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KINETIC OF BATCH PRODUCTION OF LACTIC ACID FROM CAROB PODS SYRUP

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Abstract. Lactic acid production from renewable resources such as pods of carob alternate to derived products from oil present a growing interest, in particular for the production of biodegradable polylactic acid polymer. The carob pods syrup is a by–product composed of sugars and its use as substrate of low cost in various industries is considered however, few microorganisms consume quickly and effectively sugars to give a single product. Lactic acid bacteria were screened for sugars fermentation and *Lactobacillus delbrueckii* subsp. bulgaricus showed the highest levels of lactic acid production. The physicochemical analysis of carob pods syrup showed that it presents a suitable quality. The micro–organism implemented is kind *Lactobacillus delbrueckii* subsp. bulgaricus, cultivated in bathe fermentation. Three culture mediums were tested, the carob syrup enriched with sweet cheese whey (CS+SCW), carob syrup enriched with Tween80 (CS+T80) and carob syrup enriched with sweet cheese whey+Tween80 (CS+SCW+T80), the addition of the growth factors improve the culture, that is why the production of lactate is very important improved 52.76 g/l.
***Keyword**: carob *Ceratonia siliqua* L, *Lactobacillus delbrueckii* subsp. *Bulgaricus,* lactic acid.

Introduction

The carob tree (*Ceratonia siliqua* L.), belonging to the family Cesalpiniaceae sub–family of the family Leguminoseae, is widely used in the Mediterranean regions [BATLLE and TOUS, 1999] cultivated for ornamental and industrial purposes [YOUSIF and al., 2000].

World production is estimated at about 422333.66 ton per year, and the main producers for pulp, seeds, respectively, are Spain (36 %, 28 %), Morocco (24 %, 38 %), Italy (10 %, 8 %), Portugal (10 %, 8 %), Greece (8 %, 6 %), Turkey (4 %, 6 %) and Cyprus (3 %, 2 %) of the world production.

Carob tree has an economic and environmental importance in Algeria.

It is used in reforestation of arid and degraded areas and also as for ornamental purposes.

The pulp and the seeds have some interesting properties and are often used in food and pharmacological industry [BATLLE, 1997; MARKIS et KEFALAS, 2004]. Carob pods are also characterized by high sugar content (500 g/kg) [NAS, 1979; PETIT and PINILLA, 1995; BATLLE and TOUS, 1997; MARKIS and KEFALAS, 2004].

Carob pulp is a good source of protein (2.7–7.6%) but it is poor in lipid (0.4–0.8%) [BINER et *al.*, 2007].

In Algeria, the carob tree is frequently cultivated in the Saharan Atlas and it is common in the tell, is found naturally in association with the almond tree, Olea Europea and Pistacia Atlantica in hot semiarid floors Subhumid and humid.

The pulp and the seeds are valorized in different applications, seeds powder can be used in baby foods to prevent vomiting [FAO, 2001].

The locust bean gum is also applied in pharmaceutical industry as drug delivery [SANDOLO et *al.*, 2007, BUTNARIU, *et al.,* 2011, BUTU, *et al.,* 2014b, BUTNARIU, 2012, IANCULOV, *et al.,* 2004, BUTU, *et al.,* 2014b].

Chemical composition of carob had been studied extensively for different countries of the Mediterranean area. It had been observed that this composition is depending not only on technological factors such as the extraction and analytical methodologies, but also on the genotype of the plant, the geographical origin, the climate conditions and the harvesting and storage procedures [BATLLE & TOUS, 1997; BINER et al., 2007; OWEN et al., 2003; NAGHMOUCHi et al., 2009; SIDINA et al., 2009].

However, in spite of the great interest to carob and their use in different applications, few studies are available on carob. Lactic acid production from renewable resources such as wheat carob as alternate to derived products from oil present a growing interest, in particular for the production of biodegradable polylactic acid polymer.

Carob Pods is a by–product composed of sugars and its use as substrate of low cost in various industries is considered however, few microorganisms consume quickly and effectively sugars to give a single product.

Lactic acid bacteria were screened for sugars fermentation and *Lactobacillus delbrueckii subsp. bulgaricus* showed the highest levels of lactic acid production.

Lactic fermentation is the main activity of lactic bacteria, while *Lactobacillus delbrueckii subsp. bulgaricus* is the major species used in lactic acid making. It utilizes sucrose, glucose and fructose carbon sources to produce lactic acid [CAUNII, *et al.,* 2015, BUTNARIU, *et al.,* 2015a, BUTNARIU, *et al.,* 2016, BUTNARIU, *et al.,* 2015b, BUTU, *et al.,* 2015].

Indeed, this carob is rich sugars that could be used as carbonaceous source of fermentation for the production of the biomass and lactic acid.

The objective of this study is the utilization of the Carob Pods Syrup as the substrate for the growth of La *Lactobacillus delbrueckii subsp. bulgaricus* and the production of lactic acid.

Material and methods

Vegetable material

The carob (*Ceratonia siliqua* L.) used in current experiments was harvested in the month of 2015 from the region of Mohammadia (Mascara, Algeria) (Figure 1).

Figure 1. Carob pods (*Ceratonia siliqua L.),* of the Mohammadia area (Mascara, Algeria)

The choice of this variety is justified by its availability and important nutritive value, especially the one of reducing fermentable sugars such as glucose and sucrose. 20 pods were randomly collected.

This choice was made based on the study on Morphological characteristics (whole pod weight, Weight of pulp, Seed weight, length of pod). Length (cm) of pod was measured using a measuring tape, whereas width (cm) was assessed with the Vernier caliper. Weight (g) of pods and kernels were taken using a balance.

Results of morphological characteristics of carob pod are presented in Table1.

Table 1.
Morphological characteristics of Carob pods

Parameters	Average volume
Number of sees/pod	11± 1.49
Whol pod weight (g)	12.71± 1.52
Wight of pulp (g)	5.38 ± 1.36
Seed weight(g)	0.68 ± 0.02
Lengh of pod (cm)	13.43 ± 0.760
Wigth of pod (cm)	2.16 ± 0.29
Lengh of the seed (mm)	10.3± 0.67
Width of the seed	7.9± 0.99
Thickness of the seed	0.21± 0.01
Length report/Width	6.23
Seed report/carob (%)	5.35
Report pulp / pod (%)	42.32
Pulp/seed	7.91

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Extraction and analysis of carob pods syrup

To determine the chemical composition of carob pulp, Carob pods were chopped into small particles (1–3 cm). One liter of hot water at 80–85°C was added to 200 g of carob pods, homogenized and through a cloth.

The syrup obtained was centrifuged at 15000 rpm for 10 min to separate the cellulose debris. The collected supernatant was used as culture medium. The syrup is fixed in a pH 6 and sterilized during 20 min at 120°C. The extraction parameters were obtained from method advocated by [TURHAN *et al.,* 2008, BUTU, *et al.,* 2014a, BUTNARIU, *et al.,* 2012].

pH is measured using a pH meter and density was determined by density meter. Concentration of lactic acid was determined by acidity titration with NaOH 0.1 N. Total nitrogen of carob and protein content was determined by the method of Kjeldahl digestion and distillation apparatus [AOAC, 2007, BUTNARIU and GIUCHICI, 2011, PETRACHE, *et al.,* 2014, BUTNARIU, 2014].

Total and reducing sugars were determined colorimetrically at 480 nm by Dubois method [DUBOIS *et al.,* 1956], the ash content of the carob was determined according to the AOAC official method 972.15 by incineration one gram of syrup at a temperature of 600°C during 3 h.

Moisture and dry matter were determined by drying 10 mL of syrup at 105°C during 18 h. The mineral salts are determined according to the methods advocated by [MUHAMED 1983, BUTNARIU, *et al.,* 2006, RODINO, *et al.,* 2014, BUTNARIU, *et al.,* 2005].

Leavens used: *Lactobacillus delbrueckii subsp. bulgaricus* were obtained from Giplait of Mascara (Algeria).

The strain of Lactobacillus is homo fermentative (homolactic) which means that more than 90% of metabolits are produced by the lactic fermentation.

The insulation of the lactic flora: The strain was inoculated at a rate of 1% in tubes containing 10 mL of sterile skimmed milk and incubated at 30°C until the coagulation of milk, then sown by streaking on MRS medium.

The enrichment of *Lactobacillus delbrueckii subsp. bulgaricus* was carried out using MRS broth.

Microorganism and grwth conditions

Lactobacillus delbrueckii subsp, a homofermentative lactic acid producer, was used. Stock cultures were stored in MRS medium with 25% (v/v) glycerol at –20°C. The inoculum was prepared by transferring glycerol stock culture to Erlenmeyer flasks containing 100mL of liquid MRS medium for preculture.

The flask was subsequential incubated at 38°C for 12h, the time needed for the microorganism to reach the exponential growth phase.

Then, the culture was inoculated to Erlenmeyer flasks containing the production medium. 10 % inoculums grown in the MRS medium was used in all fermentations.

Flask experiments were carried out in 250 mL Erlenmeyer containing 100 mL of production medium.

The agitation speed and culture temperature were controlled at 150 rpm and 38°C respectively.

Fermentation conditions and methods

The biochemical analysis applied on the carob pods syrup shows that it is poor in protein and fatty–acids, so the addition of these elements (growth factors) is necessary to the syrup in order to import the quantity of lactic acid.

Three culture mediums were used: carob syrup with 10 g of sweet cheese whey (CS+SCW), carob syrup with 1 mL of Tween80 (commercial form of oleic acid) (CS+T80) and carob syrup with 10 g of sweet cheese whey and 1 mL of Tween 80 (CS+SCW+T80). The biomass is determined by measurement of the optical density (OD) at 600 nm by a spectrophotometer.

The various analyses carried out allow the following time evolution of the component concentrations present in the culture medium:

[Biomass: *(OD) =f (t),* sugars: *S=f (t)* and the lactic acid: *P=f (t)]*.

From these raw data it is possible to calculate the fermentation kinetic parameters in the batch culture by the calculation of the specific rate of growth (μ in h–1), of substrate consumption (Qs in $g.g^{-1}.h^{-1}$) and lactic acid production (QL.A in $g.g^{-1}.h^{-1}$).

$$\mu = \frac{r'''x}{x}, Qs = \frac{r'''s}{x}, Ql.a\ \frac{r'''p}{x}$$

The maximal specific growth rate (μmax) was determined from the slopes of the plotted linear curve: LnX/X0=f (t).

The biomass (Yx/s) and products (Yp/s) yields are defined as the mass ratios in biomass and metabolites formed per gram of consumed carbonaceous substrate.

Results and discussion

Biochemical composition of carob pods syrup

The carob pods syrup which has been the subject of our work has high water content 83.66 % (Table 2), we agree that a product with high water content facilitates lactic acid bacteria proliferation and helps for a better substrate–enzyme contact.

Table 1.

Biochimical composition ofcarob pods syrup

Biochemical composistion of carob pods syrup	Average
Dry Matter	15 g/100mL
Moisture	83.66 %
pH	5.08
Acidities	27m.eq%
Density	2.19Kg/m^3
Total sugar	31.82g/L
Proteins	0.16g/100mL
Vitamin C	0.07g/L
Ashes	0.6 %

Carob pods syrup obtained is very rich in sugars (31.82 g/L) and vitamin C (0.07 g/L). The carob syrup is poor with protein 0.16 g/100 mL. An ash content of 0.6% indicates its richness of minerals including potassium.

The date, with a pH of 5.5; it seems to be favorable for the growth of *Lactobacillus delbrueckii subsp. bulgaricus*.

The carob syrup. An ash content of (2g/L) indicates its richness of minerals.

Results of the fermentation kinetics

The analysis applied on the carob pods syrup shows that it is poor, with protein, minerals such and fatty–acids, so the addition of these elements (growth factors) is necessary to the syrup in order to import this quantity of lactic acid.

According to [FIDAN and SAPUNDZHIEVA, 2015], carob fruit (pulps and seeds) and flour are rich in carbohydrates.

According to the literature data, many factors affect the chemical composition of the fruit as well as its mineral content, for example, temperature, dryness [NUNES *et al.*, 1992], irrigation and fertilization [CORREIA *et al.*, 1997] and salinity [El–DENGAWY *et al.*, 2011].

Finally, the biochemical analysis show that it can constitute a fermentation medium of good quality.

The results of the three fermentations, of the pods enriched with sweet cheese whey (CS+SCW), pods syrup enriched with Tween80 and pods syrup with sweet cheese whey and Tween80 (CS+SCW+T80) by *Lactobacillus delbrueckii subsp. bulgaricus* are presented in figures 1, 2, 3, which present the evolution of the concentrations in biomass, sugars and lactic acid for the pods syrup fermentation, we observe a weak initial concentration of biomass is of 0.2 then it increased after 36 h of fermentation until 1.6, after that we recorded the fall of biomass till a value of 0.5 (CS +T80).

The results clearly indicated that the highest amount of biomass and lactic acid were obtained with the syrup carob enriched with sweet cheese whey and Tween80.

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In the medium enriched with sweet cheese whey, (CS with SCW), and two components (SCW and T80), the lactic acid rate evolves gradually to achieve the end of fermentation 52.76 g/L.

The addition of Tween 80 and sweet cheese whey in carob syrup increase the lactic acid production.

However, decreasing of sugar rates is very faster in the medium supplemented with T80 and SWC.

Where the Lactobacillus *delbrueckii subsp. bulgaricus* consumes about half quantity of initial total sugars, during 35 h of fermentation from an initial quantity of 31.82 g/L of sugars, it remains 5.6 g/L which implies an amount of 27.6 g/L.

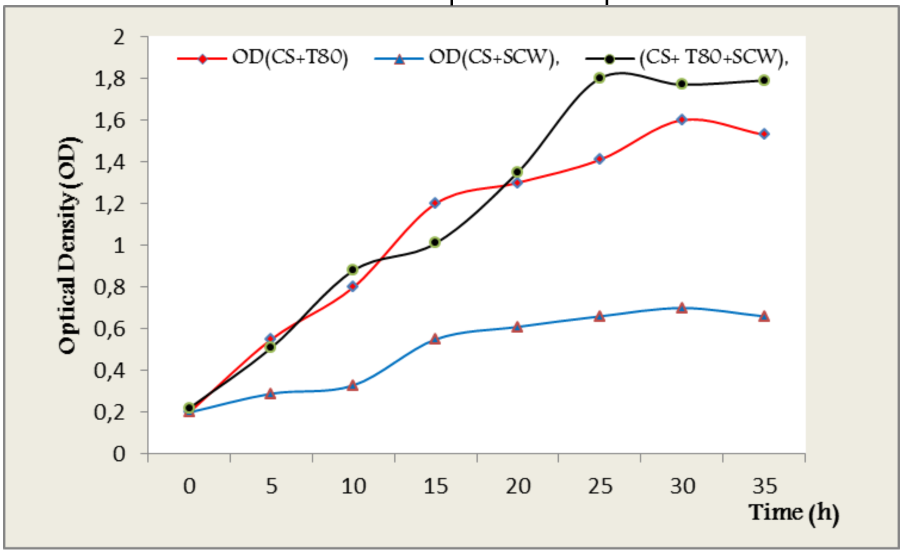

Figure 2. Evolution of OD during the culture of *Lactobacillus delbrueckii subsp. bulgaricus* on mediums containing *Carob Syrup +T80* (CS+T80), *Carob Syrup Sweet Cheese Whey* (CS+SCW) and *Carob Syrup + T80+Sweet Cheese Whey* (CS+T80+SCW).

In parallel, in the medium enriched with Tween80 and the production of the lactic acid starts from a value of 6 g/L and reach 17 g/L of lactate after 35 h of fermentation.

In parallel and since there was an evolution of biomass and lactic acid, there was a consumption of sugars; it remained at the end of fermentation 10 g sugars and 8 g/L (CS+SWC).

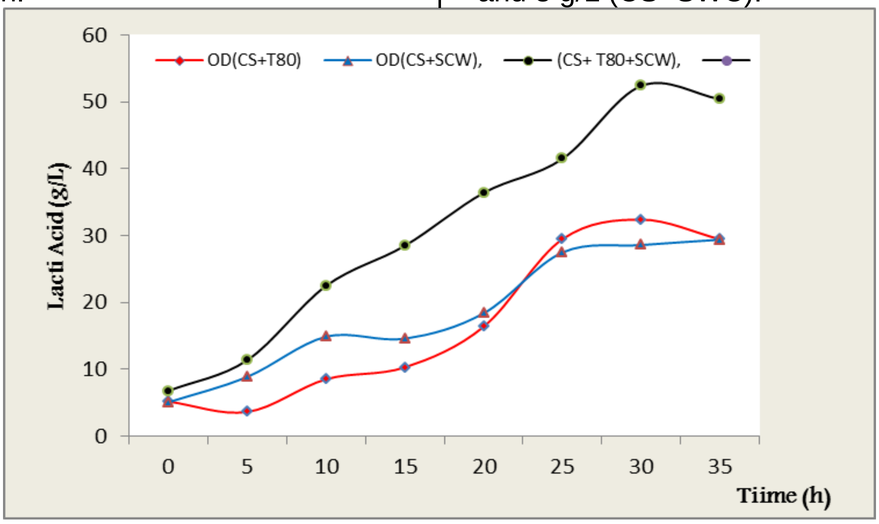

Figure 3. Evolution of lactic acid in g/L during the culture of *Lactobacillus delbrueckii subsp. bulgaricus* on mediums containing *Carob Syrup +T80* (CS+T80), *carob syrup +Sweet Cheese Whey* (CS+SCW) and *Carob Syrup + T80+Sweet Cheese Whey* (CS+T80+SCW).

However for fermentation on medium containing pods syrup with Tween80 an SCW, the optical density of *Lactobacillus delbrueckii subsp. bulgaricus* started with an initial concentration 0.2 to reach a maximum value of 1.8, then a decrease of the biomass up to a value of 0.5 was recorded.

This decreasing in biomass correspond to the phase of decline which is due to the impoverishment of nutriments from the medium and the accumulation of metabolism wastes causing important physic chemical changes and bacteria lysis.

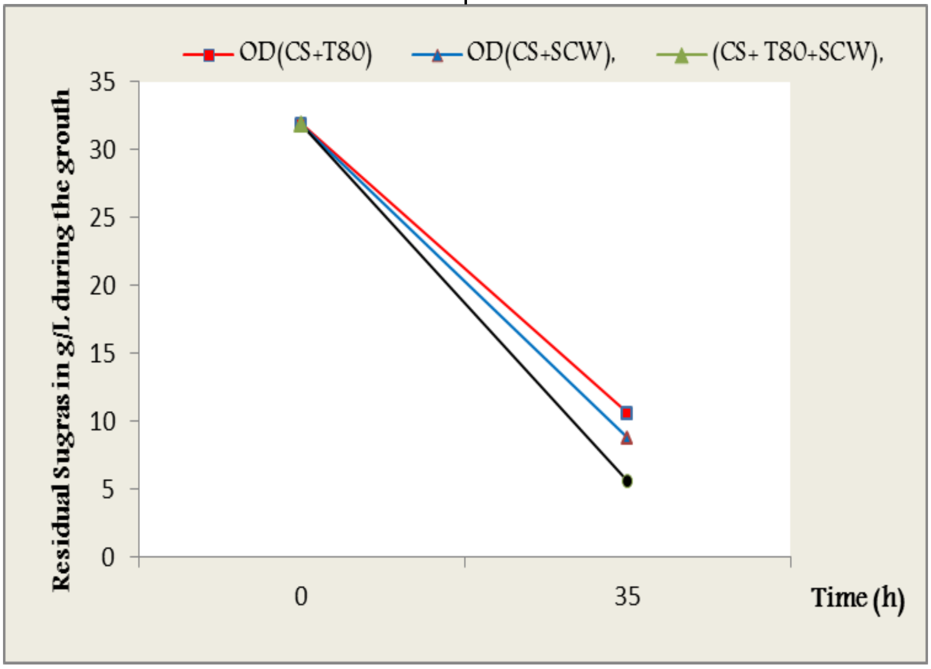

Figure 3. Evolution of residual sugars in g/L during the culture of *Lactobacillus delbrueckii subsp. bulgaricus* on mediums containing Carob Syrup +T80 (CS+T80), *Carob Syrup+ sweet cheese whey* (CS+SCW) and *Carob Syrup + T80+Sweet Cheese Whey* (CS+T80+SCW).

In the case of enriched (CS with SWC), we observe a low initial concentration of biomass and in the end of fermentation optical density reaches a maximum value 0.7.

Sweet cheese whey (source of soluble proteins) is essential for growth of lactic acid.

Comparing the results for both fermentations indicated that the addition of growth factors in culture medium has a positive and beneficial effect on fermentation, since the growth rate and lactic acid production increase after the enrichment of carob pods syrup.

The addition of Tween 80 allows better cells excretion of lactic acid by creating pores in the membrane and plays the role of surfactant which makes a good contact between bacteria and nutriments, and for more it is considered as a source of carbon and energy for electrons [BOUHADI *et al.*, 2012].

From these results, the enriched mediums present an interesting result.

Moreover, lactic acid bacteria are unable to synthesize amino acids from simple inorganic nitrogen source; *Lactobacillus delbrueckii subsp. bulgaricus* have needs glutamic acid, histidine, methionine, valine, leucine and tryptophan.

Once the amino acid exhausted, the lactic acid bacteria use the molecular weight of peptides of less than 1500 daltons.

The peptides are assimilated either by hydrolysis outside of the cell and transported form of amino acids or with active system in the absence of peptidase.

Conclusions

The present work has shown that the valorization of dates syrups, packaged industrially to 32.82g/L by the Tunisian agro–food industry, lactic acid is Possible by yeasts.

We obtained 24 g /L of Lactic Acid after 35 h of fermentation by *Lactobacillus delbrueckii subsp.* which consumes 27.22 g/L of the sugars of a culture medium based on syrup of carob.

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