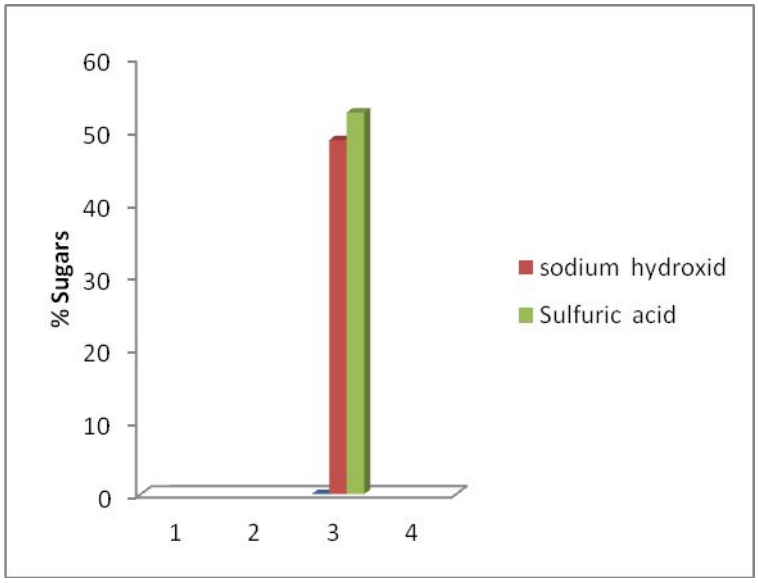

Fig.5. Hydrolysis direct of carob powder using two treatment agents at temperature100 ° C.

3.4. Rate of sugars fermented

The curves (6) and (7) represent the percentages of fermented sugars versus time. The two types of yeast have been used to the stage of fermentation, yeast (Morocco) and yeast (Indonesian) Comparing the two Figs shows that fermentation using the (yeast Morocco), is however kinetically fastest arrives at fermentation all sugars of the two yeasts.

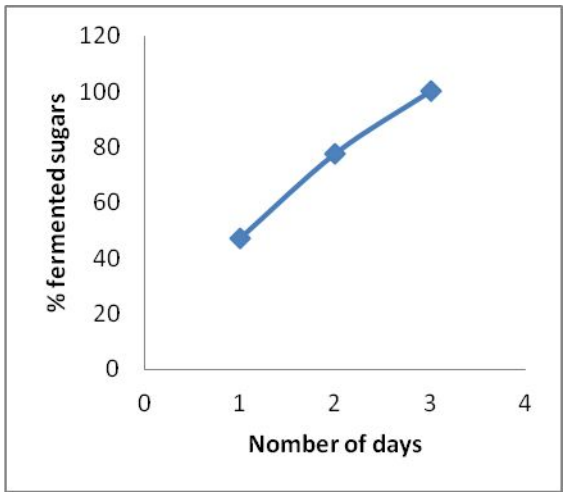

Fig.6. Fermentation aqueous solutions of carob pulp using yeast Indonesian

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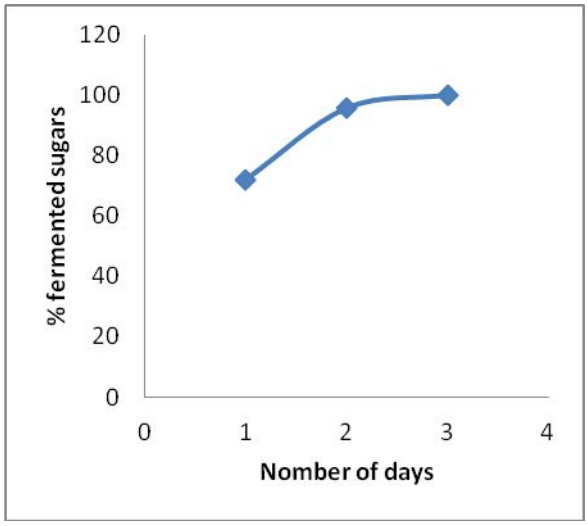

Fig.7. Fermentation aqueous solutions of carob pulp using yeast Morocco.

All the results on this preliminary study enabled us to have interesting data concerning the extraction of sugars from the pulp of the pods of the carob. As reported in the literature, the fruit of the carob tree is very rich in sugars, around 60% it is a highly a percentage higher than that of sugar cane. Which could suggest the replacement of sugar cane by the carob pods in agro-industrial applications, This eventual substitution must be buoyed by the fact that half of the amount of sugars may be extracted by a simple maceration in water at room temperature, especially in the case of a possible food industry because the maceration in water presents not health Danger since it not use dangerous chemicals. Represents the amount extracted sugars, shows that extraction is carried out in two stages, a relatively quick step and the second is slower. Durant the first six hours of maceration are obtained 77.5% on sugars, so that one must await (20h) for the remains fraction (22.5%), if we compare these percentages with those of sugars detected by HPLC, we finds that sugars obtained during the first period is close to the quantity reducing sugars and quantity of non-reducing sugars is close to that of sugars obtained during the second period (slowest) therefore, it can considered than reducing sugars are extracted more rapidly than the non-reductase sugars. Which leads us to consider what we can talk about fast and slow extraction. This difference in behavior between the two types of sugars may be due to the nature of links of these sugars in the pulp, in other terms, the sucrose is linked to the pulp more strongly than other sugars (glucose and fructose). By comparing maceration at 4 ° C and room temperature is found that the quantity of sugars extracted increases by 5%. (Fig 2) This difference shows that the temperature of the maceration may have a noticeable effect on the extraction of sugars this effect is highlighted when we examined the effect the temperature between (50-100 ° C) on indirect hydrolysis with sulfuric acid, the a percentage actually double extracts sugar from 50 to 80 ° CHowever between (80- 100 ° C) temperature has no significant effect (Fig 3).

4. CONCLUSION

For this study we are confirming the wealth of sugars of the carob pods, the percentage of sugars extracted is almost three times that of sugar cane. Half of the sugars may be extracted with a simple maceration in water at room temperature. This represents a great asset to the fruit of the carob tree in the food industry. To extract the other part of the sugars to be applied a treatment in the presence of a chemical agent such as acid or base to facilitate dissolution of the remaining sugars probably bound in the pulp in a different manner compared to those extracted by the maceration. From among the chemicals tested, oxalic acid is the most efficient. Which may suggest the replacement of the sulfuric acid, generally used in the hydrolysis of the biomass treatments, with oxalic acid. The fermentation try performed have shown that with the two yeasts used, we can transform all sugars.

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