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Effect of the Alternative Addition of Sodium Acetate and Tween 80 on the Production Curve of Lactic Acid by Lactobacillus Casei Subsp Rhamnosus from Date Variety Hmira and Carob Pods Syrups

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Abstract

Lactic acid is considered as a very important chemical compound with significant applications in pharmaceuticals, cosmetics and especially in the food industry. New applications, such as degradable plastics made from poly (lactic) acid, have the potential to greatly expand the market for lactic acid, if more economical processes could be developed. Industrial processes for the production of lactic acid typically use sucrose from cane and beet sugar, whey containing lactose, maltose and dextrose from hydrolyzed starch. Fruits of date palm (*Phoenix dactylifera* L.) are consumed throughout the world and are a vital component of the diet in most Arabian countries. The pulp of carob (*Ceratonia siliqua* L.) pods contains high contents of sugar (sucrose, fructose and glucose) and can be employed as a raw material for the production of lactic acid. In recent year, interest in carobs and date has been increasing because of a cheap source of various products by fermentation. The objective of this present study consisted in valuing the date and carob pods (very rich's in sugars and in nourishing elements) as being a medium for culture of *Lactobacillus casei subsp rhamnosus* and enhancement of the lactic acid production by addition of sodium acetate and Tween 80. The biochemical characterization of date syrup showed it richness in total sugars (42.86 ± 0.01 g/L), protein fractions (0.092 ± 0.01), ash content (0.093 ± 0.02), sodium (0.078 ± 0.002), potassium (0.80 ± 0.001) and calcium (0.22 ± 0.002). The total sugars in carob syrup is 24 ± 0.02 g/L, 0.27 ± 0.01% for protein content and 0.9 ± 0.06 % for ash percentage included sodium (83 ± 0.003), potassium (112 ± 0.001) and calcium (154 ± 0.004). Date and carob syrups have a similar values of pH (5) and dry mater content (17%). The richness of two syrups in nutriment elements and growth factors made them a medium of choice for lactic acid production and growth of *Lactobacillus rhamnosus*. The different physicochemical and biochemical analysis applied on the date and carob pod syrups showed that they were poor with minerals such as Manganese, Magnesium and fatty-acids compared with MRS medium, so the addition of these salts (growth elements) was necessary to the natural mediums (date and carob syrups) in order to import and enhancement this quantity of lactic acid. The addition combined of sodium acetate and tween 80 (growth factors) in medium, increased advantages the biomass and lactic acid production.

Keywords

Carob; Date; Fermentation ; actic acid; *Lactobacillus rhamnosus*

Introduction

Carob (*Ceratonia siliqua* L.) has an economic and environmental importance in Algeria for reforestation of arid and degraded areas [8, 16, 14]. The pulp and the seeds are used as a raw material in food industry (live stock, biscuits), cosmetics and pharmacological industry (drug delivery) and biotechnological industry[2,6,11,22,24]. The fruits have 19-92% of total dry matter and 62-67% total soluble solids, which characterized by high soluble sugars as 7-10% glucose, 10-12% fructose and 34-42% sucrose [21]. The carob is rich in fiber, calcium, antioxidants and phenolic compounds from 2 to 20% D.M. and poor in fatty acids 0.4 to 0.8% and proteins 2.7 to 7.6% [22, 26, 30]. The phenolic compounds can be used as antioxidant additive [15, 20, 29]. The carob extract can be used

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for production of interesting products by fermentation [12, 28, 31]. The number of date palms (*Phoenix dactylifera* L.) in Algeria is estimated at more than ten million [19,23], the Deglet Nour variety is in first place 46.23% followed by Deglet Beidha 32.9% [9]. Other varieties represents 30% of the national production, known as common are less quality. Theses varieties are very rich in nutriments elements (carbohydrates, vitamins, ash, and minerals salts [23]. The dates are exploited as fermentation medium for various metabolites production (yeast bread, ethanol and citric acid) [1]. The objective of this study was a determination of kinetic profile of the growth of *Lactobacillus rhamnosus* from date and carob pods syrup without and with addition of sodium acetate and tween 80 for production of lactic acid.

Material and methods

Extraction and biochemical analysis of syrups

The date Hmira and carob were collected in the month of June 2014 respectively from the area of Bechar (South-west of Algeria) and the region of Elbordj (Mascara, Algeria). The extraction protocol was obtained from the method cited by [32]. Vegetable materials were chopped into small particles and one liter of hot water 80-85°C was added to 200g of date or carob pods during 2h, homogenized and filtered through a cloth. The syrups obtained were centrifuged at 15.000 rpm for 10 min to separate the cellulose debris and the collected supernatants were used for biochemical analyzes and as culture medium. The density and pH were measured using a density meter and pH meter. Titratable aciditiy was determined by manual titration of suitable quantity 10 g with standardized 0.1 N NaOH using phenolphthalein as indicator. The volume of NaOH required to neutralize the sample was recorded and used to calculate the content of titratable acids. The protein content was determined by the method of Kjeldahl digestion and distillation apparatus [4]. Total carbohydrates were determined colorimetrically at 180 nm by Dubois method [13]. Standards were prepared with glucose solutions at different concentrations. The ash content was determined by incineration of one gram of syrup at 600°C for 3h [5]. The mineral salts were determined according to the methods advocated [17, 18]. The moisture content was determined by measuring the mass of the sample before and after water is removed by evaporation at 105°C for 24h (until constant mass was achieved).

Fermentation conditions

The *Lactobacillus rhamnosus* (LBC80 10D) strain was supplied by the Rhone Poulenc group, France (2014). This strain was maintained on MRS medium containing 10% of glycerol and preserved at -20°C. The composition of MRS medium is in g.L-1: 15 glucose, 10 Soya peptone, 10 Beef extract, 5 Yeast extract, 2 K2HPO4, 5 NaCH3CO2, 3 H2O, 2 triammonium citrate, 0.2 MgSO4, 7 H2O, 0.05 MnSO4, H2O and 1 mL Tween 80. The reactivation phase of bacteria was obtained after two successive transplantations at 42°C during 2h on liquid MRS broth [3]. All cultures were realized in a 2 L jar bioreactor (Applikon Biocontroller ADI1030). The inoculum solution was incuibated at 42°C for 12h at 300 rpm before their transfer to the bioreactor in a 10%. The culture pH was maintained at 6.25 by automatic addition of NH4OH solution during time of fermentations and the culture was sterilized at 108°C for 15 min. two fermentations control were carried by growth of *Lactobacillus rhamnosus* from date and carob syrups (DSC and CSC) at a concentration 20%. By comparison with MRS medium, carob and date syrups are poor in fatty acids, tween 80 and sodium acetate, so the addition of these elements was necessary to the natural mediums (date and carob syrups) in order to import and the enhancement of the lactic acid production by *Lactobacillus rhamnosus*. In this context, several fermentations were carried by addition of 5g of sodium acetate from date and carob medium (DSA, CSA), addition of 1 mL of tween 80 (DT80, CT80) and by addition combined of two component 5g of sodium acetate and 1 mL of tween 80 (DSAT80, CSAT80). The choice of theses concentrations was based by comparison with the composition of MRS broth. The evolution of the biomass (optical density), the lactic acid and the residual sugars were followed in regular time intervals. The optical density was measured colorimetrically at 570 nm by spectrophotometer (HITACHI 4-2000) and culture samples were centrifuged 13200g at 4°C for 5 min, diluted and filtered before to the determination of residual sugars and lactic acid. The kinetic parameters of fermentations were determinate by the calculation of the specific rate of growth μ in h^{-1}, of sugars consumption Qs in g/g.h and of lactic acid production QL.A in g/g.h [7].

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

The maximal specific rate of growth (μmax) was calculated from the slopes of the plotted linear curve: LnX/X0=f(t). The biomass (Yx/s) and lactic acid (Yl.a/s) yields are defined as the mass ratios in biomass and lactic acid formed per gram of consumed carbonaceous substrate. Theses kinetics parameters were determined from the slopes of the plotted linear curve: X2-X1=f(S2-S1) and L.A2-L.A1=f(S2-S1) [7].

Results and discussion

Biochemical analyzes of syrups

Date and carob syrups have a similar value in pH (5), moisture content (83%) and dry mater content (17%). Results are presented in the table 1. The carob syrup was very rich in total organic acids with 32 ± 0.2 m.eq % of acidities compared to the date syrup 6.4 ± 0.4 m.eq %. The protein fraction (amino

acids) and sugars can be serving a nitrogen and carbon source for growth of and for production of lactic acid. The protein content of date and carob syrups varied respectively from 0.092 ± 0.01 and 0.27 ± 0.01. Date syrup was very rich of total sugars 42.86 ± 0.01 g/L compared to carob syrup 24 ± 0.02 g/L. The ash content of 0.093 ± 0.02% for date and 0.9 ± 0.06 for carob syrups indicates its richness of minerals salts (Potassium, Sodium and Calcium). The total biochemical characterization showed the good quality of all syrups for growth of lactic acid bacteria. Our results are agreement with the work of Petit and Pinilla (1995), when report that the carob pods are also characterized by high content of sugars 20 to 50% and does not agreement with the results obtained [33,34,35] , when note that the carob pods contains 45 to 56.1% of total sugars and 13.6 to 19% of reducing sugars.

Table 1: Biochemical analyzes of date syrup and carob pods syrup (values presented are the means of triplicate analysis).

Constituents	Date Syrup	Carob pods Syrup
Moisture (%)	82.86 ± 0.2	83 ± 0.1
Dry Matter (%)	17.14 ± 0.2	17 ± 0.3
pH	5.28 ± 0.1	4.99 ± 0.3
Acidities (m.eq %)	6.4 ± 0.4	32 ± 0.2
Density (Kg.m-3)	1.068 ± 0.002	2.453 ± 0.002
Total sugars in g.L-1	42.86 ± 0.01	24 ± 0.02
Proteins in % of M.F	0.092 ± 0.01	0.27 ± 0.01
Ashes in % of M.F	0.093 ± 0.02	0.9 ± 0.06
Potassium in mg.100mL-1 of M.F	0.80 ± 0.001	112 ± 0.001
Sodium mg.100mL-1 of M.F	0.078 ± 0.002	83 ± 0.003
Calcium in mg.100mL-1 of M.F	0.22 ± 0.002	154 ± 0.004

Kinetics profile of Lactobacillus rhamnosus

For all syrups fermentations carried, there was a total absence of the lag phase indicating the perfect adaptation of *Lactobacillus rhamnosus* to the different natural medium used (date and carob). For control fermentations, the production of lactic acid in date syrup (DSC) began with an initial acidity of 24 ± 0.2 to achieve 42 ± 0.1 after 25 h of fermentation compared to the carob syrup (CSC), the initial acidity is 28.75 ± 0.1 and the final acidity obtained after 25h of fermentations is 52.39 ± 0.3 (Figure 1, Table 2). After addition of sodium acetate, the amount of acidity produced (difference between final and initial acidity) is 25.46 for DSSA and 26.13 for CSSA. The addition of tween 80 to the date and carob medium are characterized by acidities produced of 30 for DST80 and 25.46 for CST80. So, the supplementation combined of sodium acetate and tween 80 the medium improve the production of lactic acid to 40.8 for date and 30 for carob. The addition of sodium acetate and tween 80 increased advantage of lactic acid production. The optical density evolution of *Lactobacillus rhamnosus* from date syrup control (DSC) started with an initial OD 0.18 to reach a maximum 0.52 after 25 h of fermentation (Figure 2) compared to CSC, optical density initial is 0.19 and 0.50 in the end of fermentations. The fermentations characterized by addition of two combined component sodium acetate and tween 80, we observed a strong biomass production in the end of fermentation 0.78 for date and 0.68 for carob. Tween 80 is essential for growth of lactic acid bacteria and acetate is a buffer and stimulating [10]. The addition of Tween 80 allowed better cells excretion of lactic acid by creating pores in the membrane and played the role of surfactant which makes a good contact between seed and nutriments [10], and for more it is considered as a source of carbon and energy for electrons. Decreasing of sugars rate was faster in the culture characterized by addition combined of sodium acetate and tween 80 (Figure 3). The results clearly indicated that the highest amount of biomass and lactic acid were obtained with the medium enriched with sodium acetate and tween 80. From these results, the enriched medium by two components seemed the best for its interesting results. The control fermentation was compared with fermentations supplemented by sodium acetate and tween 80, in terms of QL.A max, µmax, Qsmax, Yx/S and Yl.a/S, there were significant differences (Table 2). Fermentations with enriched medium (addition of soidium acetate and tween 80) resulted in significantly higher lactic acid productivity compared to un-enriched medium.

Conclusion

The bioconversion of agricultural by- products mainly the ones rich in fermentable sugars has an economic and strategic interest. The carob and date syrups were very rich in carbohydrates which made them a substrate of choice for the development of high value added products. In producing countries, carob pods have traditionally been used as animal and human food and currently the main use is the seed for gum extraction, carob bean gum (CBG) or locust bean gum (LBG). The carob powder is being acclaimed as an ingredient with a marked nutritional value due to its high levels of dietary fiber (preventative role against heart disease) and phenol compounds (antioxidant activity). Regarding the high sugar content in date and carob pod, there have been some studies on the production of the value added product. Lactic acid can be one of these value added products due to its current and future potentials. The main aim of this work was the enhanced of the lactic acid production by *Lactobacillus rhamnosus* from date syrup and carob pods syrup by addition of sodium acetate and tween 80 (growth factors). The richness of two syrups in nutriment elements and growth factors made them a medium of choice for growth of *Lactobacillus rhamnosus*. The addition combined of sodium acetate and tween 80 (growth factors) in medium, increased advantages the biomass and lactic acid production.

Table 2: Kinetic parameters of syrup fermentations (values presented are the means of triplicate analysis).

Fermentations Parameters	Control	Sodium Acetate	Tween 80	Sodium Acetate and Tween 80
Optical Density i.	DS: 0.18±0.01 CS: 0.19±0.02	DS: 0.17±0.02 CS: 0.19±0.01	DS: 0.20±0.03 CS: 0.21±0.04	DS: 0.22±0.04 CS: 0.18±0.01
Optical Density f.	DS: 0.52±0.01 CS: 0.50±0.01	DS: 0.66±0.03 CS: 0.55±0.02	DS: 0.65±0.01 CS: 0.57±0.01	DS: 0.78±0.01 CS: 0.68±0.04
Sugars i. (g/L)	DS: 38.7±0.3 CS: 21.9±0.2	DS: 39.28±0.4 CS: 22.07±0.1	DS: 38.15±0.3 CS: 22.11±0.2	DS: 39.72±0.4 CS: 22.22±0.1
Sugars f. (g/L)	DS: 28.1±0.4 CS: 10.15±0.3	DS: 27.08±0.3 CS: 7.08±0.3	DS: 24.6±0.3 CS: 8.76±0.2	DS: 24.17±0.3 CS: 5.5±0.2
Sugars consumption (g/L)	DS: 10.6 CS: 11.75	DS: 12.2 CS: 14.99	DS: 13.55 CS: 13.35	DS: 15.55 CS: 16.72
Acidities i.	DS: 24±0.2 CS: 28.75±0.1	DS: 26.26±0.1 CS: 31±0.2	DS: 24±0.1 CS: 28.32±0.3	DS: 23.7±0.3 CS: 31±0.1
Acidities f.	DS: 42±0.1 CS: 52.39±0.3	DS: 51.72±0.4 CS: 57.13±0.3	DS: 54±0.2 CS: 53.78±0.2	DS: 64.5±0.3 CS: 61±0.4
Acidity production	DS: 18 CS: 23.64	DS: 25.46 CS: 26.13	DS: 30 CS: 25.46	DS: 40.8 CS: 30
µmax (h-1)	DS: 0.21±0.03 CS: 0.17±0.02	DS: 0.33±0.01 CS: 0.18±0.01	DS: 0.28±0.02 CS: 0.14±0.03	DS: 0.33±0.02 CS: 0.2±0.03
Qs max (g/g.h)	DS: 8.1±0.01 CS: 10.24±0.02	DS: 8.3±0.03 CS: 7.28±0.01	DS: 8.15±0.02 CS: 8.3±0.02	DS: 10.12±0.04 CS: 10.9±0.02
QL.a max (g/g.h)	DS: 2.2±0.03 CS: 15.4±0.01	DS: 3.75±0.04 CS: 25.2±0.01	DS: 2.32±0.01 CS: 16.5±0.03	DS: 2.21±0.01 CS: 25.8±0.03
Yx/s (g/g)	DS: 0.015±0.02 CS: 0.015±0.02	DS: 0.032±0.02 CS: 0.015±0.02	DS: 0.02±0.01 CS: 0.006±0.01	DS: 0.032±0.01 CS: 0.007±0.02
YL.a/s s (g/g)	DS: 2.92±0.03 CS: 1.6±0.02	DS: 6.3±0.03 CS: 3.6±0.01	DS: 1.13±0.01 CS: 2.06±0.02	DS: 3.1±0.02 CS: 3.1±0.03

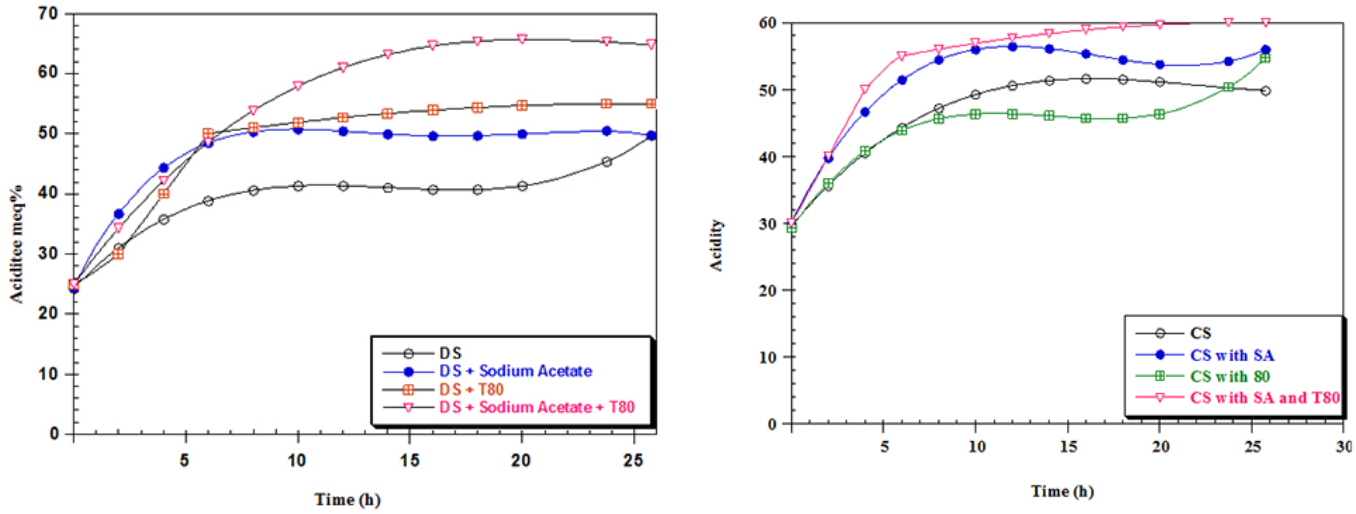

Figure 1: Evolution of acidities

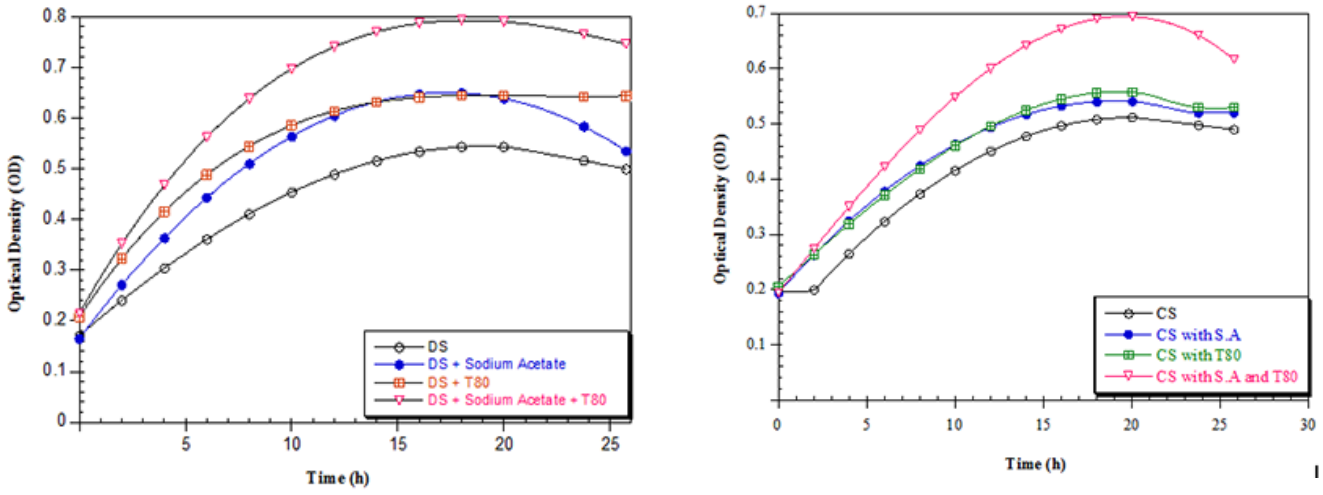

Figure 2: Evolution of optical density (OD)

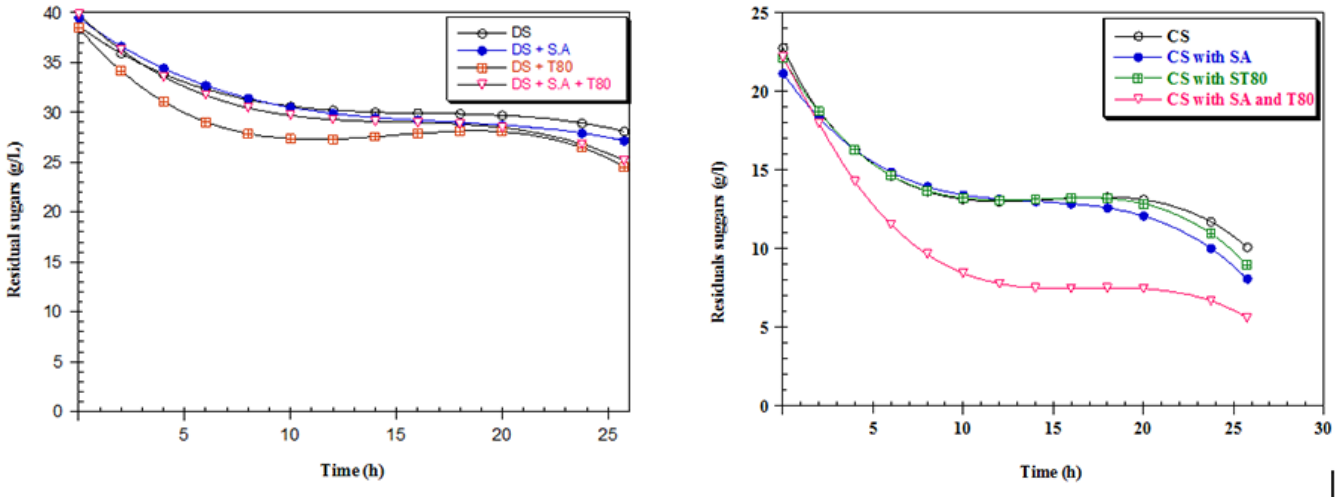

Figure 3: Evolution of residual sugars in $g.L^{-1}$.

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