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UTILIZATION OF POTATO-CHIPS WASTE FOR PRODUCTION OF HIGH ECONOMIC VALUE PRODUCTS: III - PRODUCTION OF LACTIC ACID

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ABSTRACT

The potential use of potato chips waste as a cheap substrate for lactic acid production by *Rhizopus arrhizus* NRRL 1526 in a simultaneous saccharification and fermentation was studied. Maximum lactic acid concentration of (17.10g/L) and yield (0.95 g/g starch) were produced when the fungus was cultivated at the determined optimum conditions of medium at pH 6, fermentation temperature 30 °C, starch concentration 18g/L, nitrogen concentration 0.4g/L in form of ammonium for cultivation period of 54 hr. Lactic acid used for safe human consumption in the processed meat, poultry industries, salads and confectionery.

Keywords: potato-chips waste, lactic acid, simultaneous saccharification and fermentation

INTRODUCTION

Large amounts of waste are generated yearly from potato chips industry, in Egypt. These wastes are rich in starch and other nutrients making them suitable as substrate for microbial fermentation, when these wastes not properly discharged or used, add to environmental pollution. Therefore, it is environmentally and economically significant to consider the production of lactic acid using potato chips waste.

The choice of a microorganism for the production of lactic acid primarily depends on the carbohydrate to be fermented (Bustos *et al.*, 2007). Fungal *Rhizopus* species have attracted a great interest, and have been recognized as suitable candidates for lactic acid production (Yin *et al.*, 1997).The major advantage using the fungi over the bacteria is the low costs, due to, use of raw and/or waste materials, no need specific nutrients, little pH maintenance required, as the most fungi can be tolerant to low pH environment, and easy and inexpensive separation of filamentous or pellet biomass from the fermentation broth (Soccol *et al.*, 1994; Rosenberg and Kristofikova, 1995 and Jin *et al.*, 2003). Another important aspect for using *Rhizopus* fungi is direct conversion of starch to lactic acid with both amylolytic and lactic acid producing character, these can eliminate the two-step process to make it economical (Jin *et al.*, 2003 and Ueno *et al.*, 2003).

There are four major categories for the current uses and applications of lactic acid: Food, cosmetic, pharmaceutical and chemical applications. It is widely used in almost every segment of the food industry, where it serves in a wide range of functions, such as flavouring, pH regulations, improved

microbial quality, and mineral fortification. Moreover, lactic acid is used commercially in the processed meat and poultry industries, to provide products with an increased shelf life, enhanced flavour, and better control of food-born pathogens. Due to the mild acidic taste of lactic acid, it is also used as an acidulant in salads and dressings, baked foods, pickled vegetables, and beverages. It is also used in confectionery, not only for flavour, but also to bring the pH of the cooked mix to the correct point for setting. The advantages of adding lactic acid in confectionery include its low inversion rate, ease of handling and ability to produce clear candies. Another potential application of lactic acid in the food industry is the mineral fortification of food products (Wee *et al.*, 2006).

The objective of this study was to further reduce the lactic acid production cost using potato chips wastes in place of refined costly raw materials such as glucose, starch and molasses etc., thereby decreasing the amount of potato chips wastes to protect the environment. To verify this objective, different cultivation parameters were studied.

MATERIALS AND METHODS

Materials

Potato- chips waste was obtained from a factory for potato-chips industry located in Tanta city. Wheat and rice bran were bought from local market, Ammonium sulfate, Urea, Ammonium phosphate, Sodium nitrate and Calcium carbonate were bought from Sigma. Nylon cloth was purchased from Screen Technology Group Inc., USA.

Microorganism

A Lactic acid-producing strain of *Rhizopus arrhizus* NRRL1526 was used in this study. It was obtained from Institute of Microbiology and Wine research, Gutenberg-University, Mainz, Germany, maintained on potato dextrose agar slants, stored at 4 °C and subcultured every month.

Inoculum preparation

The strain was inoculated on potato- dextrose agar slants and incubated at 30°C for 7 days. Five ml of sterile distilled water was added, spores were scraped off, suspended in water. This spores suspension was used for preparing overnight culture as follow, 100ml of medium consisted of (g/l) soluble starch 10, peptone 5, yeast extract 5, $KH2PO_4$ 0.2, $MgSO_4$, 0.2 were introduced into 250ml flask, autoclaved at 121 °C for 20 min then inoculated and incubated in a rotary shaker (200rpm) at 30°C for 12hr. The production media were inoculated with appropriate volume of overnight culture to give final concentration of 1.0×10^5 spores/ml. Spores density was checked using a hemocytometer. (Following the method of Huang *et al.*, 2005).

Preparation of production media

Lactic acid fermentation medium was prepared from waste according to the method of Jin *et al.* (2003) with some modification as follows:

The fresh waste was homogenized in kitchen blender. The resulted suspension was filtered through 106 µm (140 meshes) nylon cloth. The

chemical composition of the filtrate was determined, and the filtrate was used as basic media for lactic acid production unless stated otherwise. The fermentation was carried out in 250-ml Erlenmeyer flasks containing 100ml of the basic medium inoculated with the overnight culture and incubated in a rotary shaker (200rpm) at 28 °C for 42hr.

Analytical methods

The Micro-Kjeldahl method was used to determine the total nitrogen and thereafter its value was multiplied by the factor of 6.25 to get the crude protein content. Ether extract was determined in a Soxhlet apparatus using the petroleum ether as a solvent, and ash content was determined by ashing the samples in an electric muffle at 550°C until constant weight was maintained (A.O.A.C., 2000). Reducing sugars were estimated by 3, 5 dinitrosalicylic acid method (DNS), according to Miller (1959).The starch was determined according to the method of Kim & Hamdy (1985). Biomass was determined according to the method of Huang *et al.* (2005). Lactic acid was determined according to the method of Lawrence (1975). All values of the presented results are means of at least three separate experiments.

RESULTS AND DISCUSSION

1. Chemical composition of the potato chips wastes:

The data presented in Table (1) show that, the waste is rich in several nutritional factors especially starch, which is main component, making it suitable for lactic acid production.

Table (1): Chemical composition of potato chips waste.

Component (%)*	Whole waste (% dry bases)	Homogenized waste (% dry bases)	Homogenized waste(%wet bases)
Moisture	00.00	00.00	79.15
Starch	61.49	77.97	15.59
Crude fiber	21.84	00.91	00.93
Ash content	15.22	19.29	03.87
Reducing sugar	00.61	00.77	00.16
Crude protein	0.82	01.03	00.21

* Each value was an average of three determinations.

2. Selection of optimum conditions for lactic acid production:

2.1. Effect of medium initial pH:

To investigate the influence of medium pH on lactic acid production, the fungus was cultivated in media at different controlled pH values from 3 to 8. The results in Table (2) reveal that, the fungus could grow and produce lactic acid at all tested pH values. However, weak fungal activities in terms of starch exhaustion, biomass formation and lactic acid production were observed at pH values lower than 4 and higher than 7.These may be attributed to that, the pH values lower than 5 were unfavourable for starch saccharification, consequently other fungal performance was retarded (Haung *et al.*, 2005). pH range 5 – 6 was the favorable for lactic acid

production. Maximum lactic acid concentration (7.79 g/l) and yield (0.71g/g starch) were released at pH 6. Similar results were reported by (Miura *et al.*, 2003), who found that the highest lactic acid yield was produced by *Rhizopus* sp. MK-96-1196 at pH 6.0 - 6.5.

2.2. Effect of addition of calcium carbonate ($CaCO_3$) to the fermentation medium on lactic acid production:

In order to examine the effect of $CaCO_3$ on lactic acid fermentation, sterile $CaCO_3$ powder was added to the fermentation media after 8 hr of cultivation with different levels ranged from 0.5 to 4% w/v. As shown in Table (3), addition of $CaCO_3$ up to 1% w/v to the fermentation broth enhanced all fermentation parameters in terms of saccharification of starch, biomass formation and lactic acid production.

Table (2): Effect of medium pH on fungus growth and lactic acid production.

Medium pH values	Consumed starch (g/l)	Accumulated reducing sugar (g/l)	Biomass dry weight (g/l)	Lactic acid (g/l)	Lactic acid yield (g/g starch)
3	6.79	1.41	1.11	3.66	0.54
4	7.78	1.50	1.31	4.82	0.62
5	10.96	1.41	1.49	7.67	0.70
6	10.98	1.32	2.02	7.79	0.71
7	10.58	1.31	1.31	6.98	0.66
8	7.71	1.29	1.12	3.99	0.51

Cultivation conditions: substrate concentration 20g/l, temperature 28°C and cultivation period was 42hr.

Addition of $CaCO_3$ more than 1% w/v was followed by a decrease in all fungal activities. These may be due to the inhibition of the enzyme activities, which are responsible for the biosynthesis of lactic acid. Moreover, high concentrations of $CaCO_3$ inhibit the growth of microorganism (Kotzamanidis *et al.*, 2002).The highest lactic acid yield (0.83g/g starch) was obtained at a $CaCO_3$ concentration of 1% w/v. These results are quite similar to those of Jin *et al.*, (2003) and Huang *et al.*, (2005). Maintenance of pH is important during lactic acid fermentation to provide the optimum pH for the organism to allow it utilize maximum substrate. Calcium carbonate is one commonly used reagent to neutralize lactic acid during fermentations. (Vishnu *et al.*, 2002, Naveena *et al.*, 2005). It is clear that, a neutralizing agent $CaCO_3$ must be present in the fermentation media to achieve a maximum production of lactic acid by *Rhizopus* sp. The addition of $CaCO_3$ could neutralize H^+ released from lactic acid, which may reduce the synthesis reaction forward to lactic acid production (Rosenberg & Kristofikova, 1995, Yang *et al.*, 1995). Its low solubility in water makes it possible to neutralize lactic acid and maintain the pH at certain level (Huang *et al.*, 2005).

Table (3): Effect of addition of calcium carbonate to the fermentation broth on fungi growth and lactic acid production.

$CaCO_3$ level w/v %	Consumed starch (g/l)	Accumulated reducing sugar (g/l)	Biomass dry weight (g/l)	Lactic acid (g/l)	Lactic acid yield (g/g starch)
0.0	10.98	1.32	2.02	7.79	0.71
0.5	14.20	0.90	1.31	11.6	0.82
1.0	13.99	0.20	1.39	11.61	0.83
2.0	11.60	0.00	1.00	10.91	0.78
3.0	09.38	0.00	0.50	8.93	0.77
4.0	08.36	0.00	0.50	6.27	0.75

Cultivation conditions: substrate concentration20 g/l, pH 6, temperature 28°Cand cultivation period was 42hr.

2.3. Effect of cultural temperature on lactic acid production:

To confirm the influence of cultural temperature, fermentation was conducted at various temperatures ranged from 25 °C to 40 °C. The results presented in Table (4) show that, considerable fungus growth and lactic acid excretion were observed at all tested temperatures. Lactic acid production was increased when the temperature was increased from 25 °C to 30 °C. The highest lactic acid accumulation (11.08g/l) and yield (0.84g/gstarch) were obtained at 30 °C. However, when the temperature was increased beyond 30 °C, both growth and lactic acid formation gradually reduced. It can be also noted that, the consumption of starch was corresponded to the growth and lactic acid synthesis. Similar result was reported by **Huang *et al.*, (2003)**, who found that the optimum temperature for lactic acid production by *Rhizopus arrhizus* DAR 36017 was 30 °C.

Table (4): Effect of cultivation temperature on growth and lactic acid production.

Incubation temperature °C	Consumed starch (g/l)	Accumulated reducing sugar (g/l)	Biomass dry weight (g/l)	Lactic acid (g/l)	Lactic acid yield (g/g starch)
25	11.81	0.6	1.49	9.68	0.82
30	13.2	0.78	1.38	11.08	0.84
35	12.61	1.30	1.21	10.06	0.80
40	11.15	2.10	1.10	7.11	0.63

Cultivation conditions: substrate concentration20 g/l, pH 6 $CaCO_3$ 1% w/v and cultivation period was 42hr.

2.4. Effect of starch concentration on fungus growth and lactic acid production:

The effect of various starch concentrations (ranging from 10 to 20g/l) on lactic acid production were tested. The starch concentrations were prepared by diluting the homogenized waste with calculated volumes of distilled water. The results in Table (5) show that both fungal biomass

formation and lactic acid accumulation increased as the starch concentration increased up to 14 and 18 g/l respectively, at which the highest biomass yield (1.90g/l) and lactic acid yield (0.86g/g) were achieved. Any further increase in starch level beyond that mentioned above resulted in a decrease in fungal growth, lactic acid formation and accompanied by reduction in starch consumption. This may be due to the increase in viscosity of the fermentation broth with increasing initial starch concentration (Jin *et al.*, 2005).

2.5. Effect of enrichment of the waste different with nitrogen source:

The effect of different nitrogen sources on lactic acid production was investigated using organic and inorganic nitrogen sources. The nitrogen content of the medium was kept at level of 0.4 g/l. The results presented in Table (6) reveal that *Rhizopus arrhizus* could access all tested organic and inorganic nitrogen sources with different levels. The maximum lactic acid yield (0.88 g/g starch) was achieved with ammonium sulfate as nitrogen source while other inorganic nitrogen salts stimulated lower yield of lactic acid. This may be due to that, nitrogen form of ammonium salts can be accessed more easily for the fungal microorganisms than other forms of inorganic nitrogen such as nitrate and nitrite salts, which must first be reduced to ammonium before the nitrogen can be converted to an organic form (Prescott *et al.*, 1996).On the other hand, both wheat bran and rice bran improved lactic acid excretion with similar efficiency. Wheat bran and rice bran come in the second order after ammonium sulfate for enhancing lactic acid production. These results are in agreement with those of Jin *et al.* (2005).

Table (5): Effect of starch concentration on fungus growth and lactic acid production.

Substrate concentration (g/l)	Consumed starch (g/l)	Accumulated reducing sugar (g/l)	Biomass dry weight (g/l)	Lactic acid (g/l)	Lactic acid yield (g/g starch)
10	9.10	0.10	1.50	7.55	0.83
12	11.8	0.11	1.80	9.91	0.84
14	13.1	0.10	1.90	11.00	0.84
16	14.1	0.20	1.80	11.98	0.85
18	14.5	0.20	1.80	12.47	0.86
20	13.5	0.38	1.50	11.47	0.85

Cultivation conditions: $CaCO_3$ 1% w/v, pH 6, temperature 30 °C and cultivation period was 42hr.

Table (6): Effect of different nitrogen sources on fungus growth and lactic acid production.

Nitrogen source	Consumed starch (g/l)	Accumulated reducing sugar (g/l)	Biomass dry weight (g/l)	Lactic acid (g/l)	Lactic acid yield (g/g)
Ammonium sulfate	17.3	2.28	2.8	15.22	0.88
Urea	15.13	2.0	2.0	11.49	0.76
Ammonium phosphate	16.8	2.0	2.4	13.94	0.83
Sodium nitrate	15.9	2.1	2.3	12.40	0.78
Wheat bran extract	16.9	1.89	2.5	14.53	0.86
Rice bran extract	17.0	2.1	2.8	14.62	0.86

Cultivation conditions: starch concentration 18g/l, $CaCO_3$ 1% w/v, pH= 6, temperature 30 °C and cultivation period was 42hr.

2.6. Effect of incubation period on growth and lactic acid production:

The lactic acid production during the time course of fermentation was investigated using the favorable conditions from the previous experiments. The results presented in Table (7) show that high starch consumption rate was occurred during the first 24hr of cultivation followed by lower hydrolysis rate up to 42hr of the fermentation then it was retarded. On the other hand, maximum accumulation of reducing sugar was detected at 18hr of inoculation then gradually decreased as the cultivation time increased. This may be attributed to that the saccharification rate was greater than the fermentation rate at the early stage of the fermentation, resulting in an accumulation of reducing sugar (Huang *et al.*, 2005). Concerning biomass synthesis, it can be noted that the first 30h of the formation are a kind of growth phase within which maximum biomass was produced after 30h of the cultivation, the growth starts to decline probably due to the lysis of the fungus cells. With respect to lactic acid, it was observed that at the early stage of the fermentation (the first 12 hr) production of appreciable amount of lactic acid was delayed, followed by sharp increase of lactic acid accumulation and attained its maximum (17.10g/l) and yield (0.95g/g) at 54hr of the fermentation. Extending the cultivation period above 54hr lead to drop in lactic acid yield. From the above mentioned results it can be concluded that, the lactic acid fermentation by *Rhizopus arrhizus* NRRL1526 using potato chips waste medium was characterized by two phases, in the first phase of fermentation, biomass formation was dominated, accompanied by minimal lactic acid excretion, whereas in the second phase, lactic acid accumulation reached a maximum but biomass was low. These fermentation characteristics are in accordance with the findings of Martak *et al.*, (2003), Jin *et al.*, (2003), Jin *et al.*, (2005) and Huang *et al.*, (2005).

The quantity and accumulation rate of glucose, termed as reducing sugars in this study, may take an important role in the direct fermentation process. A high reducing sugar accumulation rate may result in high lactic acid production.

Table (7): Effect of incubation period on growth and lactic acid production.

Incubation period (hr)	Consumed starch (g/l)	Accumulated reducing sugar (g/l)	Biomass dry weight (g/l)	Lactic acid (g/l)	Lactic acid yield (g/g)
6	4.80	2.48	0.5	1.82	0.38
12	8.31	4.02	1.8	2.49	0.34
18	13.82	5.02	1.9	6.91	0.50
24	15.2	3.61	2.2	9.27	0.61
30	16.8	1.82	2.9	12.09	0.72
36	17.1	0.80	2.6	13.50	0.79
42	17.8	0.10	2.35	15.30	0.86
48	18.0	0.00	1.99	16.56	0.92
54	18.0	0.00	1.65	17.10	0.95
60	18.0	0.00	1.50	16.20	0.90

Cultivation conditions: starch concentration 18g/l, ammonium sulfate as a nitrogen source, $CaCO_3$ 1% w/v, pH 6and temperature 30 °C

On the other hand, a very high concentration of glucose may also somehow inhibit the lactic acid formation. Associated with the glucose an inhibition from the lactic acid, it self may be an important factor which affected the microbial accessibility and fungal cell growth, as well as the lactic acid formation (Huang *et al.*, 2005).

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الاستفادة من مخلفات صناعة البطاطس الشيبس في إنتاج منتجات ذات قيمة إقتصادية عالية:

III -إنتاج حامض اللاكتيك

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هدفت هذه الدراسة إلي بحث إمكانية الاستفادة من مخلفات صناعة رقائق البطاطس المحمرة في إنتاج حامض اللاكتيك باستخدام فطر *Rhizopus arrhizus* NRRL1526 لقدرته علي إفراز الإنزيمات التي تحدث تسكر للنشا بالمخلف ثم تخمير السكريات الناتجة إلي حامض لاكتيك .وذلك بغرض خفض تكاليف إنتاج حامض اللاكتيك باستخدام مواد خام رخيصة وفي نفس الوقت تجنب الأضرار التي قد تنجم عن طرح هذه المخلفات في البيئة .أوضحت الدراسة أن الفطر قادر علي الاستفادة بالمخلف في إنتاج حامض اللاكتيك وكان أقصي تركيز لحامض اللاكتيك هو ١٧,١ جم / لتر و أقصي إنتاج هو 0.95جم/جم نشا تم استهلاكه وذلك عند تنمية الفطر تحت الظروف المثلي والتي تم دراستها وهي درجة حموضة البيئة pH= 6ودرجة حرارة النمو 30درجة مئوية وتركيز مادة تفاعل 18جم/لتر و استخدام سلفات الأمونيوم كمصدر للنيتروجين عندما ينموالفطر لمدة 54ساعة .ويستخدم حامض اللاكتيك المنتج في مجال تصنيع اللحوم والدواجن والسلطات وكذلك الحلويات.