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# Lactic Acid Production and its Effect on Paper

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**Abstract:** The potential use of coarse pulp fiber as mango waste for production of lactic acid was investigated. Fermentation of this waste was carried out by Lactobacillius *bulgaricus* as a batch culture. The highest value of lactic acid, yield of lactic acid and conversion coefficient were obtained after 6 days incubation period, 10% sugar concentration, pH 6 and 40°C. Effect of freshly produced lactic acid on physical strength and water absorbance of paper was also studied.

**Key words:** pulp,lactic acid,fermentation,water absorptivity,yield.

## INTRODUCTION

Processing of fruits produced from two types of wastes-a solid waste of peel/skin, seeds, stones, etc…,a liquid waste of juice and washwaters. In some fruits the discarded portion can be very high (e.g mango 30-50%,banana 20%.pine apple 40-50% and orange 30-50%). Therefore, there often a serious waste disposal problem, which can lead to problems with flies and rats around the processing room, if not correctly dealt with.If there are no planes to use the waste it should be buried or fed to animals well away from the processing site.

Fermented waste food products offer tremendous opportunity for stimulating agro-industrial development in developing countries. Mango peel is a waste product of mango processing and is available in large quantities during the harvest season. Its disposal can cause pollution problems. Ensilage of mango peel for 6 months, the effects of ensilage on physicochemical characteristics and methane generation by an anaerobic digestion process were studied [1].

Fermentation of mango juice using yeast strains obtained from palm wine was investigated. Kinetic analysis of the resulting data is reported The fermentation rate was found to be between first and second order dependency on sugar concentration, but was relatively weakly on H+ concentration in the medium. [2].Potential use of mango peels, pulp and stones for the production of vinegar was investigated.[3,4].

It is found that the quantity of lactic acid present in the fermented mango slices could be conveniently measured, in commercial practice, by extraction with dry sulphuric ether. Though the recovery of lactic acid was low (only 74-76%),the correct value can be obtained by multiplying the measured value with a correction factor.[5]

The technical feasibility of using agricultural wastes(mango and date industry wastes) as a substratum for the cultivation of pleurotus NRRL-0366 % was evaluated.[6]

Mango wastes(mango and mango stone) were used in poultry feed. Also mango stone was used as a non-conventional source of protein. The stones of four local mango varieties were analyzed. Carbohydrates were the main components and protein concentration varied between 5.0 and 7.2%, ether extract between 10.8 and 13.6.Mango kernel proteins had high gluotamic acid, aspartic acid and leucin contents and a low concentration of sulphur amino acids and lysine [7,8].

Green mangoes were fermented in brine with 10% and 12% by weight salt, with and without acetic acid 0.1% and/or sodium benzoate 0.1% and stored for one month. Results showed that only little growth of lactic acid bacteria occurred and that the level of lactic acid produced was 0.4% to 0.7%. Most of the acid present was produced by yeast fermentation. When pickling mangoes in brine with 8% and 10% salt, with 0, 3% or 5% by weight sucrose, The growth of lactic acid bacteria was similar to that in pickled mangoes without added sucrose. For all the treatments,the final acidity was approximately 5% as lactic acid with pH of 2.9 [9].

A number of natural materials can be made into polymers that are biodegradable e.g., lactic acid.It is now commercially produced on large scales through the fermentation of sugar feedstocks obtained from sugar beets or sugar cane, or from the conversation of starch from corn, potato peels,or other starch source. It can be polymerized to produce poly (lactic acid), which is already finding commercial applications in drug encapsulation and biodegradable medical devices. It has also been used in coatings for paper and cardboard and fibers – for clothing.

Synthetic lactic acid made from petrochemical feedstocks is optically inactive while lactic acid made biochemically by fermentation is optically active and

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suitable organisms can selectively produce laevo – or dextro-rotatory enantiomers. Lactic acid is stable to heating and has smooth mild acid taste that does not mask or overpower weaker flavors. Lactic acid is the most effective acid for inhibiting Lactic acid bacteria-the spoilage microorganisms commonly found in acid products such as dressings and beverages Lactic acid play an important role in food preservation and shelf life. It helps prevent spoilage in Spanish olives.[10]So,the effect of lactic acid produced from fermented mango wastes on paper used in packaging of food was studied.

## MATERIALS AND METHODS

**Microorganism used:** The culture of *Lactobacillus bulgaricus* (20080) was obtained from Cairo Mercin, Faculty of Agriculture. Ain Shams University. This culture was stored at 4°C and serially transferred at weekly intervals. *Lactobacillus bulgaricus* (20080) was maintained on the medium which had the following constituents (g/1) [11].

Peptone 10.0, yeast extract 5, beef extract 10, glucose 20, tween 80 1ml., $KH_2PO_4$ 2, sodium acetate 5, $MnSO_4.H_2O$ 0.05. $MgSO_47H_2O$ 0.02 and distilled water 1000 ml. The inoculated slants were incubated at 40°C for 7 days to obtain luxuriant growth. The growing slants were kept in a refrigerator at 4°C

**Material:** Mango wastes (coarse pulp fiber) used as raw material in lactic acid production. This material was air dried, crushed and ground in a grinding machine. For hydrolysis to 200 g of the ground material 1000 ml. dilute $H_2SO_4$ were added. $H_2SO_4$ concentration 1.0% varied according to Abd El-Hady[12].

The mixture was autoclaved at 121°C for one hour. After hydrolysis the mixture was filtered, washed with hot water and diluted to suitable volume. This hydrolyzate was adjusted to pH 6.0 and heated to boiling cooled and filtered through filter paper. The filtered hydrolyzate was used at 4°C.

Coarse pulp fiber substrates hydrolyzate medium was used for the fermentation of lactic acid production. The following substrates were added to one liter of coarse pulp fiber substrates hydrolyzate containing 10% sugar: $CH_3CooNa$ (5.0g), Yeast extract (6.0 g), Meat extract (10.0g), $MnSO_4$ (0.09 g) $KH_2PO_4$ (2.0g) and the pH was adjusted to 6.0.

Prepared media were adjusted to pH 6.0 and 10%sugar concentration. They were put into fermentor (4.0 L. capacities) and incubated with a standard inoculum of *Lactobacillus bulgaricus* (20080) (240 ml/4.0 L) under sterile conditions at 40°C for 6 days. At the end of the incubation period, the pH values, consumed sugar and lactic acid, of the fermented cultures were determined. Yield of lactic acid and the conversion coefficient were calculated as following:

**Table 1:** Chemical composition of mango waste (coarse pulp fiber)

| Wastes | Crude protein | Crude fat | Crude fiber | Total ash | Total carbohydrate |
|---|---|---|---|---|---|
| Coarse pulp fiber | 1.39 | 2.96 | 14.33 | 2.63 | 70.70 |

Moisture =8.07 %

$$\text{* yield} = \frac{\text{Lactic acid produced}}{\text{Sugar concentrate}} \times 100$$

$$\text{** Conversion coefficient} = \frac{\text{Lactic acid produced}}{\text{Consumed sugar}} \times 100$$

The chemical composition of mango wastes is presented in Table (1). Lactic acid was determined according to Lunder[13].

The actual acid content was calculated as grams of lactic acid per 100 ml of coarse pulp fiber hydrolyzate medium.

The best incubation period was studied by measuring all the tests after each 24 hour of incubation period.

**Surface treatment of paper by lactic acid:** Two samples of hand made paper sheets composed of wood pulp with 10% rosin size and wood pulp with 10% Kaoline and Rosin size were treated with lactic acid. Paper sheets were prepared according to TAPPI Standard Method using the sheet former of AB Lorentzen and Wetter (Stockholm, Sweden). Mechanical properties and water absorptivity of paper sheets were studied.

## RESULTS AND DISCUSSIONS

Sugar of coarse pulp fiber was diluted to 6,8,10,12 and 14%. Data in Table (2) represent the effect of sugar concentration on the production of lactic acid by *Lb. bulg*.

Consumed sugar increases with increasing sugar concentration from 6 to 14%, but the amount of lactic acid produced in grams and conversion coefficient reach maximum value at sugar concentration of 10%. Data presented in Table (3) showed the effect of incubation period of coarse pulp hydrolyzate medium on the production of lactic acid by *Lb. bulga*. This experiment was carried out to detect the proper time for maximum lactic acid production. It is clear from Table 3 that consumed sugar % increased with increasing incubation period until after 144 hours. The high yield lactic acid was obtained at 144 hours incubation period. To optimize the temperature for lactic acid production coarse pulp was diluted to 10% sugar concentration, pH 6 for 144 hrs; the media were inoculated with *Lb. bulgaricus*. then incubated in fermentor at 30, 35 40, 45, 50 and 55°C for 144 hrs. Effect of different temperatures (30 to 55°C) on lactic acid production by *Lb. bulgaricus was studied* Table (4). It was important to study the effect of initial pH of coarse pulp hydrolyzate on the production

**Table 2:** Effect of sugar concentrate on lactic acid production by Lactobacillius. bulgaricus.

| Sugar concentration % | Lactic acid g/100g | Consumed sugar g/100g | Yield % | Conversion coefficient % ** |
|---|---|---|---|---|
| 6 | 1.67 | 4.21 | 28 | 40 |
| 8 | 2.86 | 6.7 | 36 | 43 |
| 10 | 4.79 | 8.43 | 48 | 57 |
| 12 | 4.76 | 9.27 | 40 | 51 |
| 14 | 4.65 | 11.39 | 33 | 41 |

**Table 3:** Effect of incubation period on Lactic acid production by *Lactobacillius bulgaricus* from coarse pulp fiber.

| Incubation period (hrs.) | Consumed sugar g/100g | Lactic acid g/100g | Yield % | Conversion coefficient |
|---|---|---|---|---|
| 24 | 2.97 | 0.83 | 8 | 28 |
| 48 | 3.69 | 1.07 | 11 | 29 |
| 72 | 5.09 | 1.66 | 17 | 33 |
| 96 | 5.52 | 2.49 | 25 | 45 |
| 120 | 6.96 | 3.29 | 33 | 47 |
| 144 | 7.99 | 4.69 | 47 | 59 |
| 168 | 7.87 | 4.26 | 43 | 54 |
| 192 | 7.91 | 4.23 | 42 | 53 |

**Table 4:** Effect of temperature on Lactic acid production by Lactobacillus bulgaricus from coarse pulp fiber.

| Temperature °C | Consumed sugar g/100g | Lactic acid g/100g | Yield % | Conversion coefficient % |
|---|---|---|---|---|
| 30 | 2.97 | 1.06 | 11 | 36 |
| 35 | 4.09 | 2.17 | 22 | 53 |
| 40 | 6.62 | 3.74 | 37 | 56 |
| 45 | 8.02 | 4.83 | 48 | 60 |
| 50 | 8.61 | 4.78 | 48 | 55 |
| 55 | 8.93 | 4.67 | 47 | 52 |

**Table 5:** Effect of pH values on Lactic acid production by Lactobacillus bulgaricus from coarse pulp fiber.

| pH value | Consumed sugar g/100g | Lactic acid g/100g | Yield % | Conversion coefficient % |
|---|---|---|---|---|
| 4 | 3.89 | 1.69 | 17 | 43 |
| 5 | 5.97 | 3.12 | 31 | 52 |
| 6 | 8.11 | 4.81 | 48 | 59 |
| 7 | 7.96 | 4.39 | 44 | 55 |
| 8 | 7.88 | 4.16 | 42 | 53 |

**Table 6:** Effect of $CH_3$ CooNa on Lactic acid production by Lactobacillus bulgaricus from coarse pulp fiber.

| $CH_3CooNa$ g/100g | Consumed sugar g/100g | Lactic acid g/100g | Yield % | Conversion coefficient % |
|---|---|---|---|---|
| 0.35 | 4.53 | 2.28 | 23 | 50 |
| 0.40 | 5.69 | 3.51 | 35 | 62 |
| 0.45 | 6.05 | 4.36 | 44 | 72 |
| 0.50 | 7.98 | 5.97 | 60 | 75 |
| 0.55 | 8.01 | 5.26 | 53 | 66 |
| 0.60 | 7.96 | 4.87 | 49 | 61 |
| 0.65 | 7.85 | 4.51 | 45 | 57 |

**Table 7:** Effect of yeast extract on Lactic acid production by Lactobacillus bulgaricus from coarse pulp fiber.

| Yeast extract g/100g | Consumed sugar g/100g | Lactic acid g/100g | Yield % | Conversion coefficient % |
|---|---|---|---|---|
| 0.3 | 5.48 | 3.18 | 32 | 58 |
| 0.4 | 5.92 | 4.23 | 42 | 71 |
| 0.5 | 6.77 | 5.02 | 50 | 74 |
| 0.6 | 8.37 | 6.79 | 68 | 81 |
| 0.7 | 8.21 | 6.23 | 62 | 76 |
| 0.8 | 8.26 | 6.11 | 61 | 74 |

**Table 8:** Effect of Meat extract on Lactic acid production by Lactobacillus bulgaricus from coarse pulp fiber.

| Meat extract g/100g | Consumed sugar g/100g | Lactic acid g/100g | Yield % | Conversion coefficient % |
|---|---|---|---|---|
| 0.7 | 5.89 | 4.05 | 40 | 69 |
| 0.8 | 6.81 | 5.48 | 55 | 80 |
| 0.9 | 6.99 | 5.76 | 58 | 82 |
| 1.0 | 8.22 | 6.66 | 67 | 81 |
| 1.1 | 8.07 | 5.78 | 58 | 72 |
| 1.2 | 8.06 | 5.39 | 54 | 67 |

**Table 9:** Effect of $MnSO_4$ on Lactic acid production by Lactobacillus bulgaricus from coarse pulp fiber.

| $MnSo_4$ g/100g | Consumed sugar g/100g | Lactic acid g/100g | Yield % | Conversion coefficient % |
|---|---|---|---|---|
| 0.003 | 5.22 | 3.43 | 34 | 66 |
| 0.006 | 5.79 | 4.21 | 42 | 73 |
| 0.009 | 7.62 | 6.09 | 61 | 80 |
| 0.012 | 7.66 | 6.08 | 61 | 79 |
| 0.015 | 7.59 | 5.93 | 59 | 78 |
| 0.018 | 7.56 | 5.48 | 55 | 72 |

of lactic acid by *Lb. bulgaricus.* Data in Table (5) show the effect of pH value of medium on lactic acid production by *Lb. bulga*. Using acidic medium gives the best production of lactic acid 4.81 g/100g and the conversion efficient 59%.

Results obtained in Table (6) indicated that the level of sodium acetate has a profound influence of growth of *Lb. bulgaricus*. Consumed sugar, lactic acid, yield of lactic acid and conversion coefficient increased with the increase in $CH_3$ CooNa in the medium up to 0.5 g/100g. Further increase resulted in progressive decreases in all parameters of lactoban.

Increasing yeast extract concentration from 0.3% to 0.8% at pH 6 has considerable effect on lactic acid production at 45°C. The yeast extract concentration were in the order 0.3% > 0.4% > 0.5% 0.6%. After yeast extract concentration 0.6% the consumed sugar and yield of lactic acid decreased. The maximum yield was at 0.6% yeast extract concentration The experiment was done to study the effect of different concentration of yeast extract on the grown of lactoban. Results recorded in Table (7) show that consumed sugar increased with increased yeast extract concentration. The suitable of yeast extract seemed to be 0.6 g/100g. This level gave the highest lactic acid production. The same trend of yeast extract concentration was observed on using meat extract at pH value 6 and 45°C Increasing the meat extract concentration from 0.7% to 1.2%, consumed sugar and yield of lactic acid increase till 1.00% then decreased Table (8). At sugar concentration 10%, pH value 6, temperature 45°C and incubation period of 144 hours. The effect of $MnSO_4$ concentration on lactic acid production was also studied. Increasing the concentration of $MnSO_4$ from 0.003% to 0.018%, the consumed sugar increased till 0.009% concentration. After this concentration it becomes constant. The best yield of lactic acid production and conversion coefficient was at 0.009 g/100g concentration Table (9).

This study was set up to investigate the most favorable concentration of $KH_2$ $PO_4$ for lactic acid production from coarse pulp fiber hydrolyzate medium by *Lactobacillius bulgaricus*.

Results obtained in Table (10) indicated that the production of lactic acid increased with the increase of $KH_2PO_4$ concentration reaching its optimum at 0.2 g/100 g level. Further increase in $KH_2PO_4$ concentration decreased the yield of lactic acid.

**Table 10:** Effect of $KH_2PO_4$ on Lactic acid production by Lactobacillus bulgaricus from coarse pulp fiber.

| $KH_2PO_4$ g/100g | Consumed sugar g/100g | Lactic acid g/100g | Yield % | Conversion coefficient % |
|---|---|---|---|---|
| 0.10 | 5.25 | 3.77 | 38 | 72 |
| 0.15 | 5.67 | 4.29 | 43 | 76 |
| 0.20 | 7.75 | 6.31 | 63 | 81 |
| 0.25 | 7.78 | 6.29 | 63 | 81 |
| 0.30 | 7.69 | 5.88 | 59 | 76 |
| 0.35 | 7.56 | 5.49 | 55 | 73 |

**Table 11:** Optimizing condition for Lactic acid production by Lactobacillus bulgaricus

| Incubation period (hrs.) | Consumed sugar g/100g | Lactic acid g/100g | Yield % | Conversion coefficient % |
|---|---|---|---|---|
| 24 | 3.36 | 2.19 | 22 | 65 |
| 48 | 4.49 | 3.41 | 34 | 76 |
| 72 | 5.60 | 4.26 | 43 | 76 |
| 96 | 6.31 | 4.88 | 49 | 77 |
| 120 | 7.29 | 5.77 | 58 | 79 |
| 144 | 8.93 | 7.53 | 75 | 84 |
| 168 | 8.66 | 6.79 | 68 | 78 |
| 192 | 7.87 | 6.11 | 61 | 77 |

**Table 12:** Effect of lactic acid treatment on mechanical properties of wood pulp paper.

| Mechanical properties | Wood pulp + Rosin Size1 | | Wood pulp + Kaolin + Rosin2 | |
|---|---|---|---|---|
| | untreated | treated | untreated | treated |
| T.s,kg | 2.5 | 1 | 15 | 2.1 |
| Tear,g | 56 | 1.1 | 48 | 3.5 |
| Burst,kg | 12 | 0.32 | 8 | 0.32 |

**Table 13:** Water absorptivity of wood pulp paper treated with lactic acid.

| Time | Wood pulp + Rosin Size1 | Wood pulp + Kaolin + Rosin2 |
|---|---|---|
| 5 minute | 116.9 | 63.5 |
| 10 minute | 121.8 | 67.1 |
| 25 minute | 136.6 | 72.9 |
| 35 minute | 155.55 | 80.2 |
| 2 hours | 206.89 | 85.6 |
| 24 hours | 107.1 | 45.3 |

From the optimization fermentation conditions for lactic acid production from coarse pulp fiber hydrolyzate medium (containing 10% sugar 0.5% $CH_3CooNa$ 0.6% yeast extract, 1.0% Meat extract, 0.009% $MnSO_4$ and 0.2% $K_2HPO_4$) by *Lactobacillius bulgaricus* as a batch culture in the fermentor which are presented in Table (11). It can be generally concluded from the above results that the highest values of lactic acid, yield of lactic acid and conversion coefficient were obtained by lactobaccillius bulgaricus grown on coarse pulp fiber hydrolyzate after 6 days incubation periods, 10% sugar concentration, pH 6 and 40°C. They were 7.53g/100g, 75% and 84%, respectively.

**Effect of lactic acid on properties of paper:** Tensile strength is a measure of the ability of paper to resist breaking under tension, which is dependent on the strength of fibers, their surface area and length and the bonding strength between them. Table (12) indicated that TS decreased as a result of treatment by lactic acid. The decrease in TS of treated paper is mainly due to swelling of cellulose fiber by lactic acid penetration and may be partially due to the fact that lactic acid is impregenated into the cellulose structure of paper and interfere with fiber – to – fiber interaction.

Water resistance of treated paper was measured through impregenating paper in water. Again it is shown from Table (13) that the water absorptivity increased as water contact time increased.. This is may be due to lactic acid itself rather than paper absorbed water and swelled considerably. After 24 hours, water absorptivity decreased.This decrease in water uptake may be due to the leaching of lactic acid from the paper surface. This may be help for the storage and distribution of high moisture foods such as fresh agriculture produce.[14]

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