

## Lactic Acid Production Vis-à-Vis Biowaste Management Using Lactic Acid Bacteria

Preeti Chanalia, Dimpi Gandhi, Raman Kumar, Pooja Attri and Suman Dhanda

Department of biochemistry, Kurukshetra University, Kurukshetra, 136119, India

**Abstract:** Lactic acid production by three lactic acid bacteria (LAB) viz. *Pediococcus acidilactici*, *Lactobacillus plantarum* and *Lactobacillus brevis* was optimized using whey. Lactic acid production by liquid and solid state fermentation was also studied with different biowastes. *P. acidilactici*, *L. plantarum* and *L. brevis* produced 21.3, 26.5 and 25.4 g/L of lactic acid respectively after 48 h with whey. *P. acidilactici* produced maximum lactic acid at 32°C, pH 6.5 and yeast extract concentration of 10 g/dm<sup>3</sup>, *L. plantarum* at 32°C, pH 6.0 and yeast extract concentration of 7.5 g/dm<sup>3</sup> and *L. brevis* at 37°C, pH 5.5 and yeast extract concentration of 7.5 g/dm<sup>3</sup>. Inoculum size of 2 % was optimum for all lactic acid bacteria. The results were validated by employing a central composite design and response surface methodology. Fermentation resulted in significant production of lactic acid and waste disposal. Apple pomace resulted in maximum yield (>30 g/L). **Practical Applications:** In this study, lactic acid production from whey was optimized using three lactic acid bacteria (LAB) viz. *Pediococcus acidilactici*, *Lactobacillus plantarum* and *Lactobacillus brevis*. Lactic acid production from sugarcane juice and biowastes including sugarcane bagasse, apple pomace and grape pomace was also studied under optimized conditions. Biowastes were used without pretreatment. All the studied substrates efficiently produced lactic acid and apple pomace resulted in maximum yield (>30 g/l). Therefore, fermentation of biological wastes serves dual purpose of lactic acid production and waste disposal by using LAB which are generally regarded as safe (GRAS).

**Key words:** lactic acid • *Lactobacillus brevis* • *Lactobacillus plantarum* • *Pediococcus acidilactici* • pomace

### INTRODUCTION

Lactic acid (2-hydroxy propanoic acid) and its derivatives have gained prominence because of their applications in food, pharmaceutical, textile, cosmetic and chemical industries [1-3]. It is used for fortification of food products and controls food borne pathogens. Being mildly acidic in taste, it is used as an acidulant in salads, dressings, pickled vegetables, beverages and baked foods. In confectionary, lactic acid contributes to aroma and also used for setting desirable pH of cooked foods. In chemical industries, it serves as a precursor for several chemicals [4].

Consumption of lactic acid has recently increased because of its use in production of polylactic acid which is biodegradable and well known sustainable bioplastic material. The production of lactic acid is 80, 000 tons year<sup>-1</sup> throughout the world. The global demand of lactic acid is also expected to rise further [5].

Lactic acid can be produced by chemical synthesis and microbial fermentation. 90% of the total lactic acid

produced worldwide is produced by bacterial (lactic acid bacteria) fermentation because chemical synthesis yields its racemic mixture. LAB produces DL or D(-) or L(+) form. L(+)-Lactic acid is preferred by food industry because it is metabolized in humans. By using selective LAB strain(s), L(+)-Lactic acid can be produced. Commercial production of LA is preferably carried out by using homofermentative LAB because they exclusively produce lactic acid from sugar. In the present study, use of homofermentative LAB viz. *L. brevis*, *L. plantarum* and *P. acidilactici* for producing lactic acid by fermentation of biowaste (whey, sugarcane bagasse, apple pomace and grape pomace) and sugarcane juice is being reported.

Whey is the major byproduct of dairy industry and retains 55% of milk's nutrients including lactose, soluble proteins, lipids and minerals that makes it suitable substrate for fermentation by LAB [6, 7]. It also poses the major disposal problems because of its volume and high biochemical oxygen demand.

Apple and grape pomace are also waste materials of juice industry and studies revealed them to be rich in

some nutrients [8-10]. Likewise sugarcane bagasse is also a waste material available in abundance in sugarcane industry. Sugarcane juice was also used as substrate for production of lactic acid. All the substrates used in the present study pose a challenge for waste management. Therefore the objective of present work is to draw the dual benefit for production of valuable product (i.e., lactic acid) by fermentation of industrial biological wastes that are posing disposal problem by using LAB which are generally regarded as safe (GRAS).

## MATERIALS AND METHODS

**Microorganisms:** *Pediococcus acidilactici*, *Lactobacillus plantarum* and *Lactobacillus brevis*, all LAB were purchased from National Dairy Research Institute (NDRI) Karnal, India. The cultures were revived in MRS (de Mann Rogosa Sharpe) medium and were subcultured for three successive generations.

**Maintenance and Cultivation of Cultures:** The bacterial cultures were revived in MRS broth (pH 6.2±0.2). The cultures were activated by transferring them after every 48 h upto three generations. The cultures were maintained on MRS slants by subculturing, aseptically at fortnight intervals and stored at 4°C, until further use.

**Preparation of Starter Cultures:** The bacterial cultures were grown in 50 ml of MRS medium in 250 ml flask. After sterilization, the medium was inoculated with a loopful of cells from agar slant and incubated at 37°C and 150 rpm for 24 h.

**Liquid State Fermentation:** Liquid state fermentation was carried out using whey and sugarcane juice as a substrate. Whey was purchased from a dairy in Kurukshetra. Whey clarification was carried out by protein precipitation by heating the whey at 90°C for 20 min that precipitated proteins. Precipitated proteins were removed by centrifugation at 4,000 rpm for 20 min and supernatant (whey) was supplemented with yeast extract (0.5%), MnSO<sub>4</sub> (50 mg/l) and CaCO<sub>3</sub> (0.5%). This whey was sterilized at 121°C for 20 min and used as fermentation medium for the production of lactic acid using all the three different strains of LAB. All the fermentation conditions were optimized using whey as substrate. Yeast extract was not added while optimizing yeast extract concentration. Sugarcane juice was purchased from local market and was used as substrate in the same way as described for whey.

**Solid State Fermentation (SSF):** SSF was carried out using industrial wastes i.e., sugarcane bagasse (SB), apple pomace (AP) and grape pomace (GP) as substrates. These wastes were obtained from the juice corners of local market.

Substrates for SSF were oven dried and grounded to powder form. Powdered substrates (12.5 g) were moistened with 50 ml of 4X sucrose medium (sucrose (31g/100ml), MnSO<sub>4</sub> (50mg/l), CaCO<sub>3</sub> (0.5%), CuSO<sub>4</sub> (40mg/l), NH<sub>4</sub>NO<sub>3</sub> (1%) and (0.5%) yeast extract) and autoclaved. This was used as fermentation medium by inoculating with LAB.

**Fermentation:** Fermentation was carried out in flasks, in comparison to control in which medium composition was kept same but not inoculated with strains. Fermentation medium was inoculated and incubated. Then the medium was centrifuged at 3000 rpm for 30 min to pellet out bacterial cells and the supernatant was used for lactic acid estimation. In case of SSF, after incubation, the lactic acid was extracted with 25 ml of water at 40°C for 1 h and supernatant was collected after centrifugation at 3000 rpm for 30 min and used for estimation of lactic acid.

**Lactic Acid Estimation:** Lactic acid produced by fermentation was determined using titration method. 25ml of culture broth of LAB isolates was transferred into 100ml flask. One ml of phenolphthalein indicator (0.5% in 0.5% alcohol) was added to this and titrated against 0.1 N NaOH. Lactic acid concentration (g/l) was calculated according to Fortina *et al.*, 1973 [11] with slight modifications using the following formula:

$$\text{Lactic acid (g/l)} = \frac{X \cdot N \cdot 90.08}{Y}$$

X = volume (ml) of NaOH used

N = Normality of NaOH used

90.08 = Molecular weight of lactic acid

Y = Volume of titrant used

**Experimental Design and Statistical Analysis:** RSM (Response Surface Methodology) was used to analyze and validate the conditions for lactic acid production from whey using three different lactic acid bacteria. A central composite design was employed with four variables viz., temperature, pH, time and yeast extract concentration. The minimum and maximum values were set for all the variables. RSM provided 30 runs for lactic acid

production. All the experiments carried out orderly and analyzed the best condition for lactic acid production. The analysis of variance (ANOVA) was applied to validate the model. Table 1 shows the experimental and coded levels of four variables.

**Optimization of Different Parameters Using Whey as Substrate;** Different process parameters such as pH (5.0-6.5), temperature (32-42°C), yeast extract (0-10 g/dm<sup>3</sup>) and inoculum size (1-5% v/v), were varied over a range during fermentation to optimize these parameters for maximal lactic acid production.

## RESULTS

**Optimization of Process Parameters for Lactic Acid Production Using Whey as Substrate:** LAB produce lactic acid from different sugars by the process of fermentation. Lactic acid can also be produced by chemical synthesis. But due to environmental concerns and limited variety of petrochemicals, it is preferably produced by microbial fermentation. To achieve the cost effective lactic acid production, the fermentation conditions need to be optimized.

**Effect of pH:** The effect of pH on lactic acid production was evaluated by adjusting the pH of fermentation medium in the range of 5.0-6.5 in shake flasks. Study revealed that pH of 6.5 is optimum for lactic acid production using *P. acidilactici* (Fig 1a) and it produced 16.21 g/L of lactic acid after 48 h. Whereas for both *L. plantarum* and *L. brevis* studied pH range of fermentation medium has no significant effect on lactic acid production and pH 6.0 was considered optimum for further studies (Fig 1b and Fig 1c) and 15.9 g/L and 15.5 g/L of lactic acid for *L. plantarum* and *L. brevis* respectively after 48 h of incubation.

**Effect of Temperature:** The effect of temperature on lactic acid production was evaluated by incubating whey medium (after inoculation with different LAB) over a temperature range of 32-42°C. The temperature of 32°C was optimum for *P. acidilactici* (Fig 2a) and *L. plantarum* (Fig 2b) and 14 and 16.2 g/L of lactic acid was produced respectively by *P. acidilactici* and *L. plantarum* after 48 h of incubation. Optimum temperature was found to be 37°C for *L. brevis* (Fig 2c) and it produced 14.4 g/L of lactic acid at 37°C after 48 h. At 42°C, all the three studied LAB produced comparatively very low levels of lactic acid.

**Effect of Yeast Extract Concentration:** Yeast extract supplementation of fermentation medium is the key factor in lactic acid production. However, the high cost of yeast extract imposes limits to its usage in fermentation reaction, therefore, in industrial processes the concentration of yeast extract is mostly suboptimal. Therefore, to find the optimum concentration the effect of yeast extract was examined in this study by conducting fermentation in shake flasks with different concentration of yeast extract (0, 2.5, 5.0, 7.5 and 10 g/dm<sup>3</sup>).

Yeast extract concentration significantly affected lactic acid production for all three LAB. For *P. acidilactici* lactic acid production was maximal (14.41 g/L after 48 h) at yeast extract concentration of 10 g/dm<sup>3</sup> while without yeast extract, very less amount of lactic acid was produced even after 72 h of incubation (Fig 3a). For *L. plantarum* and *L. brevis* yeast extract concentration of 7.5 g/dm<sup>3</sup> and 10 g/dm<sup>3</sup> resulted in same amount of lactic acid production (14 g/L and 13 g/L after 48 h by *L. plantarum* (Fig 3b) and *L. brevis* (Fig 3c) respectively. Therefore, yeast extract at a concentration of 7.5 g/dm<sup>3</sup> was used for further studies.

**Effect of Inoculum Size:** Influence of inoculum size was studied by adding cultures with different inoculum volumes (1-5 % v/v) to the fermentation medium and lactic acid production was measured after 48 h. In the present study lactic acid production increased with increase in inoculum size upto 2 % for all the three strains. Lactic acid production remains same with 2-5 % (v/v) inoculum size for all the three strains (Fig 4). Therefore, an inoculum size of 2 % (v/v) was considered optimum for lactic acid production using 24 h old bacterial culture for all the three studied strains. Time of incubation for optimization of fermentation parameters was 48 h in our studies with all three LAB.

**Experimental Design and Statistical Analysis:** Response surface methodology and Central Composite Design were used to standardize the extraction parameters. Experimental values obtained for lactic acid production from whey using *P. acidilactici*, *L. plantarum* and *L. brevis* are summarised in Table 1, 2 and 4 respectively. The results are shown in 3-D graphs (Figure 5, 6 and 7). The expected value lactic acid obtained using RSM were compared with observed values. Both these values were approximately very close to each other. This indicates that the response surface methodology validate the experimental data.

**Biowastes and Sugarcane Juice as Substrates for Lactic Acid Production:** Studies of lactic acid production were extended using various biological wastes. LAB produce lactic acid from different sugars by the process of fermentation. Lactic acid production using purified sugars like glucose, sucrose etc. is very expensive and thus different dairy/agro/food/juice industry products form cheaper alternatives for lactic acid production. Sugarcane bagasse (SB), apple pomace (AP), grape pomace (GP) and sugarcane juice were used for fermentative production of lactic acid using all three LAB viz. *P. acidilactici*, *L. plantarum* and *L. brevis*. Fermentations with these substrates were carried out under the similar conditions as optimized for whey i.e in shake flasks using 2 % v/v inoculum size for 48 h. Fermentations was carried out at 32°C for *P. acidilactici* and *L. plantarum* and at 37°C for *L. brevis* in incubator shaker operated at 150 rpm. In the present study all the three strains effectively utilized the studied substrates for the production of lactic acid. The concentration of lactic acid produced from whey was 21.3, 26.5 and 25.4 g/L after 48 h of incubation by *P. acidilactici*, *L. plantarum* and *L. brevis* respectively. With sugarcane juice 24, 14.6 and 19.5 g/L, with sugarcane bagasse 22.5, 14.5 and 17.9 g/L, with apple pomace 31.8, 33.6 and 33.6 g/L and with grape pomace 23.2, 25.6 and 21.3 g/L lactic acid was produced by *P. acidilactici*, *L. plantarum* and *L. brevis* respectively (Fig 8).

## DISCUSSIONS

**Optimization of Process Parameters for Lactic Acid Production Using Whey as Substrate:** Lactic acid holds an important place in different industries. It is produced by fermenting different sugars using lactic acid bacteria (LAB) and also by chemical synthesis. But rising environmental concerns and limited of petrochemicals, it is preferably produced by microbial fermentation. Cost effective lactic acid production is achieved under standardized fermentation conditions. By using response surface methodology, the satisfactory conditions of operating variables were obtained for lactic acid production.

**Effect of pH:** pH is an important parameter that influences the microbial growth and functioning of microbial enzymes. It also affects transport of nutrients into the cell and affects protein and RNA synthesis. Increase in lag phase is observed if microorganisms are not grown at their optimum pH. Our studies of optimum pH of 6.5 for *P. acidilactici* and 6.0 for both *L. plantarum* and *L. brevis*

are in agreement to earlier studies of *L. casei* that produces lactic acid in the pH range of 6.0-6.5 [12, 13]. pH of 5.5 was used for lactic acid production using *L. helveticus* [14] with glucose as substrate, *Lactobacillus plantarum* and *Pediococcus cerevisiae* produced lactic acid at a pH of 5.5 [4].

**Effect of Temperature:** Temperature is an important factor that affects activity of metabolic enzymes. Enzymes are most active at optimum temperature and any decrease or increase in temperature results in altered cell metabolism and thus productivity. LAB exhibits optimum growth in the temperature range of 20-45°C. Optimum temperature of 32°C for *P. acidilactici* and *L. plantarum* and 37°C for *L. brevis* fall within the range. The studies of all the three studied strains are in agreement to studies from other bacteria. Similar reports are also available for different LAB. Lactobacilli produced lactic acid between 30-44°C [15]. *L. delbrueckii* and *L. bulgaricus* can ferment at a temperature of 45°C or even higher. For *L. helveticus* and *L. acidophilus* temperature range of 37-45°C was optimum. Kruschke *et al.*, 1991 used 37°C temperature for lactic acid production using *L. casei*.

**Effect of Yeast Extract Concentration:** Yeast extract is a key supplement of fermentation media for lactic acid production. However, its high cost extract imposes limits to its usage and thus in industrial processes its concentration is mostly suboptimal. In present study, yeast extract concentration significantly affected lactic acid production for all three LAB. Guoqiang *et al.*, [16] reported increase in lactic acid production with increase in yeast extract concentration (0-10 g/dm<sup>3</sup>). Hujanen and Linko, [17] reported that type and initial concentration of nitrogen source influenced lactic acid production. In batch fermentation of whey permeate by *L. casei* subsp *casei*, best growth occurred with a combination of yeast extract (5 g/dm<sup>3</sup>) and hydrolysed whey retentate (50 g/dm<sup>3</sup>) [12]. Increase in lactic acid conversion was observed by increasing yeast extract concentration from 0.0 to 3.0 g/dm<sup>3</sup> [18]. Yeast extract concentration of 5 g/dm<sup>3</sup> was found optimum for batch production of lactic acid from whey by *L. casei* [19].

**Effect of Inoculum Size:** Inoculum size has a great impact on the bacterial fermentation process. Inoculum size of 2 % for all the three strains was considered optimum for lactic acid production using 24 h old bacterial culture for all three strains. Inoculum size of 2 % (v/v) was also used for lactic acid production by other workers [20, 21].

Inoculum size of 3 % (v/v) was used for lactic acid production using *L. helveticus* [22]. Inoculum size of 2 % was optimum for production of lactic acid using *L. casei* from whey [13]. Whereas Cock *et al.*, 2006 [23] reported that production of lactic acid increased at 10 % size of inoculum in *P. cerevisiae* and *L. plantarum*.

Lactic acid production by fermentation of whey was also reported earlier using *L. casei* (33.73 g/L after 36 h of incubation) [13] and *L. casei* (NRRL B-491) [19]. Time of incubation for optimization of fermentation parameters was 48 h in our studies with all three LAB.

**Biowastes and Sugarcane Juice as Substrates for Lactic Acid Production:** Lactic acid production was done by fermenting biological wastes. Since lactic acid production using purified sugars like glucose, sucrose etc. is very expensive and thus different dairy/agro/food/juice industry products form cheaper alternatives for lactic acid production. Sugarcane bagasse (SB), apple pomace (AP), grape pomace (GP) and sugarcane juice were fermented under the same conditions as standardised for lactic acid production from whey.

Though agro-industrial residues are rich in carbohydrates but their utilization is limited due to low protein content and poor digestibility [24]. Annually approximately 3.5 billion tons of agroindustrial residues are produced worldwide and they are used as specific carbohydrate feed stock. In the present study all the three strains effectively utilized all the studied substrates for the production of lactic acid. There are several reports on lactic acid production from cellulosic substrates. Defatted rice bran, filterpaper, paper mil sludge, pretreated cardboard, sugarcane bagasse cellulaose has been studied as substrates for lactic acid production using bacterial strains *L. delbrueckii* IFO 3202, *L. coryniformi* ATCC 25600, *L. paracasei*, *L.coryniformi* 25600, *L. delbrueckii* Uc-3 respectively [25-28]. The media used in these studies was pretreated with enzymes. The potential of apple pomace (pretreated with enzymes) for lactic acid production has been studied using *L. rhamnosus* [29]. SB, AP and GP were used as substrates for solid state fermentation and these substrates were not pretreated with enzymes. Saccharification and fermentation of these substrates using the studied strains can be further explored for the production of lactic acid.

Studied LAB efficiently fermented whey and other media to produce lactic acid. Using purified sugars in fermentation medium is expensive but use of biowastes can be cost effective. Complex composition of whey and sugarcane juice also adds to cost of its purification. Solid

state fermentation is a cheaper alternative. Biowastes were used without pretreatment. Further studies will be focused on using the biomass to produce cellulose, then sugar which can be fermented to lactic acid. Identification of the L (+)-lactic acid producing strains and/or purification of L (+)-lactic acid from racemic mixture is very important and will be focused for further studies.

## AKNOWLEDGEMENTS

Preeti Chania is thankful to University Grant Commission for providing Rajiv Gandhi National Fellowship. We are thankful to Raman Kumar, Department of Biochemistry, K.U.K. for providing help to employ response surface methodology.

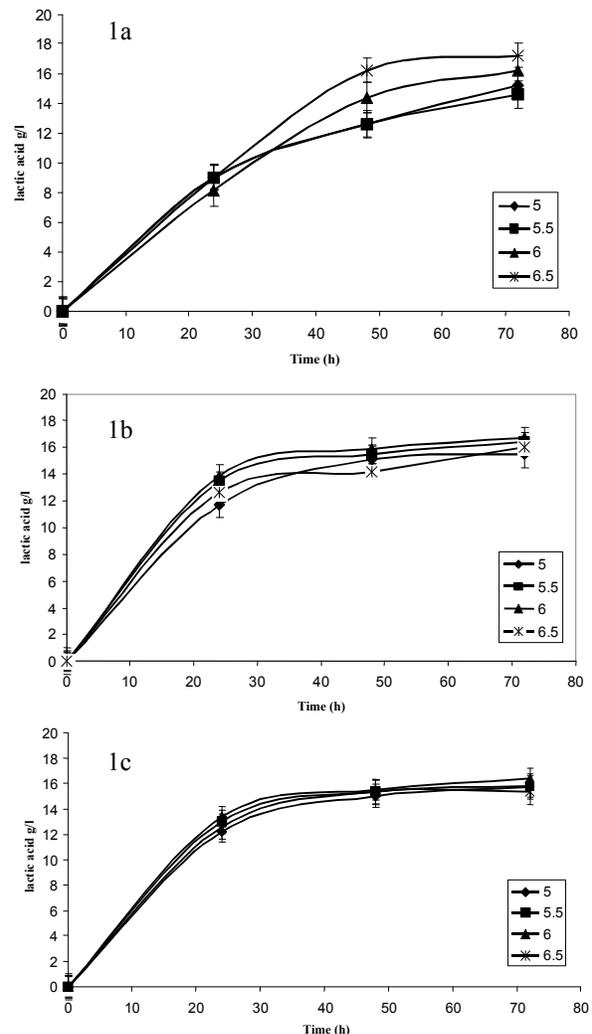


Fig. 1: Lactic acid production in whey as a function of pH by *P. acidilactici* (1a), *L. plantarum* (1b) and *L. brevis* (1c).

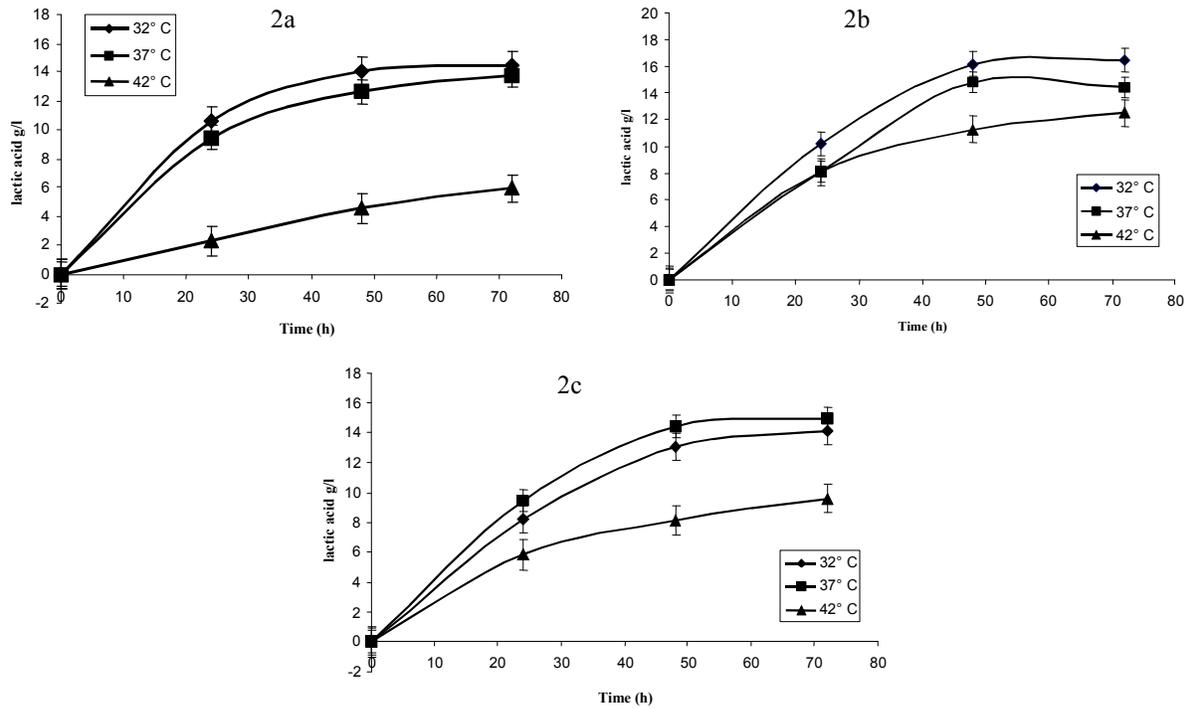


Fig. 2: Lactic acid production in whey as a function of temperature by *P. acidilactici* (2a), *L. plantarum* (2b) and *L. brevis* (2c).

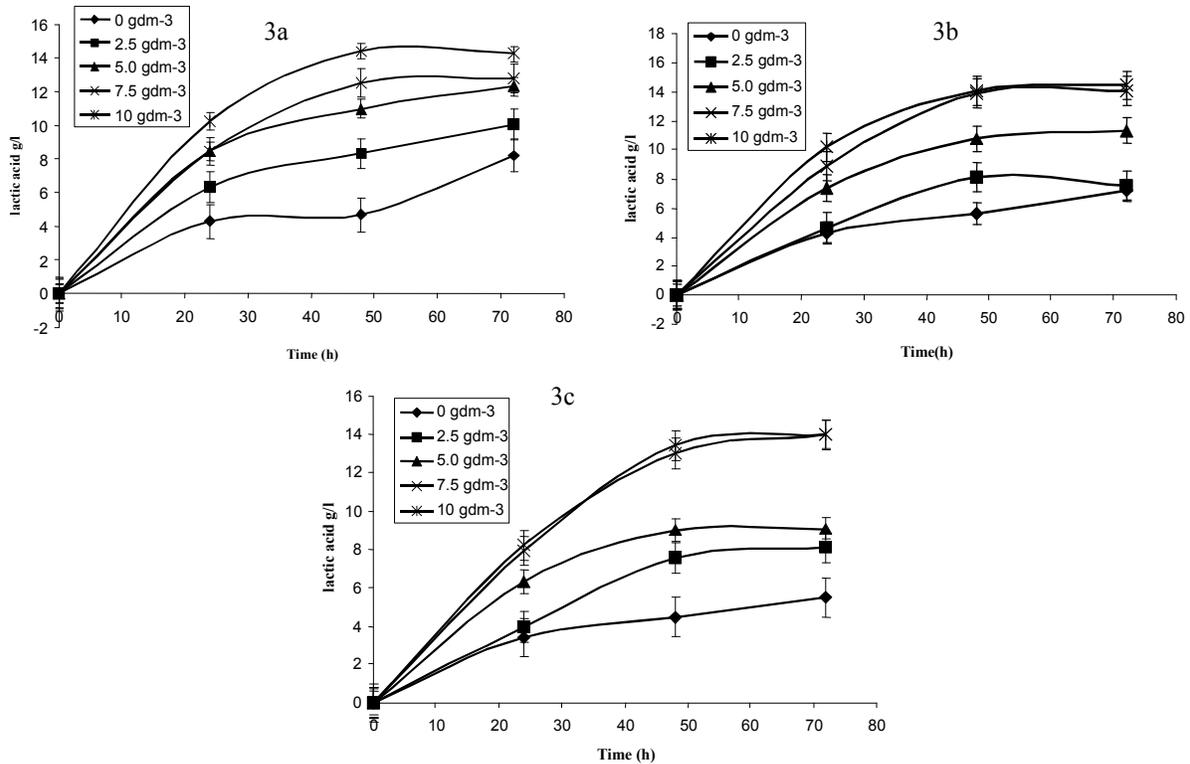


Fig. 3: Lactic acid production in whey as a function of yeast extract concentration by *P. acidilactici* (3a), *L. plantarum* (3b) and *L. brevis* (3c).

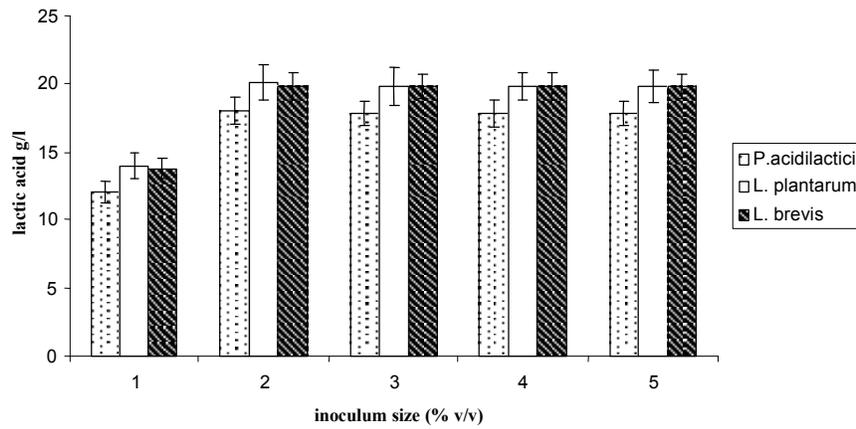


Fig 4: Lactic acid production in whey by *P. acidilactici*, *L. plantarum* and *L. brevis* as a function of inoculum size.

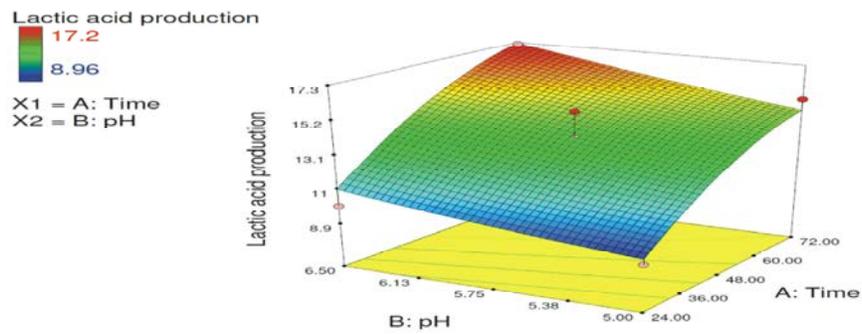


Fig. 5(a): Effect of pH and time on lactic acid production using whey by *P. acidilactici*

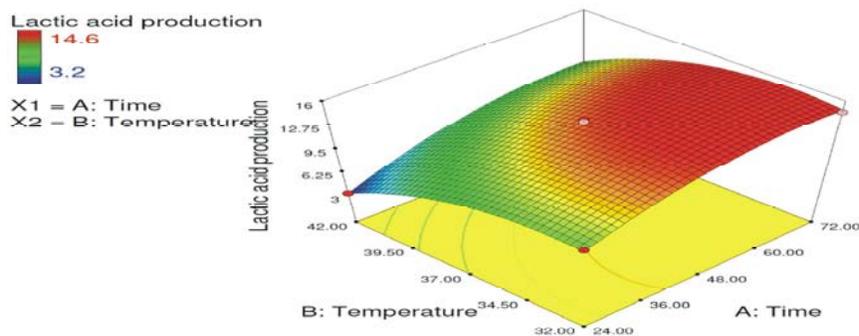


Fig. 5(b): Effect of temperature and time on lactic acid production using whey by *P. acidilactici*

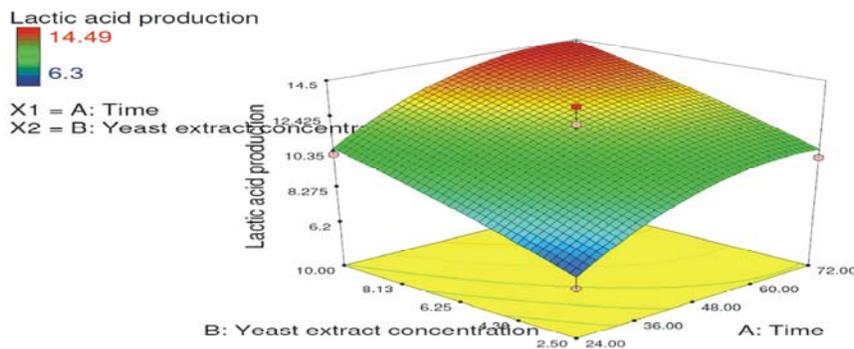


Fig. 5(c): Effect of yeast extract concentration and time on lactic acid production using whey by *P. acidilactici*

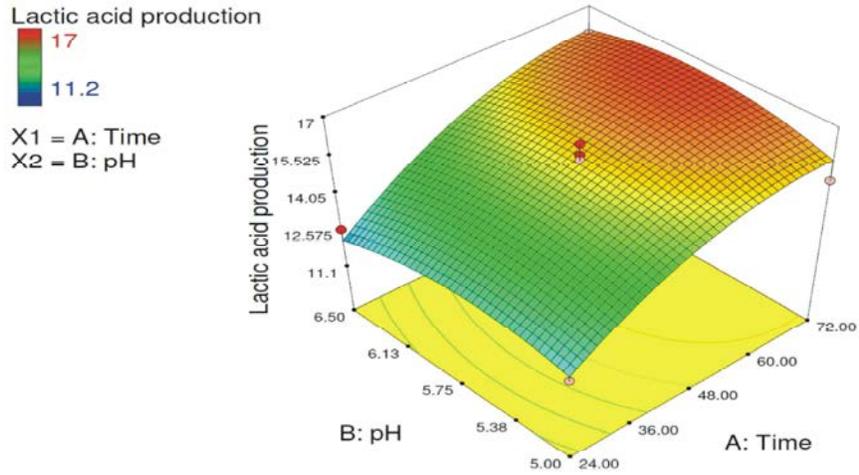


Fig. 6(a): Effect of pH and time on lactic acid production using whey by *L.plantarum*

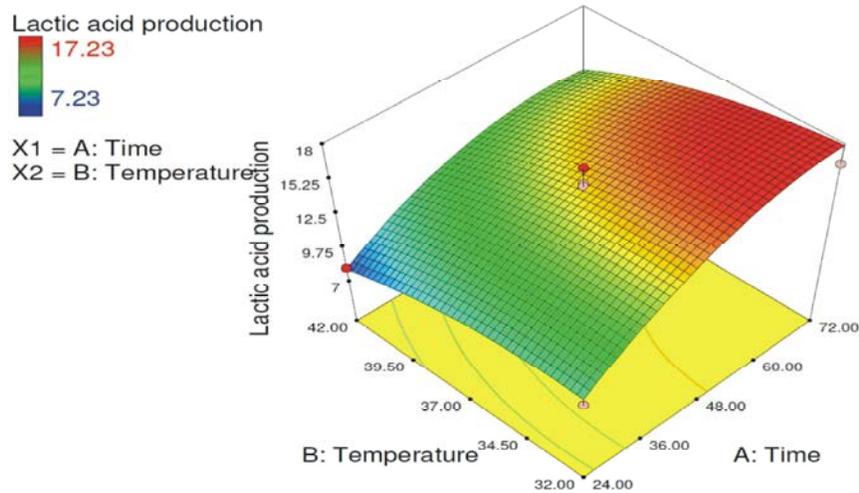


Fig. 6(b): Effect of temperature and time on lactic acid production using whey by *L.plantarum*

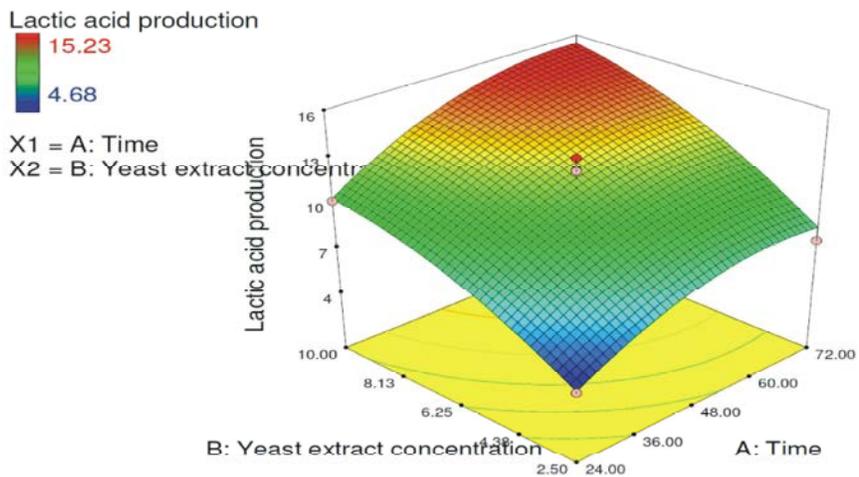


Fig. 6(c): Effect of yeast extract concentration and time on lactic acid production using whey by *L.plantarum*

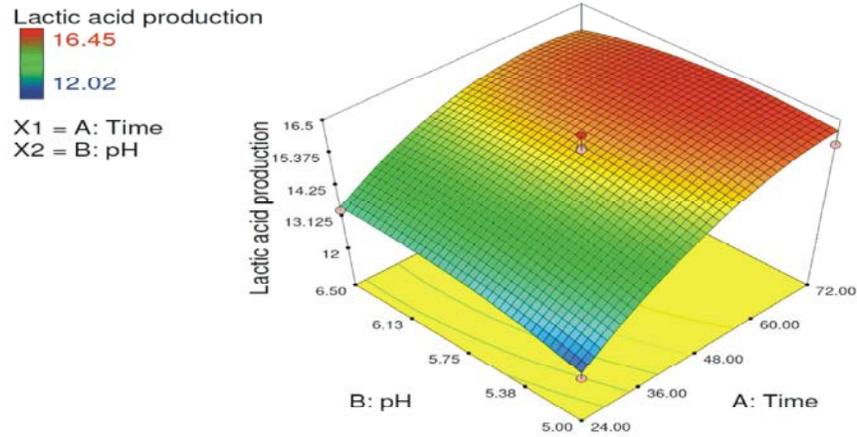


Fig. 7(a): Effect of pH and time on lactic acid production using whey by *L.brevis*

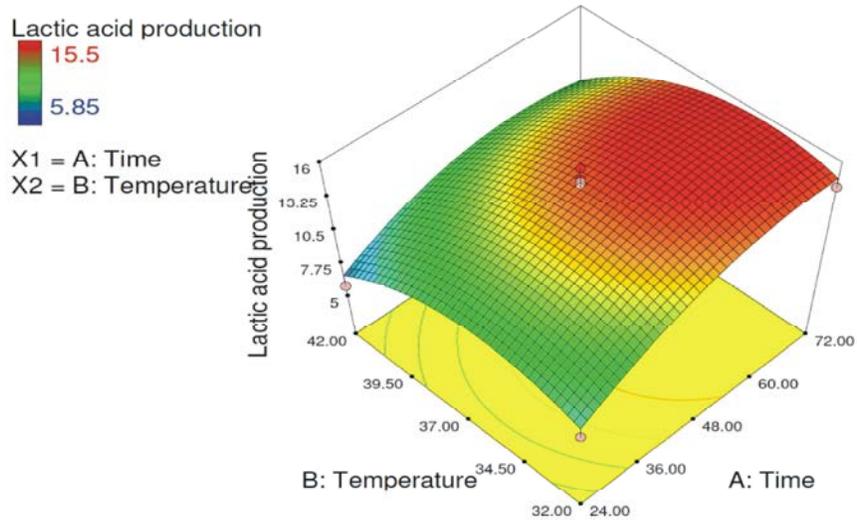


Fig. 7(b): Effect of temperature and time on lactic acid production using whey by *L.brevis*

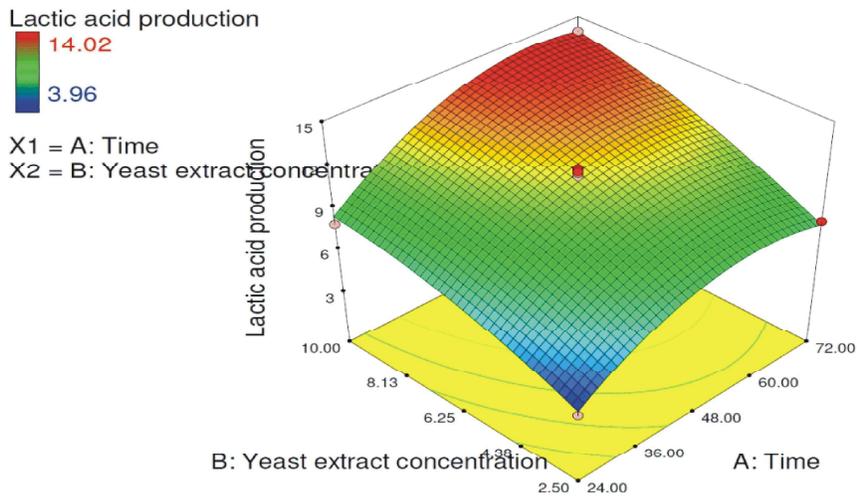


Fig. 7(c): Effect of yeast extract concentration and time on lactic acid production using whey by *L.brevis*

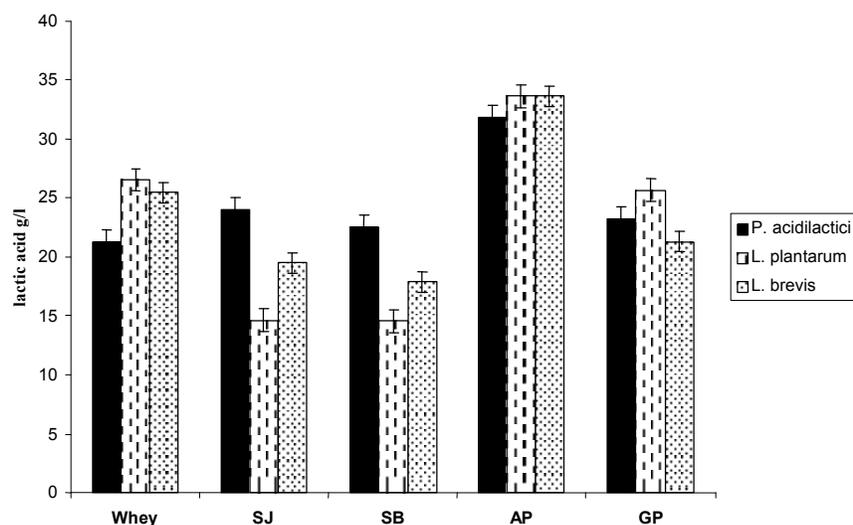


Fig. 8: Lactic acid production from whey, sugarcane juice (SJ), sugarcane bagasse (SB), apple pomace (AP) and grape pomace (GP) using *P.acidilactici*, *L. plantarum* and *L.brevis*

#### REFERENCES

- Vickroy, T.B. 1985. Lactic acid. In: Moo-Young A ed. Comprehensive Biotechnology, 3: 761-776.
- Dimerci, A., A.L. Pometto and K.E. Johnson, 1993. Lactic acid production in a mixed culture biofilm reactor. Appl. Environ. Microbiol., 59: 203-207.
- Senthuran, A., V. Senthuran, B. Mattiasson and R. Kaul, 1997. Lactic acid fermentation in a recycle batch reactor using immobi-lized Lactobacillus casei. Biotechnol. Bioeng., 55: 843-853.
- Sheeladevi, A. and N. Ramanathan, 2011. Lactic acid production using Lactic acid bacteria under optimized conditions. International Journal of Pharmaceutical and Biological Archives, 2(6): 1686-1691.
- Hofvendahl, K. and B. Hahn-Hagerdal, 2000. Factors affecting the fermentative lactic acid production from renewable resources. Enzyme Microbiol. Technol., 26: 87-107.
- Marwaha, S.S. and J.F. Kennedy, 1988. Review: whey pollution problem and potential utilization. Intl. J. Food Sci. Technol., 23: 323-336.
- Mawson, A.J., 1994. Bioconversions for whey utilization and waste abatement. Biores. Technol., 47: 195-203.
- Shi, J., J. Yu, J.E. Pohorly and Y. Kakuda, 2003. Polyphenolics in grape seeds-biochemistry and functionality. J. Med. Food., 6: 291-299.
- Louli, V., N. Ragoussis and K. Magoulas, 2004. Recovery of phenolic antioxidants from wine industry bi-products. Bioresource Technology, 92: 201-208.
- Maghsoud, B., T. Akbar, J. Hossein and M.G. Ali, 2008. Evaluation of some byproducts using In situ and In vitro Gas production Techniques. American Journal of Animal and Veterinary Sciences, 3(1): 7-12.
- Fortina, M.G., C. Parini, P.R. Rossi and P.H. Magnhim, 1973. Mapping of three plasmids from Lactobacillus helveticus ATCC 15009. Letters in App. Microbial., 17: 303-306.
- Krischke, W., M. Schroder and W. Trosch, 1991. Continuous production of L-lactic acid from whey permeate by immobilized Lactobacillus casei subsp. casei. Appl. Microbiol. Biotechnol., 34: 573-578.
- Panesar, P.S., J.F. Kennedy, C.J. Knill and M. Kosseva, 2010. Production of L(+) lactic acid using Lactobacillus casei from whey. Braz. Arch. Boil. Technol., 53(1): 1-9.
- Ghaly, A.E., M.S.A. Tango, N.S. Mahmood and A.C. Avery, 2004. Batch propagation of Lactobacillus helveticus for production of lactic acid from lactose concentrated cheese whey with microaeration and nutrient supplementation. World J. Microbiol. Biotechnol., 20: 65-75.
- Linko, Y.Y. and P. Javanainen, 1996. Simultaneous liquefaction, saccharification and lactic acid fermentation on barley starch. Enzyme Microb. Technol., 19: 118-123.
- Guoqiang, D., R. Kaul and B. Mattiasson, 1991. Evaluation of alginate-immobilized Lactobacillus casei for lactate production. Appl. Microbiol. Biotechnol., 36: 309-314.

17. Hujanen, M. and Y.Y. Linko, 1996. Effect of temperature and various nitrogen sources on L(+)-lactic acid production by *Lactobacillus casei*. *Appl. Microbiol. Biotechnol.*, 45: 307-313.
18. Aeschlimann, A. and U. Von Stockar, 1990. The effect of yeast extract supplementation on the production of lactic acid from whey permeate by *Lactobacillus helveticus*. *Appl. Microbiol. Biotechnol.*, 32: 398-402.
19. Buyukkileci, A.O. and S. Harsa, 2004. Batch production of L(+) lactic acid from whey by *Lactobacillus casei* (NRRL B-441). *J. Chem. Technol. Biotechnol.*, 79: 1036-1040.
20. Roy, D., J. Goulet and A. Leduy, 1986. Batch fermentation of whey ultrafiltrate by *Lactobacillus helveticus* for lactic acid production. *Appl. Microbiol. Biotechnol.*, 24: 206-213.
21. Gandhi, D.N., R.S. Patel, B.K. Wadhwa, N. Bansal, M. Kaur and G. Kumar, 2000. Effect of agro-based by-products on production of lactic acid in whey permeate medium. *J. Food Sci. Technol.*, 37: 292-295.
22. Chiarini, L., L. Mara and S. Tabacchioni, 1992. Influence of growth supplements on lactic acid production in whey ultrafiltrate by *Lactobacillus helveticus*. *Appl. Microbiol. Biotechnol.*, 36: 461-464.
23. Cock, L.S. and A.R. De Stouvenel, 2000. Lactic acid production by a strain of *Lactococcus lactis* subsp *lactis* isolated from sugarcane plants. *Electron J. Biotechnol.*, 9(1): 1-7.
24. John, R.P., K.M. Nampoothiri and A. Pandey, 2007. Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives. *Appl. Microbiol. Biotechnol.*, 74: 524-534.
25. Tanaka, T., M. Hsshina, S. Tanabe, K. Sakai, S. Ohtsubo and M. Taniguchi, 2006. Production of D-lactic acid from defatted rice bran by simultaneous saccharification and fermentation. *Bioresource Technology*, 97(2): 211-17.
26. Yanez, R., A.B. Moldes, J.L. Alosa and J.C. Parjo, 2003. Production of D(-)-lactic acid from cellulose by simultaneous saccharification and fermentation using *Lactobacillus coryniformis* subsp. *torquens*. *Biotechnol. Lett.*, 25(14): 1161-1164.
27. Yanez R., J.L. Alonso and J.C. Parajo, 2005. D-lactic acid production from waste cardboard. *J. Chem. Technol. Biotechnol.*, 80: 76-84.
28. Adsul, M.G., A.J. Varma and D.V. Gokhale, 2007. Lactic acid production from waste sugarcane bagasse derived cellulose. *Green Chem.*, 9: 58-62.
29. Gullon, B., R. Yanez, J.L. Alonso and J.C. Parjo, 2008. L-lactic acid production from apple pomace by sequential hydrolysis and fermentation. *Bioresource Technology*, 99(2): 308-319.