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Enhancement of Lactic Acid Production by Utilizing Liquid Potato Wastes

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ABSTRACT

A viable process based on a low cost production media is desired to enhance the economics of fermentative production of l-lactic acid. Attempts were made to exploit Liquid Potato Wastes (LPW) excluded in the potato processing industry (chips), as a substrate for lactic acid production. Aiming at maximum lactic acid productivity, the screening of strains, components of media and cultivation conditions were varied. The production of a notable and highly effective lactic acid, by the most efficient strain *Lactobacillus casei* EMCC 11093, utilizing the abundant Liquid Potato Wastes (LPW) was achieved in 4-day cultures, at temperature and pH of 32°C and 3.5, respectively. The LPW with MRS medium (with absence of peptone, yeast extract and glucose, and presence of malt extract, galactose and manganese sulphate) represented the most preferable nutritional conditions for obtaining maximum production of lactic acid (16.09 g L⁻¹).

Key words: *Lactobacillus casei*, Liquid Potato Wastes (LPW), lactic acid, MRS optimization

INTRODUCTION

Potato starch obtained from waste waters of chips manufacturing was used as a fermentation substrate for yeast protein enrichment. Among 18 yeast strains, 6 strains were screened according to their biomass yield and protein content after fermentation for 16 h at 30°C in an aerated glucose-based liquid media (4.5 Ls). Using concentrated media (25% solids) made from potato starch pre-hydrolyzed with malt flour and batch-fermented for 20 h at 26°C under aerobic conditions, *Candida utilis* ATCC 9256 was the most efficient protein-forming strain. Scaled-up at the 100 Ls level, the aerobic batch process was improved under fed-batch conditions with molasses supplementation (Gelinias and Barrette, 2007).

Potato annual world production is around 300 million tons and areas planted cover more than 18 million ha. Major producing countries (and the world's share of production) are China (20), Russia (12), India (8) and United States (8%) (Miranda and Aguilera, 2006).

In Egypt potato is one of the most important crops grown for local consumption, export and processing. The area cultivated with potatoes about 212,000 acres producing about 2.2 million tons, with an average of 10.5 tones per acre (Hegazy, 2009).

Large amounts of agro industrial residues are generated from diverse economic activities that represent one of the energy rich resources available on the planet and when not properly discharged or used, add to environmental pollution (Francis *et al.*, 2003). Biotechnology industries demand potato (*Solanum tuberosum*) the best raw materials to prepare growth media for the fermentative processes (Liu and Yan, 2008). The selection of a specific raw material depends mainly on its availability, composition and price.

Liquid potato waste containing natural materials (like starch) are used for production of lactic acid. The starchy materials consisted of glucose are easily hydrolyzed into fermentable sugars.

Lactic acid is a valuable product with many practical applications: as a preservative, pH regulator and taste-enhancer in food industry, for implants and suture in the medical practice, as a reagent for polylactic and polyacrylic acids synthesis for biodegradable polymers (Wee *et al.*, 2006). The reduction in emulsion pH by the addition of LA and/or GDL significantly ($p < 0.05$) influenced the processing and quality parameters of pork sausages (Thomas *et al.*, 2008).

Biotechnological production is primarily carried out by bacterial fermentation of simple sugars, and bacterial species *Lactobacillus* and *Lactococcus* have received a worldwide interest in industrial processes because of their high growth rates and product yields (Hofvendahl *et al.*, 1999; Gonzalez-Vara *et al.*, 2001). Moreover, the improvement of the biomass yield is interested because biomass from Lactic Acid Bacteria (LAB) fermentation is widely used in food and pharmaceutical industry (Lee *et al.*, 2007).

Further, agricultural resources such as barley, wheat, and corn were hydrolyzed by commercial amylolytic enzymes and fermented into lactic acid by *Enterococcus faecalis* RKY1. Although no additional nutrients were supplemented to those resources, lactic acid productivities were obtained at $>0.8 \text{ g L}^{-1} \text{ h}$ from barley and wheat (Oh *et al.*, 2005). Simultaneous saccharification with glucoamylase effectively improved lactic acid production with *L. casei* alone, with the highest lactic acid concentration of 120 g L^{-1} (yield 667 g kg^{-1} barley flour fermented) on barley flour treated with barley malt without any additional nutrients (Linko and Javanainen, 1996). Different nutritional and process parameters influencing lactic acid production by *Lactobacillus casei* were studied by Senthuran *et al.* (1999).

In this study, we have characterized the chemical composition of liquid potato waste to evaluate its suitability as raw materials for lactic acid production. To achieve more rapid and cost-effective lactic acid production from liquid potato wastes, we have attempted direct fermentation using the most lactic acid producer, *Lactobacillus casei* EMCC11093 and examined the influence of nutritional and environmental conditions in LPW-MRS medium on lactic acid produced by this strain and discussed its yield and feasibility.

MATERIALS AND METHODS

Chemical composition of liquid potato wastes: The liquid potato wastes samples were obtained from the Chips's Company for food and analyzed according to standard methods industries in 2008, Assiut, Egypt, (AOAC, 1990). Elemental analysis was carried out using atomic absorption spectrophotometer (model GBC 906 AA) in the laboratories of Agricultural College, Assiut University, Egypt.

Microorganisms, culture conditions and inoculum preparation: Six homofermentative l (+) lactic acid bacteria, *Lactobacillus delbrueckii* subsp. *bulgaricus* EMCC 11102, *Lactobacillus casei* EMCC 11093, *Lactobacillus acidophilus* ATCC 4356, *Lactobacillus lactis* subsp. *lactis* EMCC 11552, *Streptococcus thermophilus* EMCC 11044 and *Streptococcus thermophilus* SMU 3855, were obtained from Cairo Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

The cultures were maintained in MRS agar slabs at 4°C and subcultured fortnightly. The MRS medium composition was as follows g L^{-1} : Bacteriological peptone, 10; yeast extract, 5; beef extract,

10; glucose, 20; dipotassium phosphate, 2; sodium acetate, 5; diammonium citrate, 2; MgSO₄·7H₂O, 0.02; MnSO₄·4H₂O, 0.05 g. Tween (1 mL) was added. The medium was purchased from Biolife Co., Milano, Italy.

The inoculum for the experiments was prepared from fresh MRS slants. A loopful of culture was inoculated into 25 mL MRS medium in 250 mL Erlenmeyer flask and incubated at 37°C for overnight. The 18-h old bacterial culture having 10⁹ CFU mL⁻¹ was used as the inoculum.

Fermentation conditions: Fermentation was performed in 250 mL Erlenmeyer flasks containing 80 of LPW with 20 mL of MRS. The medium was sterilized by autoclaving at 121°C for 20 min. Flasks were inoculated with 1 mL of actively growing culture *Lactobacillus casei* EMCC11093 and incubated at 37°C under static conditions for seven days as experimental design. At the end of fermentation, the lactic acid, protein content and final pH were determined. Each experiment was performed in triplicate.

Extraction and estimation of lactic acid: After fermentation, the complete lactic acid produced in each flask was extracted and clarified by squeezing through dampened cheese cloth (Ramesh and Lonsane, 1990), followed by cold centrifugation at 5000 rpm for 20 min and the supernatant was used for lactic acid estimation according to the method of Taylor (1996). The pH of the extract was measured by a pH-meter equipped with a glass electrode, using a solid-liquid ratio of 10% (w/v) with distilled water. Protein are quantified using the Folin-Ciocalteu method (Lowry *et al.*, 1951) using Bovine Serum Albumin (sigma) as a standard.

RESULTS AND DISCUSSION

Chemical composition of liquid potato wastes: The composition of liquid potato wastes is presented in Table 1. The average contents of dry matter, total carbon and organic matter were 8.8 mg, 0.40 and 0.68%, respectively. While the ash content, TSS and protein were 227 mg, 1 and 0.14%, respectively. It demonstrated that LPW from potato process industry containing a less concentration of useful materials. These materials are much lower than in, food wastes (Ohkouchi and Inoue, 2006) and waste activated sludge (Maeda *et al.*, 2009).

As shown in Table 2, LPW containing the highest mineral values (100 and 91.2 ppm mg L⁻¹) were in Ca and Mg, respectively, the middle values (35, 40 and 58 ppm mg L⁻¹) were in K, Na and

Table 1: Percentage composition of liquid potato wastes

Components	Average content in LPW (%)
Dry matter ^a	8.8 mg
Total carbon content ^b	0.394%
Organic matter ^b	0.68
Ash ^c	227 mg
TSS	1
Reducing sugars	0.44
Non reducing sugars	3.08
Starch	1.68
Protein	0.14
pH	4.66-4.67

^aCalculated in 1 mL extract; ^b Calculated in 25 mL⁻¹ extract; ^c Calculated in 1 g dry matter

Table 2: Analysis of minerals content in liquid potato wastes

Mineral	Fe	Mn	Cu	Zn	Ca	Mg	Na	K	S
Concentration (ppm mg L ⁻¹)	0.234	0.180	0.223	0.460	100	91.2	39.99	34.99	58

Table 3: Screening of six lactic acid bacterial strains on LPW and LPW- MRS

Bacteria strain	LPW- MRS			LPW		
	Lactic acid concentration (g L ⁻¹)	Final pH	Protein content (%)	Lactic acid concentration (g L ⁻¹)	Final pH	Protein content (%)
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> EMCC 11102	6.65	3.91	0.98	2.55	4.84	0.65
<i>Lactobacillus casei</i> EMCC 11093	7.78	3.67	1.029	2.67	4.76	0.70
<i>Lactobacillus acidophilus</i> ATCC 4356	6.19	3.76	0.957	2.32	4.84	0.55
<i>Lactobacillus lactis</i> subsp. <i>lactis</i> EMCC 11552	5.65	3.99	0.87	2.24	4.82	0.44
<i>Streptococcus thermophilus</i> EMCC 11044	6.34	3.69	0.98	1.85	4.82	0.19
<i>Streptococcus thermophilus</i> SMU 3855	6.03	3.78	0.93	2.28	4.84	0.40

S, respectively and the lowest values (0.180, 0.223, and 0.460 ppm mg L⁻¹) were in Mn, Cu and Zn, respectively. Ohkouchi and Inoue (2006) studied on the composition of minerals in culture medium with food wastes compared with MRS medium universally used for cultivation of *Lactobacillus* sp. and pointed out that, the values of Ca content in culture medium with food wastes exhibited large deviations, while, K and Mn were less than MRS medium and Mn was especially deficient in culture medium with food wastes (contained 14.6±0.8 µM versus 208 µM in MRS medium), hence the effect of Mn concentration on lactic acid production was examined.

Screening of different lactic acid bacteria strains for their lactic acid production:

Preliminary experiments were made on screening of cost effective MRS concentration with LPW for lactic acid production using six lactic acid bacteria (data not shown). The last experiment resulted in the lowest MRS's concentration should be added with LPW, to enhance lactic acid production was in a ratio of 20: 80% (v/v) respectively (Table 3).

To compare the lactic acid production aptitude of six available strains, cultivation was conducted in flasks, in an LPW medium, compared with MRS- LPW (as ratio above). The results showed in all cases a higher use of the LPW-MRS medium, higher production of lactic acid and protein content, and lower level of pH values, and vice versa in cultures on LPW only. With the exception of the strain *Lactobacillus casei* EMCC 11093, where, this culture had the highest production of lactic and protein content, 7.78 g L⁻¹ and 1.03%, respectively, as well as the lowest final pH 3.67 (Table 3). Consequently, was selected for lactic acid production using of this study using LPW-MRS medium.

Lactobacillus casei was the organism of choice as it produces predominantly the isomer of lactic acid (Senthuran *et al.*,1999).

The amounts of lactic acid produced in the fermentation medium containing only date juice as carbon and nitrogen sources were smaller than those produced in the MRS broth medium

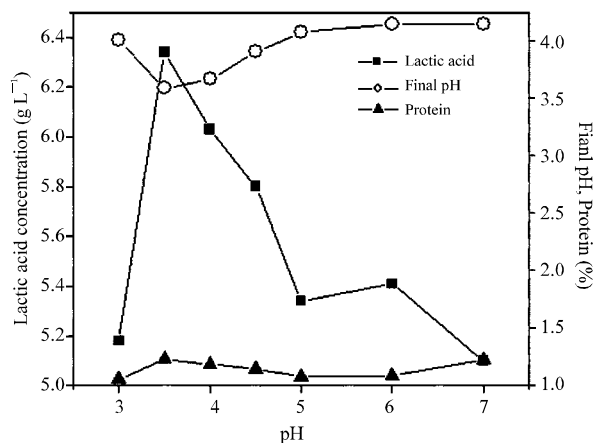


Fig. 1: Influence of cultivation pH on lactic production by *L. casei*

(Nancib *et al.*, 2001). Petrov *et al.* (2008) studied the maximum lactic acid productivity, components of the media and the cultivation conditions by *Lactococcus lactis* subsp. *lactis* B84 utilizing starch as a sole carbon source, and found that, MRS-starch medium (with absence of yeast and meat extracts), at 33°C, agitation 200 rpm and pH 6.0 for 6 days, a complete starch hydrolysis occurred and 5.5 g L⁻¹ lactic acid were produced from 18 g L⁻¹ starch. Pintado *et al.* (1999), noted that on Mussel Processing Wastes (MPW) lactic acid is lower than with MRS-starch. Ogunbanwo and Okanlawon, 2009 studied the influence of nutrients utilization and cultivation conditions on the production of lactic acid by homolactic fermenters, and indicated that, all the *Lactobacillus* species isolated produced little quantity of lactic acid when grown at 30°C in normal De Man Rogosa Sharpe (MRS) broth. However, a temperature of 40°C at initial pH of 5.5 in constituted MRS medium with 6% (w/v) carbon concentration of D-glucose and 4% (w/v) nitrogen concentration of yeast extract fermented for 48 h supported lactic acid production optimally with *Lactobacillus acidophilus* producing 18.4 ± 0.01 g L⁻¹ of lactic acid.

Therefore, on using LPW, MRS broth medium must be added into the fermentation to support both microbial growth to study the optimum environmental conditions for lactic acid formation.

pH for cultivation: The different initial pH values in LPW-MRS medium of *L. casei* culture was controlled at pH 3.0, 3.5, 4.0, 4.5, 5.0, 6.0 and 7.0 and lactic acid production were monitored. The initial pH values at culture were adjusted with 5 N hydrochloric acid-5 N sodium hydroxide.

As shown in Fig. 1, LPW-MRS medium with initial pH 3.5, higher production was observed, and the final amount of lactic acid was decreased obviously compared with initial pH 3.5. At this particular pH, *L. casei* had the highest production of lactic and protein content, 6.34 g L⁻¹ and 1.0%, respectively, as well as the lowest final pH 3.34.

These results were differ from those obtained with, *Lactobacillus rhamnosus* (Maizirwan *et al.*, 2006; Mel *et al.*, 2007), *Lactobacillus casei* (Senthuran *et al.*, 1999), *L. lactis* subsp. *lactis* B84 (Petrov *et al.*, 2008) and *Lactobacillus manihotivorans* LMG18011 (Ohkouchi and Inoue, 2006) which had an optimum pH for growth at 6.0 and from *L. amylophilus*, which could produce lactic acid from starch over a pH of 5.0-6.8 (Yumoto and Ikeda, 1995).

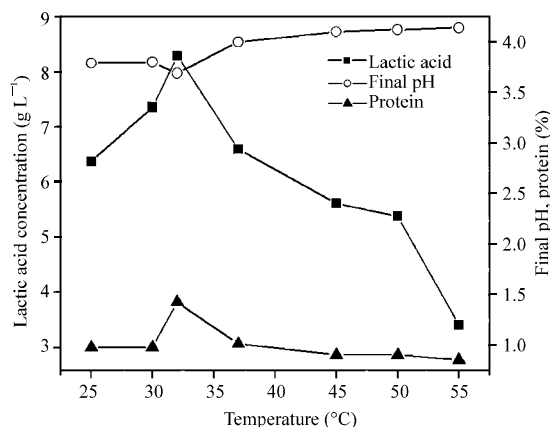


Fig. 2: Influence of fermentation temperature on lactic acid production

Fermentation temperature: We examined the effect of incubation temperature on lactic acid production to reveal which temperature is the best fermentation condition.

The impact of the cultivation temperature on the LPW-MRS medium, adjusted at pH 3.5, was investigated by controlling the growth temperatures at 25, 30, 32, 37, 45, 50 and 55°C. The results from measuring the lactic acid indicated that there was an increase in its as the temperature increased from 25 to 32°C and a further increase than 32°C resulted in a slight improvement for production of lactic acid *L. casei* culture (Fig. 2). At this particular temperature 32°C, *L. casei* had the highest production of lactic acid and protein content, 8.28 g L⁻¹ and 1.43%, respectively, as well as the lowest final pH 3.69 (Fig. 2). Lactic acid concentration was in the middle concentration at temperature range of 37-50°C, except low lactic acid concentration at 55°C (Fig. 2). In agreement to this result, Hujanen and Linko (1996) and Petrov *et al.* (2008) have reported the optimal incubation temperature for production of lactic acid by *L. casei* and *L. lactis* subsp. *lactis* B84, respectively, was at 33°C. Also, In contrast, a very high range of temperature, 37°C, was detected for lactic acid production with *L. casei* (Linko and Javanainen, 1996). Consequently, 32°C appeared to be an optimum cultivation temperature for lactic acid production by the *L. casei* in LPW-MRS medium.

Incubation period: To study the reaction time required for lactic acid production, LPW-MRS *L. casei* culture medium were taken at 0, 1, 2, 3, 4, 5, and 6 day. The reactions were carried out in 250 mL Erlenmeyer flasks each containing 100 mL of the mixed suspension at pH and temperature, 3.5 and 32°C, respectively.

As shown in Fig. 3, the lactic acid concentration gradually increased with the increase in the incubation time. The maximal concentration of lactic acid and protein content, 6.57 g L⁻¹ and 1.01%, respectively, following the lowest final pH 3.30 was obtained at 4 day, and there was no further increase with an increase in incubation time (Fig. 3). In the present study it was observed that incubation period has remained, 4 days.

Our results are comparable to those obtained with, *Lactobacillus amylophilus* GV6 (Naveena *et al.*, 2005; Altaf *et al.*, 2006); *Lactobacillus delbrueckii* (John *et al.*, 2006), which had an optimal incubation period for production of lactic acid after 5 days and with *Lactococcus lactis* subsp. *lactis* B84 (Petrov *et al.*, 2008) after 6 days.

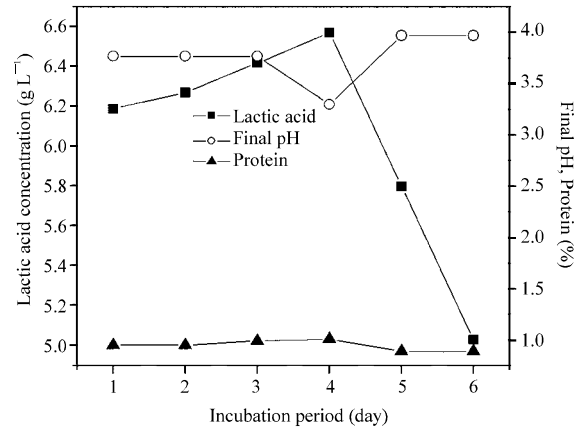


Fig. 3: Influence of incubation period on lactic acid production

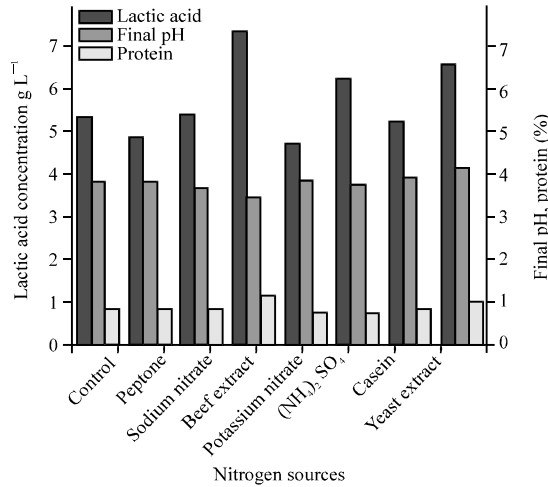


Fig. 4: Influence of nitrogen source on lactic acid production

Nitrogen source: Various nitrogen sources (sodium nitrate, potassium nitrate, ammonium sulfate and casein comparable to control) were compared with peptone, beef and yeast extract in terms of their efficiency for lactic acid production in MRS medium used above (maintaining the same level of elemental nitrogen).

Replacing the peptone and yeast extract containing MRS medium with beef extract based LPW-MRS medium resulted in a higher lactic acid and protein content 7.35 g L⁻¹ and 1.13%, respectively, followed by final pH 3.44 (Fig. 4). None of these nitrogen sources gave lactic acid concentrations as high as that obtained with beef extract.

An attempt to reduce its amount, different concentrations had added with MRS in basal LPW-MRS medium and the results pointed out that the highest lactic acid yield was obtained at 1.6 g L⁻¹ beef extract (data not shown). *L. amylophilus* GV6 has shown good efficiency in utilizing red lentil flour and yeast cells as substituents to commercial peptone and yeast extract in SSF using wheat bran as substrate to reduce the cost of fermentation medium (Altaf *et al.*, 2006). Preliminary

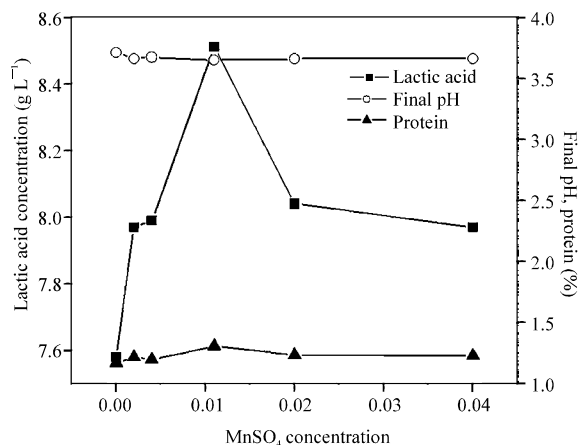


Fig. 5: Influence of MnSO₄ concentration on lactic acid production

studies were made on screening of cost effective nitrogen sources, of which red lentil flour and yeast cells were selected to replace costly commercial peptone and yeast extract in the modified MRS medium (Altaf *et al.*, 2005).

Senthuran *et al.* (1999) revealed that hydrolyzed whey protein constituted a richer source of nitrogen influencing lactic acid production by *Lactobacillus casei*.

Mel *et al.* (2008) indicated that the optimum concentration of glucose and peptone for optimum bacterial growth rate and lactic acid production in shake flask were 9.80 and 9.98 g L⁻¹, respectively. The optimum productivity of the lactic acid was 0.630 g L⁻¹ h which correspond to optimum growth rate of the bacteria at 0.341 h.

MnSO₄ concentration: Effect of MnSO₄ concentration on lactic acid production was determined by adding different concentration of MnSO₄ g L⁻¹ 0.002, 0.004, 0.011, 0.02 and 0.04, with MRS medium (eliminating MnSO₄) in based LPW-MRS medium. In *L. casei* culture medium deficient in MnSO₄, the concentration of lactic acid production was lower than in MnSO₄-supplemented medium. As shown in Fig. 5, the concentration of lactic acid and protein content reached their maximum rate 8.51 g L⁻¹ and 1.31%, respectively, followed by lower final pH 3.65 in the presence of 0.011 g L⁻¹ MnSO₄. Differ to this results, Ohkouchi and Inoue (2006) pointed out that no decrease in lactic acid productivity occurred in manganese deficient medium, when cells grown in a medium supplemented *L. manihotivorans* LMG18011 can therefore presumably accumulate manganese. Archibald and Fridovich (1981) reported that some lactobacilli strains required high concentration of manganese for growth. For example, *L. plantarum* accumulated manganese to 30 mM (Archibald and Duong, 1984). It had been known that lactobacilli strains were deficient in catalase activity, but could grow in the presence of oxygen. Manganese has been recognized to act as a scavenger of toxic oxygen species such as superoxide anion (O₂⁻) or hydrogen peroxide by Gonzalez *et al.* (1989) also recognized its ability to act as an inducer of MnSO₄ activity. In both cases, manganese provides a positive effect for the growth of *L. manihotivorans* LMG18011 in a microaerophilic condition, to acquire protection against reactive oxygen species.

Based on our chemical characterization of liquid potato waste (Table 2). However, our result indicated that Mn was an only deficient element, and concluded that Mn was an essential fermentation factor for *L. casei*. When MnSO₄ was added to MRS medium with LPW at least 0.011 g L⁻¹, lactic acid production was relatively high.

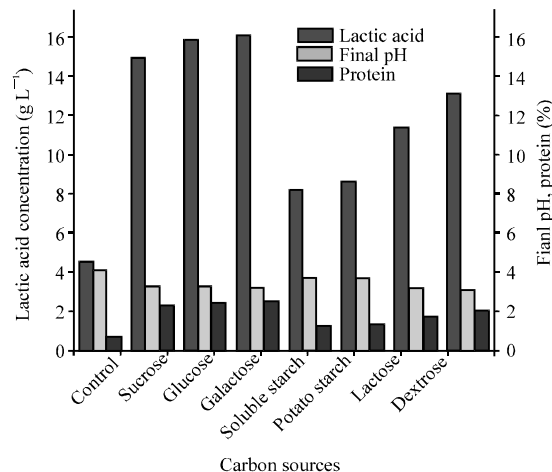


Fig. 6: Influence carbon source on lactic acid production

Carbon source: The effect of some carbon sources as sucrose, glucose, galactose, soluble starch, potato starch, lactose and dextrose at the concentrations of 1.0% on lactic acid production by *L. casei* were examined in LPW-MRS medium as previously mentioned. The results in Fig. 6 showed that *L. casei* preferred galactose as carbon source for lactic acid production, followed by glucose and sucrose, while soluble and potato starch were poorly utilized. The maximum lactic acid concentration and protein content in the culture supernatant was, 16.09 g L⁻¹ and 2.24%, respectively, as well as the lowest final pH 3.48 in the presence of galactose (Fig. 6).

This result was differ from those obtained by Senthuran *et al.* (1999) and Ohleyer *et al.* (1985), where lactose and glucose was the preferred substrate by *L. casei* and *Lactobacillus delbreuckii*, respectively, moreover, Lu *et al.* (2010) reported that, the acorn powder was the most excellent one for *L. rhamnosus* HG 09 among the four carbon sources as it could enhance the L(+)-lactic acid production with a satisfied productivity and yield. The results of the present work indicated that a lower nitrogen, MnSO₄ and carbon concentrations in MRS result in a higher productivity of L(+)-lactic acid for *Lactobacillus casei* EMCC 11093 and further reduction in production cost could be achieved.

CONCLUSIONS

In this study, liquid potato wastes were investigated as a nutrient resource for l (+)-lactic acid production. In the liquid potato wastes, the average contents of dry matter, total carbon and organic matter were 8.8 mg, 0.40 and 0.68%, respectively. While the ash content, TSS and protein were 227 mg, 1 and 0.14%, respectively and this result demonstrated that LPW from potato process industry containing a less concentration of useful materials and could become a key point for direct production of lactic acid from LP wastes.

To reduce the production cost, preliminary experiments were made on screening of cost effective MRS concentration with LPW for lactic acid production using six lactic acid bacteria and resulted in the lowest MRS's concentration should be added with LPW, to enhance lactic acid production was in a ratio of 20: 80% (v/v) respectively using the most efficient strain *Lactobacillus casei* EMCC 11093, where, this culture had the highest production of lactic and protein content, 7.78 g L⁻¹ and 1.03%, respectively, as well as the lowest final pH 3.67.

The production of a notable and highly effective lactic acid, by *L. casei* EMCC 11093, was achieved in 4-day cultures, at temperature and pH of 32°C and 3.5, respectively. Under experimentally controlled conditions, the maximum yield of l (+)-lactic acid reached up to 16.09 g L⁻¹ from LPW- MRS medium (with absence of peptone, yeast extract and glucose and presence of malt extract, galactose and manganese sulphate) which represented the most preferable nutritional conditions and indicated the feasibility of direct l (+)-lactic acid production from LPW.

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