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Research Article

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Comparison between the productions curves of lactic acid from carob pods syrup by different lactic acid bacteria

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Abstract The main aim of this present study was to comparison between the kinetic growths of the lactic acid bacteria (*Lactococcus lactis cremoris, Lactobacillus bulgaricus* and *Streptococcus thermophilus*) from the carob pods syrup. The syrup has a 94.44% of moisture, 30.69 $g.L^{-1}$ of reducing sugars, 0.25% of protein fraction and 0.2% of ash content. The different physicochemical and biochemical analyzes show the richness of carob pods syrups in nutritional elements that makes them favorable to the lactic acid fermentation. All experiments were carried out in a 2 L jar fermenter with an initial volume of 1.5 L at 45°C for growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and 30°C for *Lactococcus lactis cremoris*. According to the obtained results, it is observed that the *Lactococcus lactis cremoris, Lactobacillus bulgaricus* and *Streptococcus thermophilus* growth's for the fermentations are characterized by a short duration of the latency phase which indicates that the inoculated cells were in full exponential phase and the perfect adaptation of strains to the carob pods syrup medium. The results of fermentation clearly indicated that the highest amount of biomass (OD: 1.62) and lactic acid (32.3 $g.L^{-1}$) were obtained with fermentation made by *Lactococcus lactis cremoris*.

Keywords Carob syrups, Fermentation, Lactic acid bacteria.

Introduction

Carob (*Ceratonia siliqua L.*), which has been widely grown in the Mediterranean region, belongs to the Caesalpinaceae subfamily of the family Leguminoseae [1]. 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 [2, 3]. 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. [3, 4]. 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 [5]. Carob pods are also characterized by high sugars content 500 $g.kg^{-1}$ [6]. Carob also contains phenolic compounds from 2 to 20% D.M. [7]. 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 [8]. Carob pulp is a good source of polyphenols (mainly tannins 16-20%) [3, 7] 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 [9]. The obtained carob extract has been utilized for production of value added products, especially by fermentation as suggested [10, 11]. 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 [12]. It and its derivatives are widely used in food, pharmaceutical, leather and textile industries [13]. 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 [14, 15]. 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 [16, 17]. The objective of our study was to comparison between the productions of lactic acid in carob pods syrup by different lactic acid bacteria.

Material and Methods

Vegetable material: The carob (*Ceratonia siliqua* L.) 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.

Biologic Material: The biologic material used is cultures of *Lactococcus lactis cremoris*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* derived from Orolait of Mascara (Algeria). All strains used in this study were grown in MRS broth (*Lactobacillus bulgaricus*) at 45°C for 24-48 h and M17 broth (*Lactococcus lactis cremoris* and *Streptococcus thermophilus*) at respectively 30°C and 45ºC for 24- 48 h in anaerobic jars. All strains were maintained at 4˚C (in aerobic condition) and renewed every week for short term preservation. The long-term conservation of the purified isolates was carried out in MRS or M17 broth with sterile glycerol (15%) and stored at -80ºC [18].

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 *et al.* [19]. Total nitrogen and protein content was determined by the method of Kjeldahl digestion and distillation apparatus [20]. Reducing sugars were determined colorimetrically at 480 nm by Dubois method [21]. 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 [22]. Moisture and dry matter were determined by drying 10 mL of syrup at 105°C during 18 h.

Fermentation conditions and methods: All experiments were carried out in a 2 L jar fermenter (Applikon Biocontroller ADI1030) with an initial volume of 1.5 L at 45°C for growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and 30°C for *Lactococcus lactis cremoris*. The agitation speed was set at 300 rpm to insure complete mixing of the fermentation medium. The inocula were incubated at 45°C (or 30°C) for 12 h at 300 rpm before their transfer to the fermenter in a 10%. The culture pH was maintained at 6.1 by automatic addition of 25% (w/w) NH_4OH solution using a computer coupled peristaltic pump during the 40 hours of fermentation. The samples were withdrawn at desired intervals and frozen for further analysis. The biomass is determined by measurement of the optical density (OD) at 600 nm by a spectrophotometer HITACHI 4-2000. Culture samples were centrifuged (13200 g at 4°C for 5 min), diluted and filtered. Residual sugars were determined colorimetrically at 480 nm by Dubois method 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. 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 [23]. 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 [5]. 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 [3, 4, 24]. Petit and Pinilla, report that the carob pods are also characterized by high sugar content 200-500 g/kg [6]. According to the results obtained by Yousif and Alghzawi [1] and by Vaheed *et al*. [25], carob pod contains 45 to 56.10% total sugar and13.60 to19.00% reducing sugar. Vaheed *et al.* [25] show that the carob pods powder contained 9.09 moisture, 56.10 total sugars, and 19.00 reducing sugars (all as weight %). 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 [26], 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 [27], irrigation and fertilization [28] and salinity [29]. 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.

Lactic acid fermentations of Carob pods syrup: This study was designed to evaluate the potential of carob pods syrups for lactic acid production by *Lactococcus lactis cremoris, Lactobacillus bulgaricus* and *Streptococcus thermophilus*. According to the obtained results, it is observed that the *Lactococcus lactis cremoris, Lactobacillus bulgaricus* and *Streptococcus thermophilus* growth's for the fermentations are characterized by a short duration of the latency phase which indicates that the inoculated cells were in full exponential phase and the perfect adaptation of strains to the carob pods syrup medium. The biomass evolution of *Lactococcus lactis cremoris, Lactobacillus bulgaricus* and *Streptococcus thermophilus* in the carob pods syrup starts with an initial concentration respectively (OD=0.25, 0.26 and 0.1) and evolves gradually to achieve after 40 h of fermentation respectively a maximum value OD=1.62, 0.65 and 0.7. The specific rate of *Lactococcus lactis cremoris* growth (µ) in the carob pods syrup starts at 0.33 h^{-1} and reaches its maximum value 0.67 h^{-1} after 5 hours of fermentation, then it decreases down to 0 h^{-1} after 22 hours of culture. For the *Lactobacillus bulgaricus* and *Streptococcus thermophilus* fermentations, µ starts respectively with an initial

value of 0.02, 0.2 h^{-1} increasing to 0.07 and 0.54 h^{-1}. This means that the fast increase in the bacterial mass during fermentation is due to the substrate consumption which is very important during this phase (figure 1, table 2).

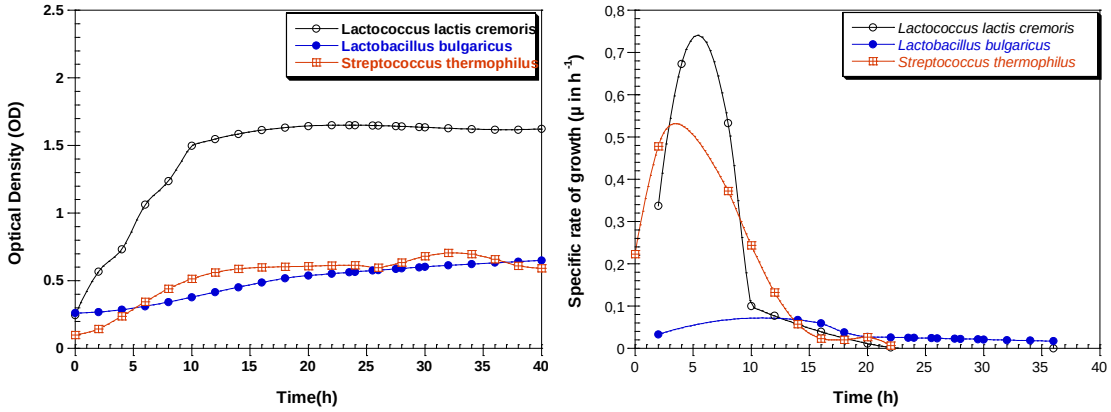

Figure 1: Evolution of optical density (OD) and specific rate of growth μ (in h^{-1}) during batch fermentations

Table 2: Kinetic parameters during batch fermentations (i: initial, f: final, L.a: Lactic acid)

Parameters	***Lactococcus lactis cremoris***	***Lactobacillus bulgaricus***	***Streptococcus thermophilus***
Optical Density *i.*	0.25	0.26	0.1
Optical Density *f.*	1.62	0.65	0.7
Sugars *i.* ($g.L^{-1}$)	27.12	27.13	28.05
Sugars *f.* ($g.L^{-1}$)	8.4	13.16	14.71
Sugars consumption ($g.L^{-1}$)	18.72	13.97	13.34
Lactic acid *i.* ($g.L^{-1}$)	11.32	8.04	1.286
Lactic acid *f.* ($g.L^{-1}$)	32.27	13.84	5.3
μmax (h^{-1})	0.67	0.07	0.54
Qs max ($g.g^{-1}.h^{-1}$)	3.27	2.5	5.9
QL.a max ($g.g^{-1}.h^{-1}$)	5.53	2.28	5.64

However, decreasing of sugar rates is very faster in the fermentation made by *Lactococcus lactis cremoris*. Where the strain consumes about major quantity of initial total sugars, during 40h of fermentation from an initial quantity 27.12 $g.L^{-1}$ of sugars, it remains 8.4 $g.L^{-1}$ which implies an amount of 18.72 $g.L^{-1}$. In parallel, 13.97 and 13.34 $g.L^{-1}$ as the quantity of sugars consumed by *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* Results are shown in the figure 2 and table 2.

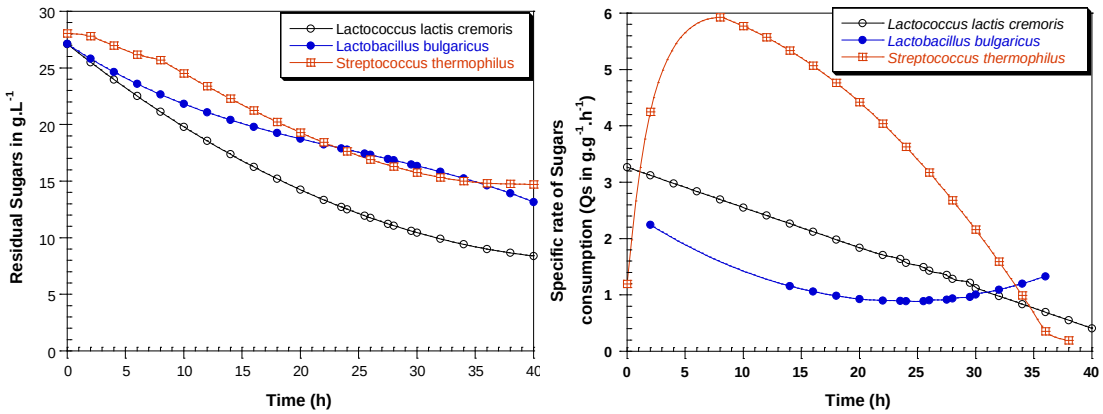

Figure 2: Evolution of residual sugars in $g.L^{-1}$ and specific rate of sugars consumption Qs in ($g.g^{-1}.h^{-1}$) during batch fermentations.

The maximal sugar consumption specific rate (Qs max) is respectively of the order of 3.27, 2.5 $g.g^{-1}.h^{-1}$ for the *Lactococcus lactis cremoris* and *Lactobacillus bulgaricus* cultures and decrease down to 0.4 and 0.9 $g.g^{-1}.h^{-1}$ after 40 hours of fermentation. For the *Streptococcus thermophilus* culture, the maximal sugar consumption specific rate (Qs max) is 5.76 $g.g^{-1}.h^{-1}$ obtained after 8 hours of fermentation and decrease gradually to achieve 0.2 after 40 hours of culture. It seems that the majority of the sugar is used for the cellular maintenance and reproduction and the remainder for the lactic acid production. The production curve of lactic acid by *Lactococcus lactis cremoris* begins with an initial concentration of 10 to achieve 32.3 after 40 h of fermentation. In parallel, the production of lactic acid by *Lactobacillus bulgaricus* and *Streptococcus thermophilus* begins with an initial value respectively of 8 and 1.3 to achieve 13.8 and 5.3 in the end of fermentations. The results clearly indicated that the highest amount of lactic acid was obtained by *Lactococcus lactis cremoris*. The maximal (QL.a.max) lactic acid production specific rate is of the order of 5.53 $g.g^{-1}.h^{-1}$ for the fermentation made by *Lactococcus lactis cremoris*. However it decreases considerably down to 2.28 and 5.64 $g.g^{-1}.h^{-1}$ in the *Lactobacillus bulgaricus* and *Streptococcus thermophilus* cultures (figure 3, table 2).

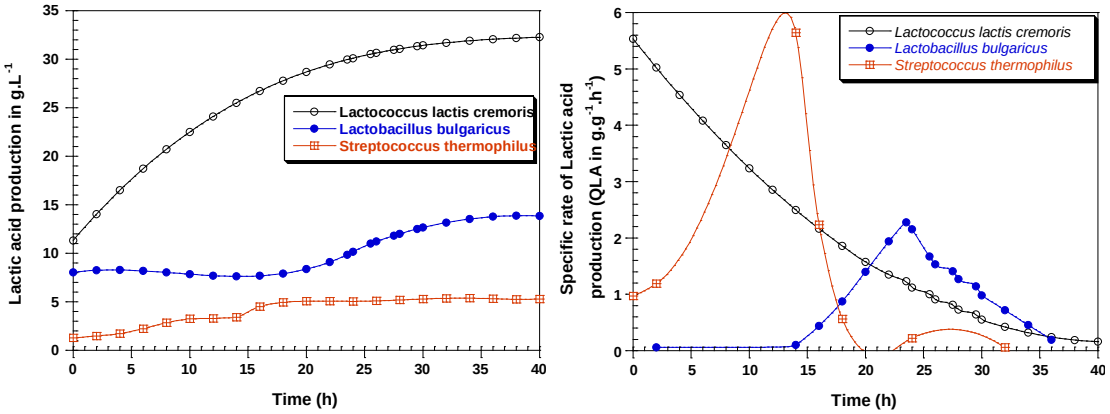

Figure 3: Evolution of lactic acid in $g.L^{-1}$ and specific rate of lactic acid production

Conclusion

The bioconversion of agricultural by- products mainly the ones rich in fermentable sugars has an economic and strategic interest. The main aim of this study was to comparison between the productions curves of lactic acid in carob pods syrup by different lactic acid bacteria (*Lactococcus lactis cremoris, Lactobacillus bulgaricus* and *Streptococcus thermophilus*). By its biochemical composition, the Carob pods syrup is very rich in carbohydrates and nutritional elements. Which make it a substrate of choice for the development of high value added products. According to the obtained results, it is observed that the *Lactococcus lactis cremoris, Lactobacillus bulgaricus* and *Streptococcus thermophilus* growth's for the fermentations are characterized by a short duration of the latency phase which indicates that the inoculated cells were in full exponential phase and the perfect adaptation of strains to the carob pods syrup medium. The results clearly indicated that the highest amount of lactic acid was obtained by *Lactococcus lactis cremoris*.

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