CONVERSION OF MALIC ACID INTO LACTIC ACID IN ALOEVERA BY USING LACTIC ACID BACTERIA

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SUMMARY

Malic acid is an excellent indicator of gel freshness. This acid is produced naturally in the leaves of aloes and other succulents whose cells contain large, water-filled vacuoles. Under poor handling conditions in the presence of bacteria, malic acid can be broken down to form lactic acid. Gram-negative rod bacteria, which grow in some aloe gels, assimilate malic acid and free glucose and produce other organic acids such as lactate or lactic acid. Lactic acid is one of the major products of carbohydrate breakdown by lactic acid bacteria during malolactic fermentation (MLF). Malolactic enzyme demonstrated to be present in most lactic acid bacteria but not in other bacteria, catalyzes the reaction:

\[ 1 \text{ malate} + \text{H}^+ \rightarrow \text{1lactate} + \text{CO}_2. \]


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1. Introduction

\textit{Aloe vera}, also known as the Medicinal Aloe, is a species of succulent plant that probably originated in northern Africa. The species does not have any naturally occurring populations, although closely related Aloes do occur in northern Africa. \textit{Aloe vera} grows in tropical climates and is widely distributed in Africa, Asia and other tropical areas. The species is frequently cited as being used in herbal medicine. Extracts from \textit{A. vera} are widely used in cosmetics and alternative medicine, being marketed as having rejuvenating, healing, and soothing properties. There have been many scientific studies of the use aloe Vera, some of it conflicting. Despite these limitations, there is some preliminary evidence that \textit{A. Vera} extracts may be useful in the treatment of diabetes and elevated blood lipids in humans. These positive effects are thought to be due to the presence of compounds such as polysaccharides, mannans, anthraquinones and lectins. \textit{A. Vera} is a stemless or very short-stemmed succulent plant growing to 60–100 cm (24–39 in) tall, spreading by offsets.

Fig 1. \textit{Aloe vera}

Scientific classification in \textit{Aloe vera}:

\begin{itemize}
  \item \textbf{Kingdom:} Plantae
  \item \textbf{Family:} Liliaceae
  \item \textbf{Genus:} Aloe
  \item \textbf{Species:} Barbadensis
\end{itemize}

The stems, thick and fleshy, green to grey-green, with some varieties showing white flecks on the upper and lower stem surfaces. The margin of the stem is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90 cm
(35 in) tall, each flower pendulous, with a yellow tubular corolla 2–3 cm (0.8–1.2 in) long. Like other Aloe species, A. Vera forms arbuscular mycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil.

Chemical properties

Amino acids:
- Provides 7 of 8 essential amino acids they are
- Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Valine
- Amino acids like Aspartic acid, Glutamic acid, Alanine, Cystine, Glycine, Histidine, Hydroxyproline, Proline, Serine and Tyrosine.

Enzymes
- Provides 8 enzymes:
  - Aliase, Alkaline phosphatase, Amylase, Carboxypeptidases, Catalase, Cellulase, Lipase, Peroxidase.

Minerals
- Calcium, Phosphorus, Potassium, Iron, Sodium, Chlorine, Manganese, Magnesium, Copper, Chromium, Zinc.

Monosaccharide and polysaccharides:
- D-Arabinose, D-Glucose, D-Galactose, D-Mannose, L-Rhamnose, D-Xylose, Cellulase, Aldopentose, Acemannan.

Vitamins:
- Vitamin A (Retinol), B1 (Thiamine), B2 (Riboflavin), Niacinamide, Vitamin B6 (Biotin), Vitamin B12 (Cyanocobalamin), Vitamin C (Ascorbic acid), Vitamin E (Tocopherol).

Aloe emodin
- Aloe emodin is an anthraquinone present in aloe latex, an exudate from the aloe plant. It has a strong stimulant-laxative action.

Free sugars
- Carbohydrates are a group of organic compounds containing carbon, hydrogen and oxygen in the general proportions of 1:2:1.
- Monosaccharide can be defined as the simplest form of these carbohydrates. The analysis of mixtures of carbohydrates is important in various industries, especially those concerned with pharmacology and the preparation and stabilization of food and drink.

Examples of common monosaccharide are:
Aloin
Aloin, also known as Barbaloin [Reynolds, Aloes - The genus Aloe, 2004], is a bitter, yellow-brown colored compound noted in the exudates of at least 68 Aloe species at levels from 0.1 to 6.6% of leaf dry weight (making between 3% and 35% of the total exudates) [Groom & Reynolds, 1987), and in another 17 species at indeterminate levels [Reynolds, 1995b]. It is used as a stimulant-laxative, treating constipation by inducing bowel movements.

Aloe resin
It contains C-glycosides and resins, anthroquinone glycosides (including aloe-emodin and aloin A and aloin B).

Fatty acids helpful in tissue maturation
Linoleic, linolenic, myristic, caprylic, oleic, palmitic, stearic

Antiseptic
Cinnamic acid, lupeol (a natural salicylic acid), phenol, sulfur, urea nitrogen

Analgesic
Lupeol, magnesium (as lactate)

Anti-inflammatory
Brady kinase, B-sitosterol, campesterol, beneficial HDL cholesterol

Natural defense system enhancers
A newly discovered component of the Aloe Vera plant, called acemannan, shows preliminary evidence of strengthening the body's natural defences. Alkaline phosphatase, creatine phosphatase, creatinine, glucose, lactate, sodium, triglycerides
Malic acid:
Malic acid is an excellent indicator of gel freshness. This acid is produced naturally in the leaves of aloes and other succulents whose cells contain large, water filled vacuoles such plants have the crassulacean acid metabolism (CAM); an additional photosynthetic pathway where malic acid is produced naturally.

2. Materials and Methods
Washing glassware
Glassware were first soaked in chromic acid cleaning solution (10%Potassium dichromate solution 25%sulphuric acid) for few hours and washed. Thoroughly in tap-water after second washing in detergent solution they were again washed thoroughly in tap-water and rinsed in distilled water.
Glass were generally used is conical flasks, burette, pipette, test tubes, measuring cylinder.

**Sterilization**

Sterilization of culture media was carried out in an autoclave at 121°C for 15 minutes. The glassware was sterilized at 160°C in a hot air oven for 1 hr.

**Collection of Aloe vera leaf:**

In aseptic conditions Aloe Vera raw leaf was collected from the farm and from different places of Thanjavur.

**Harvesting of sample**

*Aloe vera* leaf was washed with distilled water and make extract in aseptic conditions. 1ml sample was collected and transferred it in to the FL broth (Fluid lactose broth) and kept incubation for 24 hrs at 32°C. FLB medium was prepared and Mix all the ingredients and autoclave for 15 minutes at 121°C. Checked its pH after sterilization the medium was Cool immediately. *Aloe vera* powder was collected in aseptic conditions. 5 gms of Aloe Vera powder was transferred in to the TSB broth (Tryptone Soya broth) and kept incubation for 24 hrs at 32°C. Leaves were harvested and filleted. Fresh aloe gel was used for the experimental work. Samples used for the individual parameter screening were left at room temperature for three days and then re-tested to detect possible changes associated with poor handling conditions.

**Isolation of lactic acid bacteria by pour plate method**

*Lactobacillus* was collected from Aloe Vera powder organism, Leaf surface organism.

Aloe Vera 1gm powder was taken and suspended with 50 ml TSB (Tryptone Soya broth) and from that 1ml was plated and added to lactobacillus MRS Agar medium and similarly to SDA medium. After adding to the medium the plates are kept for incubation for 24 hrs at 32°C.

**Identification of lactic acid bacteria by gram staining method**

*Lactobacillus* can be identified by using gram staining technique. Petri plates with the colonies of micro organism are taken. Gram stain requirements, Glass slides, loop, microscope, burner Gram stain reagents crystal violet, grams iodine, grams decolorized solution and, safranin was required for staining. Glass slide is cleaned with the distilled water. With the sterile loop thin smear was made on the slide and then heat fixed the slide. Few drops of crystal violet is added to the smear. After 30-60 seconds slide is washed with distilled water then grams iodine is added to the slide and washed with water, following gram’s decolorized solution and countered with safranin.

**Identification of malic and lactic acid value in aloe vera by titremetric method**

*Aloe vera* was collected in aseptic conditions and crushed at room temperature.50 ml of Aloe Vera extract was taken. This sample was titrated against 0.1N NaOH. By using phenolphthalein as indicator to find out the total acid concentration present in the solution.

Juice was kept for 3days at room temperature. And 50 ml of sample was taken titrated against 0.1N NaOH by using phenolphthalein as indicator.
Biochemical identification of lactic acid bacteria

Catalase test

Catalase test is used to identify the catalase producing micro-organisms. In this test H$_2$O$_2$ is added to the culture to test the presence of catalase enzyme.

Acidic Determination (pH)

The pH of aloe gel was determined using a calibrated pH meter (crison micro pH 2000). The samples were once again analyzed fresh and after being stored for three days at room temperature.

Quantification and identification of malic and lactic acid by hplc method

A Shimadzu LC-6AD HPLC system equipped with a Waters differential refract meter R401 was used. Fresh aloe gel was passed through a Cameo 0.22 mm nylon filter and 20 μl injected directly into the system. Reference samples of pure acids (4 mg/mL) were used as external standards. Peaks generated from the aloe gel were identified by comparison of their retention times and co-injections. Concentrations of organic acids were calculated using peak areas generated from the simultaneous analysis of organic acids and sugars. Samples were analyzed fresh (directly from the fridge) and once again three days after being kept at room temperature.

3. Result

Morphological characters of lactic acid bacteria in different media

Morphological characters of lactic acid bacteria on MRS Agar medium:

After incubation of 72 hrs at 32°C Lactobacilli appear as large, white pointed colonies on the surface of the Agar medium was observed.

Morphological characters of lactic acid bacteria on TSA medium:

Large, spreaded, irregular, white colored colonies are observed on tryptone Soya agar medium.

Morphological characters of lactic acid bacteria on Tomato Juice Agar medium:

Colonies are large, spreaded, irregular colonies are observed. Cultural characters was observed after 40-48 hrs at 35 ± 2°C.

Morphological characters of lactic acid bacteria on Fluid Lactose broth:

Growth in the broth medium is indicated by the presence of Turbidity compared to an un-inoculated control after 48 hrs incubation at 32°C.

Microscopic observation

In gram staining violet colored, rod shaped bacteria was observed, by this it is conformed as gram-positive bacillus.
Malic and lactic conversion in fresh aloe vera gel and three days older gel

By the effect of lactic acid bacteria malate converting to lactate. By this Lactic acid concentration was increased at 0.40% in three days old gel.

Table 1: Malic and lactic acid levels in fresh and three days old gel

<table>
<thead>
<tr>
<th>Aloe species</th>
<th>Malic acid (mg/ml)</th>
<th>Lactic acid (mg/ml)</th>
<th>Malic acid (mg/ml)</th>
<th>Lactic acid (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.barbadensis</td>
<td>0.71</td>
<td>0.96</td>
<td>0.151</td>
<td>1.546</td>
</tr>
</tbody>
</table>

pH

In fresh Aloe Vera gel and after 3 days old gel shows difference in old Aloe Vera gel. pH was decreased to 4.81, and in fresh gel pH was observed as 5.6.

Table 2: pH levels in aloe gels and changes associated with storage

<table>
<thead>
<tr>
<th>Aloe species</th>
<th>Acidity (pH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Extract</td>
<td>5.6</td>
</tr>
<tr>
<td>3 days at room temperature</td>
<td>4.81</td>
</tr>
<tr>
<td>A.barbadensis</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Biochemical identification

Lacto bacillus was confirmed by its catalase reaction in catalase test bacteria possess catalase that is catalase positive.

Leaf surface organisms

These are gram negative bacillus bacteria observed on the surface of the gel.

HPLC method for quantification and identification of malic and lactic acid

Powder organisms

These are the gram positive bacillus bacteria which are observed in Aloe Vera Powder and Identified as Lactobacillus.

Malic and Lactic acid

Table 3: Malic and Lactic acid

<table>
<thead>
<tr>
<th></th>
<th>Malic acid</th>
<th>Lactic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder organisms</td>
<td>15.82 mg/g</td>
<td>30.80 mg/g</td>
</tr>
<tr>
<td>Leaf surface organisms</td>
<td>9.50 mg/g</td>
<td>26.38 mg/g</td>
</tr>
</tbody>
</table>
Table 4 Malic and lactic acid levels in powder sample (89 + 90 a) at 24 and 48 hrs

<table>
<thead>
<tr>
<th>Medium / organism</th>
<th>Powder Sample (89+90A)</th>
<th>24 hrs</th>
<th>48 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM WATER BLANK</td>
<td>M.A. = 0.21</td>
<td>M.A. = 0.23</td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>L.A. = 0.29</td>
<td>L.A. = 0.30</td>
<td></td>
</tr>
<tr>
<td>DM WATER BLANK</td>
<td>M.A. = 0.13</td>
<td>M.A. = 0.21</td>
<td></td>
</tr>
<tr>
<td>TEST</td>
<td>L.A. = 0.18</td>
<td>L.A. = 0.29</td>
<td></td>
</tr>
<tr>
<td>LEAF SURFACE ORGANISM</td>
<td>M.A. = 0.20</td>
<td>M.A. = 0.21</td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>L.A. = 0.27</td>
<td>L.A. = 0.29</td>
<td></td>
</tr>
<tr>
<td>LEAF SURFACE ORGANISM</td>
<td>M.A. = 0.06</td>
<td>M.A. = 0.09</td>
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</tr>
<tr>
<td>TEST</td>
<td>L.A. = 0.09</td>
<td>L.A. = 0.12</td>
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<tr>
<td>POWDER ORGANISM</td>
<td>M.A. = 0.20</td>
<td>M.A. = 0.21</td>
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</tr>
<tr>
<td>CONTROL</td>
<td>L.A. = 0.27</td>
<td>L.A. = 0.29</td>
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<tr>
<td>POWDER ORGANISM TEST</td>
<td>M.A. = 0.17</td>
<td>M.A. = 0.06</td>
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</tr>
<tr>
<td></td>
<td>L.A. = 0.23</td>
<td>L.A. = 0.09</td>
<td></td>
</tr>
</tbody>
</table>

*M.A - Malic acid; L.A - Lactic acid

Table 5 Malic and lactic acid levels in powder sample (89 + 90 b) at 24 and 48 hrs

<table>
<thead>
<tr>
<th>Medium / organism</th>
<th>Powder Sample (89+90B)</th>
<th>24 hrs</th>
<th>48 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM WATER BLANK</td>
<td>M.A. = 0.32</td>
<td>M.A. = 0.32</td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>L.A. = 0.43</td>
<td>L.A. = 0.43</td>
<td></td>
</tr>
<tr>
<td>DM WATER BLANK</td>
<td>M.A. = 0.27</td>
<td>M.A. = 0.29</td>
<td></td>
</tr>
<tr>
<td>TEST</td>
<td>L.A. = 0.36</td>
<td>L.A. = 0.40</td>
<td></td>
</tr>
<tr>
<td>LEAF SURFACE ORGANISM</td>
<td>M.A. = 0.29</td>
<td>M.A. = 0.29</td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>L.A. = 0.40</td>
<td>L.A. = 0.40</td>
<td></td>
</tr>
<tr>
<td>LEAF SURFACE ORGANISM</td>
<td>M.A. = 0.16</td>
<td>M.A. = 0.23</td>
<td></td>
</tr>
<tr>
<td>TEST</td>
<td>L.A. = 0.21</td>
<td>L.A. = 0.30</td>
<td></td>
</tr>
<tr>
<td>POWDER ORGANISM</td>
<td>M.A. = 2.25</td>
<td>M.A. = 0.29</td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>L.A. = 4.34</td>
<td>L.A. = 4.40</td>
<td></td>
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<tr>
<td>POWDER ORGANISM TEST</td>
<td>M.A. = 2.47</td>
<td>M.A. = 2.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L.A. = 6.63</td>
<td>L.A. = 6.10</td>
<td></td>
</tr>
</tbody>
</table>

*M.A - Malic acid; L.A - Lactic acid
4. Discussion

There is a change in pH after the aloe gel has been stored at room temperature for three days. However, the pH values do not exhibit a general increase or decrease but rather erratic changes. Lactic acid formation is associated with ageing of aloe gel, resulting in a concentration decline of malic acid levels caused by bacterial infection. A possible explanation for this relationship is that glucose (free from) can also be converted into lactic acid which can result in an increase of free ions or conductivity with in decaying aloe gels. Increases in pH values are expected, as lactic acid is a weaker acid than malic acid. The degradation of malic acid during MLF to lactic acid results in an increase in pH. Some species studied showed the ‘expected’ increase in pH, but some showed a decrease in pH.
5. Conclusion
The lactic acid bacteria have efficiency to convert the malate to lactate in 72 hrs incubation time.
The result indicates that the amount of malate to lactate conversion was same as 1 malate to 1 lactate and producing CO₂. The amount of malic and lactic conversion was depending on the incubation time. Malic acid in Aloe Vera gel determined the freshness of the gel. Lactate was produced by Lactic acid bacteria in normal conditions.

References
Park J.H et al. 1995. Chemical constituents of Aloe caseains sagengyak hakhocchi.26, 244.
Johansen, H.N., Glitso, V. and Knudsen, K.E.B. 1996. Influence of extraction solvent and temperature on the quantitative determination of


