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KINETICS STUDY OF THE GROWTH OF *Lactobacillus bulgaricus* FROM CAROB PODS SYRUP

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Hariri AHMED[1]*, Ouis NAWEL[2], Bouhadi DJILALI[1] and Ould Yerou KARIMA[1]

*[1]Bioconversion Laboratory, Microbiology Engineering and Health Safety, Faculty of Science the Nature and Life, University of Mascara, (UN 2901), BP.763, 29000, Sidi Said, Mascara, **ALGERIA***
*[2]Laboratory of Physical Chemistry of Macromolecules and Biological Interfaces, Faculty of Science the Nature and Life, University of Mascara, (UN 2901), BP.763, 29000, Sidi Said, Mascara, **ALGERIA***
Corresponding author: haririahmed@yahoo.fr

Abstract. The objective of this work consists in valuing the carob pods (very rich in sugars and in nourishing elements) as being a medium of culture for growth of *Lactobacillus bulgaricus* and comparison this natural syrup with MRS medium for lactic acid production curve. By its biochemical composition, the carob syrup is very rich in carbohydrates 16 g/L, protein 0.25 %, ash 0.8% including potassium (110 mg/100 mL of MF), sodium (80) and calcium (150). Kinetics study of growth of *Lactobacillus bulgaricus* in MRS and carob pods syrup showed that high biomass and lactic acid were obtained in the end of carob fermentations respectively (5.51 and 13.8) compared to MRS fermentation (4.57 and 10.9). This study clearly showed that carob pods have potential to be used as feedstock for lactic acid production by *Lactobacillus bulgaricus* using batch culture.
Keyword: Carob pods syrup, fermentation, *Lactobacillus bulgaricus*.

Introduction

Carob (*Ceratonia siliqua L.*), which has been widely grown in the Mediterranean region, belongs to the Caesalpinaceae subfamily of the family Leguminoseae [YOUSIF and ALGHZAWI, 2000].

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 [GIROLAMO and LAURA, 2002; BINER *et al.*, 2007]. Several products are produced from its seed and pod. The pod of the carob has a high energy value 17.5 $kJ.g^{-1}$ D.M. [BINER *et al.*, 2007; AVALLONE *et al.*, 1997].

When the fruits are ripe enough, they have 91–92 % total dry matter and 62–67% total soluble solids, which consist of 34–42% sucrose, 10–12% fructose, and 7–10 % glucose [KARKACIER and ARTIK, 1995]. Carob pods are also characterized by high sugars content 500 $g.kg^{-1}$ [PETIT and PINILLA, 1995, IANCULOV, *et al.*, 2004, BUTNARIU, *et al.*, 2015b, BUTU, *et al.*, 2014c, BUTNARIU, *et al.*, 2006].

Carob also contains phenolic compounds from 2 to 20 % D.M. [OWEN *et al.*, 2003]. These phenolic compounds present opportunities chemo–preventive interesting against certain cancers, especially those of the gastrointestinal tract. These phenolic compounds are mainly composed of gallic acid, syringic, p–coumaric, m–coumaric, benzoic acid and hydroxytyrosol [FADEL *et al.*, 2011].

Carob pulp is a good source of polyphenols (mainly tannins 16–20%) [BINER *et al.*, 2007; OWEN *et al.*, 2003] and protein (2.7–7.6 %) but it is poor in lipid (0.4–0.8 %). The pulp and the seeds are valorized in different applications.

The pod fiber content plays a role in hypocholesterolemic and hypoglycemic regulation, whereas phenolic compounds can be used as antioxidant additive. Moreover, the locust bean gum (additive E 410) extracted from the endosperm of seeds is used as stabilizer and thickening agents in food industry [NEUKOM, 1989, BUTNARIU, *et al.*, 2015a, BUTU, *et al.*, 2014b, BUTNARIU, *et al.*, 2016, BUTU, *et al.*, 2015, CAUNII, *et al.*, 2015].

The obtained carob extract has been utilized for production of value added products, especially by fermentation as suggested [ROUKAS, 1994; TURHAN *et al.*, 2010]. Lactic acid can be one of these value-added products due to its current and future potentials. Lactic acid is a natural organic acid that can be

produced by chemical synthesis or fermentation [SINGH *et al.*, 2006].

It and its derivatives are widely used in food, pharmaceutical, leather and textile industries [HUJANEN and LINKO, 1996].

Furthermore, since lactic acid has an excellent reactivity that stems from the fact that it possesses both carboxylic and hydroxyl groups, it can undergo a variety of chemical conversions into potentially useful chemicals such as propylene oxide, propylene glycol, acrylic acid, 2,3–pentanedione and lactate ester [LITCHFIELD, 1996; YUN *et al.*, 2003].

The production of lactic acid using fermentation has several advantages compared to chemical synthesis because of low–cost substrates and low energy consumption. Recently, there has been an increased interest in L–lactic acid production because it could be used as a raw material for the production of polylactic acid, a polymer used as special medical and environmental–friendly biodegradable plastic, and hence a substitute for synthetic plastics derived from petroleum feedstocks [DATTA *et al.*, 1995; WEE *et al.*, 2006, [BUTNARIU, 2012, PETRACHE, *et al.*, 2014, BUTNARIU, *et al.*, 2005].

The objective of our study was to comparison between the productions of lactic acid in carob pods syrup by different lactic acid bacteria.

Purpose of the research consists in valuing the carob pods (very rich in sugars and in nourishing elements) as being a medium of culture for growth of *Lactobacillus bulgaricus* and comparison this natural syrup with MRS medium for lactic acid production.

Material and methods

Vegetable material and extraction: The carob used in current experiments was harvested in the region of ELBORDJ (Mascara, Algeria).

The species was identified by Mr Kada RIGHI, from SNV faculty, University of Mascara and the voucher specimen of the plant has been retained in the Department of Biology. 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 fructose [BUTNARIU and CORADINI, 2012, FERENCZ, *et al.*, 2012, BUTNARIU, *et al.*, 2012, RODINO, *et al.*, 2014].

Extraction and biochemical analysis of carob pods syrup: Carob pods were chopped into small particles (1–3 cm). One liter of hot water at 85–90°C was added to 200 g of carob pods (20%), homogenized and through a cloth. The syrup obtained was centrifuged at 15.000 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 and collab. [TURHAN et al., 2008]. Total nitrogen and protein content was determined by the method of Kjeldahl digestion and distillation apparatus [AOAC, 2007]. Reducing sugars were determined colorimetrically at 480 nm by Dubois method [DUBOIS, 1956, BUTNARIU and GIUCHICI, 2011, BUTU, *et al.*, 2014a, BUTNARIU, 2014, BARBAT, *et al.*, 2013, BUTNARIU, *et al.*, 2011].

Standards were prepared with glucose solutions at different concentrations. The ash content 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 [AOAC, 2006].

Moisture and dry matter were determined by drying 10 mL of syrup at 105°C during 18 h.

Bacterial strain and media: *Lactobacillus delbrueckii subsp. bulgaricus* were obtained from Giplait of Mascara (Algeria). It was maintained on MRS medium in the presence of 10% glycerol and preserved at –20°C.

The MRS medium used in growth and inoculum preparations contained the following components (in $g.L^{-1}$): 10 Soya peptone, 10 beef extract, 5 yeast extract, 2 K_2HPO_4, 5 $NaCH_3CO_2$, 3 H_2O, 2 triammonium citrate, 0.2 $MgSO_4$, 7 H_2O, 0.05 $MnSO_4$, H_2O, 15 glucose and 1 mL Tween 80. The pH was adjusted to 6 prior to sterilization at 108°C for 15min. The reactivation phase is realized after two successive transplantations at 42°C during 2 hours on liquid MRS medium [AMRANE and PRIGENT, 1994].

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Fermentation conditions and methods: All experiments were carried out in a 2L jar fermenter (Applikon Biocontroller ADI1030). The agitation speed was set at 200 rpm, pH at 6 and the inocula were incubated at 42°C for 12hr at 200 rpm before their transfer to the fermenter in a 10 %. The fermentation batch (in MRS) made by *Lactobacillus bulgaricus* for production of lactic acid at a glucose concentration of 15 g.L^{-1} was carried out. Other culture led in the same conditions was also prepared in carob pod syrup at concentration 20%. The biomass is determined by measurement of the optical density (OD) at 570 nm by a spectrophotometer HITACHI 4–2000.

Culture samples were centrifuged (13200 g at 4°C for 5 min), diluted and filtered. Residual glucose and lactic acid concentrations were determined by Multi parameter Medical Analyzer.

The enzymatic kit used for the lactic acid dosage is the PAP Ref–61 192 and for the glucose dosage it is the Elitech diagnosis ref–GPSL–0500. The various analyses carried out allow the following time evolution of the component concentrations present in the culture medium.

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 sugars consumption Qs in g.g^{-1}.h^{-1} and of lactic acids production Ql.a in g.g^{-1}.h^{-1}.

$$\mu = \frac{\frac{dX}{dt}}{X} \qquad Qs = \frac{-\frac{dS}{dT}}{X} \qquad Ql.a = \frac{\frac{dP}{dt}}{X}$$

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

Results and discussion

Biochemical composition of carob pods syrup: The carob pods syrup has a 94.44 % of moisture, we agree that a product with high water content facilitates lactic acid bacteria proliferation and helps for a better substrate–enzyme contact since free water is the nutriments carrier [BOUHADI *et al.*, 2012]. Results are shown in the table 1.

Table 1.

Biochemical composition of carob pods syrup.

Biochemical composition of carob pods syrup	Average
Moisture (%)	94.44 ± 0.33
Dry Matter (%)	5.56 ± 0.33
pH	5.25 ± 0.08
Reducing sugars in g.L^{-1}	30.69 ± 0.78
Proteins in % of M.F	0.25 ± 0.02
Ashes in % of M.F	0.2 ± 0.017
Potassium in mg.100 mL^{-1} of M.F	18.5 ± 0.17
Sodium mg.100 mL^{-1} of M.F	1.9 ± 0.13
Calcium in mg.100mL^{-1} of M.F	69 ± 0.26

Karkacier and Artik report that the fruits are ripe enough; they have 91–92 % total dry matter and 62–67% total soluble solids, which consist of 34–42 % sucrose, 10–12 % fructose, and 7–10 % glucose [KARKACIER and ARTIK 1995].

Reducing sugars (30.69 g.L^{-1}) are a carbon source that can satisfy the lactic acid bacteria requirements. The content of sugars found a high-energy value 17.5 kJ/g D.M. for pod of the carob [BINER *et al.*, 2007; AVALLONE *et al.*, 1997; DAKIA *et al.*, 2007].

Petit and Pinilla report that the carob pods are also characterized by high sugar content 200–500 g/kg [PETIT and PINILLA 1995]. According to the results obtained by Yousif and Alghzawi and by Vaheed and collab., carob pod contains 45 to 56.10% total sugar and13.60 to19.00% reducing sugar [YOUSIF and ALGHZAWI 2000, VAHEED *et al.*, 2014]. Vaheed and collab. show that the carob pods powder contained 9.09 moisture, 56.10 total sugars, and 19.00 reducing sugars (all as weight %) [VAHEED *et al.*, 2014].

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 [FIDAN and SAPUNDZHIEVA, 2015], carob fruit (pulps and seeds) and flour are rich in carbohydrates, proteins and also are a good source of K, Ca, Na, Fe, and Mg.

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 and MARTINS–LOUCAO, 1997] and salinity [EL–DENGAWY *et al.*, 2011]. The protein fraction are considerable (0.25 %); therefore it can serve as a nitrogen source. An ash content of 0.2 % for carob syrup indicates its richness of minerals including potassium, sodium and calcium.

The different biochemical analyzes show the richness of carob pods syrups in nutritional elements that makes them favorable to the lactic acid fermentation.

Fermentation kinetics: According to the obtained results (figures 1 to 3), it is observed that the *Lactobacillus bulgaricus* growth for the batch fermentations is characterized by a short duration of the latency phase which indicates that the inoculated cells were in full exponential phase.

Table 2.

Kinetics parameters of MRS and carob pods syrup fermentations.

Parameters	MRS	Carob pods syrup
Biomass max ($g.L^{-1}$)	4.57	5.51
Lactic acid max ($g.L^{-1}$)	10.9	13.8
Residual glucose ($g.L^{-1}$)	0.008	0.007
μmax (h^{-1})	0.17	0.22
Qsmax ($g.g^{-1}.h^{-1}$)	1.56	1.50
Q.l.a max ($g.g^{-1}.h^{-1}$)	0.61	0.61
Yx/s ($g.g^{-1}$)	0.84	0.41
Yp/s ($g.g^{-1}$)	0.71	0.87

For the fermentation in MRS medium, it is observed a weak biomass initial concentration of 1.1 $g.L^{-1}$ which increases after 8 hours of fermentation until 4.57 $g.L^{-1}$ (at the end of the exponential phase).

The growth end is due to the glucose exhaustion in the culture medium which reaches a final value of 0 $g.L^{-1}$ after 24 hours of fermentation.

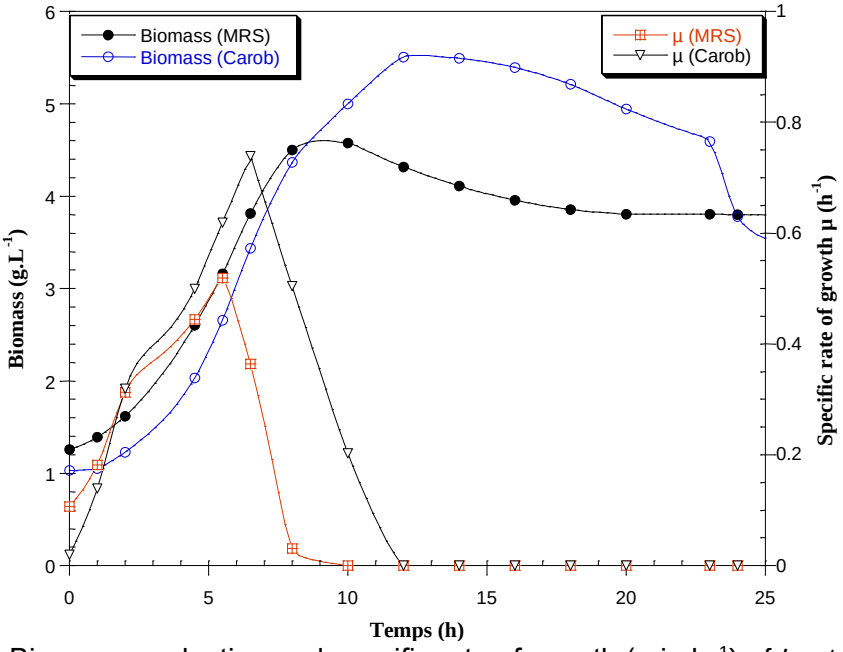

Figure 1. Biomass production and specific rate of growth (μ in h^{-1}) of *Lactobacillus bulgaricus* during two batch fermentations (MRS medium and Carob pod syrup medium).

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During the stationary phase, the strain consumes the glucose and uses it only for the cellular maintenance. In parallel, the lactic acid production starts from a value of 3 g.L^{-1} and reaches 10.9 g.L^{-1} of lactic acid after 24 hours of fermentation. However for the carob pods syrup fermentation, the *Lactobacillus bulgaricus* biomass starts with an initial concentration of 1.1 g.L^{-1}.

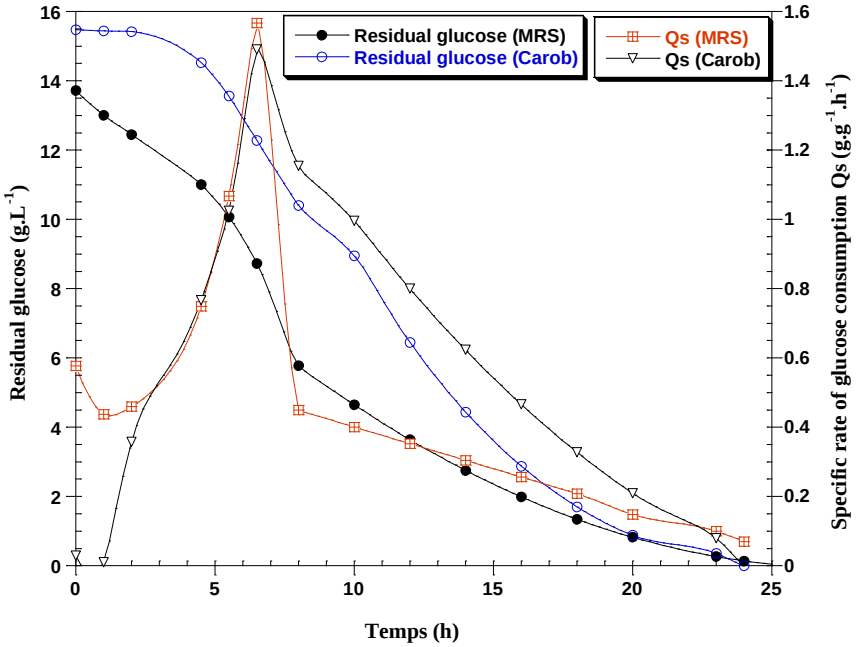

Figure 2. Residual glucose and specific rate of glucose consumption (Qs in g.g^{-1}.h^{-1}) by *Lactobacillus bulgaricus* during two batch fermentations (MRS and Carob pod syrup).

After 10 hours of fermentation, cellular concentration increases at a value of 5.5 g.L^{-1}. For the production of lactic acid the *Lactobacillus bulgaricus* culture reaches the maximum value of 13.8 g.L^{-1} after 24 hours of fermentation. In parallel and since there is an increase in the biomass and lactic acid amounts, there is sugar consumption and it remains at the end of the fermentation 0 g.L^{-1} of glucose.

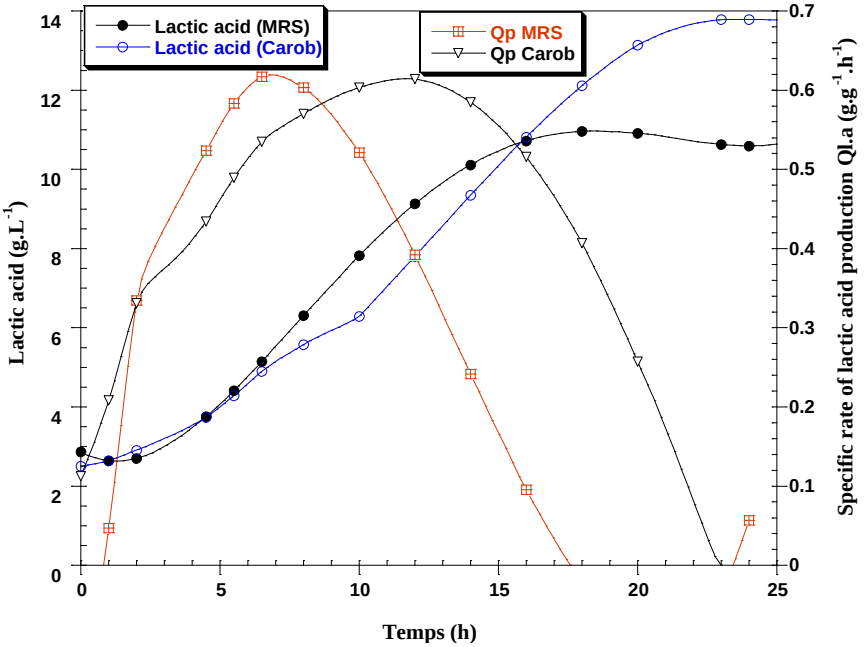

Figure 3. Lactic acid production and specific rate of Lactic acid production (Ql.a in g.g^{-1}.h^{-1}) by *Lactobacillus bulgaricus* during two batch fermentations (MRS and Carob pod syrup).

The specific rate of *Lactobacillus bulgaricus* growth (μ) in the MRS medium starts at 0.1 h^{-1} and reaches its maximum value 0.5 h^{-1} after 6 hours of fermentation, then it decreases down to 0 h^{-1} after 10 hours of culture. For the carob pods syrup fermentation, μ starts with an initial value of 0.02 h^{-1} increasing to 0.76 h^{-1} after 6 hours of fermentation.

The maximal sugar consumption specific rate (Qs max) is of the order of 1.5 $g.g^{-1}.h^{-1}$ for the MRS and carob medium and the maximal (Ql.a.max) lactic acid production specific rate is of the order of 0.6 $g.g^{-1}.h^{-1}$ for the MRS as well as carob cultures.

The growth kinetics may be characterized by a maximal growth specific rate (μmax) which is equal to 0.17 h^{-1} in the MRS medium and increases up to 0.22 h^{-1} in the carob medium. For the MRS fermentation, the yield of sugar conversion into biomass is 0.86 $g.g^{-1}$ and 0.7 $g.g^{-1}$ into lactic acid (Table 2).

Conclusions

The bioconversion of agricultural by products mainly the ones rich in fermentable sugars has an economic and strategic interest. The objective of this work consists in valuing the carob pods as being a medium of culture for growth of *Lactobacillus bulgaricus* and comparison this natural syrup with MRS medium.

By its biochemical composition, the carob syrup is very rich in carbohydrates. Which make it a substrate of choice for the development of high value substances. Overall, this study clearly showed that carob pods have potential to be used as feedstock for lactic acid production by *Lactobacillus bulgaricus* using batch culture.

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