

## *Comparative of fermentation in red and yellow watermelon juice by lactic acid bacteria*

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### **Abstract**

Red and yellow watermelon were evaluated as a substrate for production of probiotic watermelon juice by three strains of lactic acid bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus plantarum*). Microbial population, pH, titrable acidity and total soluble solids (Brix) were carried out during fermentation. All bacteria were grown well in both fruit juices and produced lactic acid. The amount of pH reduced and titrable acidity increased during fermentation but *L.plantarum* could not grow as well as other strains in yellow watermelon juice. All bacteria were reached to maximum amount after 12 hours of fermentation. Brix was decreased slightly. Both fruit juices was proved to be a suitable media for production of a fermented probiotic drink.

Key words: Probiotic, Lactic acid bacteria, Fermented, Watermelon .

### **Introduction**

Study has shown that probiotic bacteria have beneficially affected on host by improving its intestinal microbial balance [11]. Probiotics are describe as live microorganisms which have health benefits on gastrointestinal

infection antimicrobial activity improvement in tolerance lactose, reduction in serum cholesterol, antimutagenic properties, anti-carcinogenic properties, anti-diarrheal properties, immune system stimulation, improvement in inflammatory bowel disease and suppression of Helicobacter

pylori infection by adding of selected strains to food products [1, 5, 10, 13].

Lactic acid bacteria have been used for variety of dairy products such as fermented milks and yogurt but two major important defects related to ferment dairy products are lactose intolerance and cholesterol content [17, 7]. In last two decades, consumers demand for non-dairy products has increased [16]. Fruit and vegetable juices have nutritional and biological potential for growing lactic acid bacteria. Probiotic juices are obtained by control fermentation with choose species of lactobacillus [9].

In this study red watermelon and yellow watermelon have been used as a substrate for producing fermented fruit juices with three strains of lactic acid bacteria (*Lactobacillus acidophilus*, *Lactobacillus casei* and *Lactobacillus plantarum*). Both fruits are good sources of vitamins and mineral element and are very low in saturated fat, cholesterol and sodium.

Watermelon is really a vegetable (*Citrullus lanatus*) and native to tropical Africa.

Red and yellow watermelons have carotenoids pigments, Lycopene and Lutein are their pigments respectively. Carotenoids are tetraterpenoid pigments, where they contribute to the red, orange and yellow color of many flowers and fruits. They have antioxidant properties and fundamental contribution to human health [14]. The aim of this study was to

comparative the suitability of red and yellow watermelon as a raw material for production of fermented probiotic watermelon juice by *L. acidophilus*, *L. casei* and *L. plantarum*.

## Materials and methods

### Raw material

Both watermelons (*Citrulluslanatus*) were purchased from a local store in Tehran. At first, the peels of fruits were washed and the juice of watermelon was prepared by slicing, followed by separation of the juices in a juice maker, then it was removed by filtration with a cotton cloth. Before using, the juices were pasteurized at 85°C for 10 minutes.

### Strains and culture

*L. acidophilus* DSMZ 20079, *L. plantarum* DSMZ 20147 and *L. casei* DSMZ 20011 (Probiotic lactic acid bacteria) were provided by the Deutsche Sammlung von Mikroorganismen and Zellkulturen GmbH, Germany). All bacterial cultures were stored frozen at -20 °C in MRS medium (Merck, Germany) containing 20% glycerol. The strains were reactivated on MRS when needed.

### Fermentation of probiotic watermelon juice

The inoculums was prepared by growing the culture at 30°C for 24 h in MRS broth. Fermentation experiments were conducted in 250 mL flask, each containing 100 mL of sterile red and yellow watermelon juices that were pasteurized for 10 min at 85°C. All sample were inoculated with a 12-h culture (>10<sup>5</sup> CFU/mL) and incubated at 37°C for 48 h. Samples were taken at 0, 3, 6, 9, 12, 15, 18, 24, 36 and 48 hour for determining viable cell count by the standard plate method with MRS agar medium after 48 h of incubation at 37°C and sample were taken at 0, 12 h for chemical analyses.

There were 6 types of juices:

- 1: Red watermelon juice was fermented by *L. acidophilus* (A).
- 2: Red watermelon juice was fermented by *L. plantarum* (P).
- 3: Red watermelon juice was fermented by *L. casei*(c)
- 4: Yellow watermelon juice was fermented by *L. acidophilus*(A).

5: Yellow watermelon juice was fermented by *L. plantarum*(P).

6: Yellow watermelon juice was fermented by *L. casei*(c)

## Chemical and microbial analysis

The pH of probiotic watermelon juice was measured with a pH meter (WTW, German). Total acidity, expressed as present citric acid, was determined by titrating with titrazol 0.1N NaOH to pH 8.2. The brix was measured with the laboratory refractometer (RX7000α-ATAGO). Microbial population was measured by standard plate count method.

## Statistical analysis

The experiments were carried out in triplicate. The results are expressed as mean ± SD (standard deviation). The two way analysis of variance (ANOVA) was used to analyze the experimental data SAS (V. 9.1) software (SAS, 1998). Mean analysis using Duncan's multiple range test was carried out if needed.

## Result and discussion

The increase in viable cell count corresponds to the decrease in pH and enhanced in acidity during fermentation. According to Fig. 1, all bacteria come to logarithmic phase at initials hours of fermentation. Microorganism passed lag phase in pre cultures. At first the growth of bacteria were slow but rate of growth after 3 hours of fermentation were fast. At 12 h of fermentation viable cell count reached to maximum amount. The microbial viability decreased after passing 14 h of fermentation in red and yellow watermelon juices. *L. plantarum* didn't growth as well as other strain in both culture exactly in yellow watermelon. After 48 h of fermentation viable cell counts of *L. acidophilus*, *L. casei* and *L. plantarum* were decreased to 8.16±0.27, 8.29±0.2, 6.98±0.24 Log CFU/mL in red and 8.2±0.028, 8.32±0.21, 6.91±0.16 Log CFU/mL in yellow watermelon respectively. Result indicated that lactic acid

bacteria growth well in watermelon juices without adding any nutrient material (Fig. 1)

Zhou *et al* (2009) reported the cell viability of *L. casei* increased until 14 h of fermentation and it reached  $8.56 \pm 0.04$  Log CFU/mL after 24 h in mare milk. Some others reported that the decrease in the pH of the culture and production of lactic acid, diacetyl, and acetaldehyde from growth and fermentation are the main factors for viability loss of probiotics added to milk [4].

All strain could decrease the pH of both fermented watermelon juices. It is indicated they able to produced acid expect of *L. plantarum* that couldn't decrease pH in yellow watermelon as well as others Fig 2. The reason of redusing pH is duo to the bacteria metabolism in substrate. Guo *et al* (2009) reported that during fermentation *L. casei* reduced pH of milk to 5.59 after 24 h and after 28 days the pH reached 4.60. Some authors showed that pH and acidity decreased and increased in fermented juices, respectively [16, 17]. Probiotic bacteria could tolerate acid medium and survive during fermentation process [8]. High acid of medium cause the bacteria come in to lag phase so decrease the growth rate [15]. The brix of both of them were decreased slightly during fermentation Filg 3. In yeelow watermelon brix decreased more than red watermelon.

## Conclusion

This study showed that red and yellow watermelon are suitable substrate for producing healthy juices by lactic acid bacteria. All strain could growth well in juices. The viable cell of *L. plantarum* was less than other strains after 48 h. Experimental condition showed that sugar was consumed an acid produced during fermentation. The amount of pH and acidity proved its.

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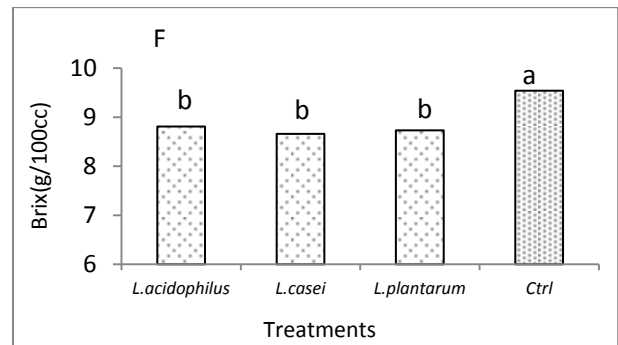
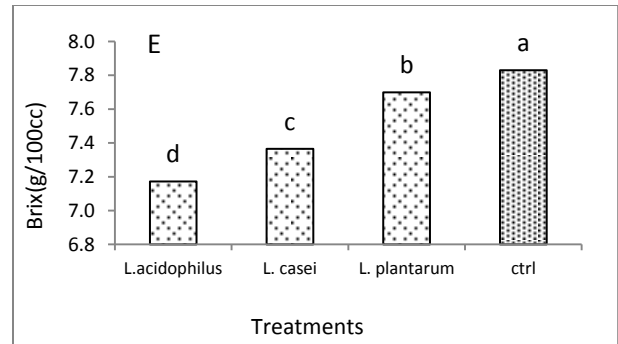
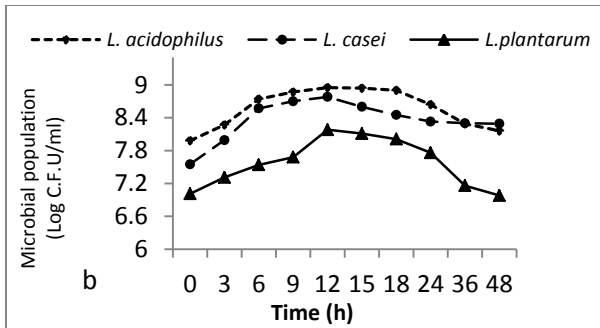
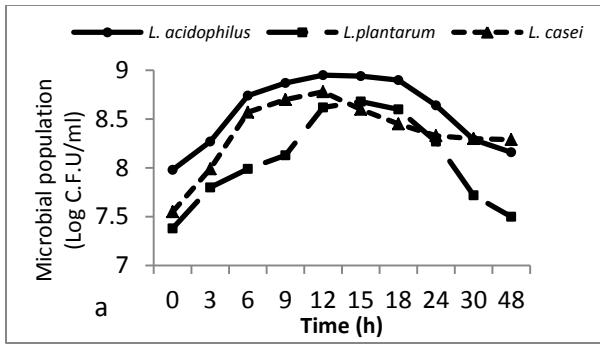


Fig1. Growth kinetic of *L. acidophilus*, *L. casei* and *L. plantarum* during fermentation in red (a) and yellow watermelon juice (b) at 37°C.

Fig. 3. Brix of yellow watermelon (e), and red watermelon (f) during fermentation by three lactic acid bacterial.

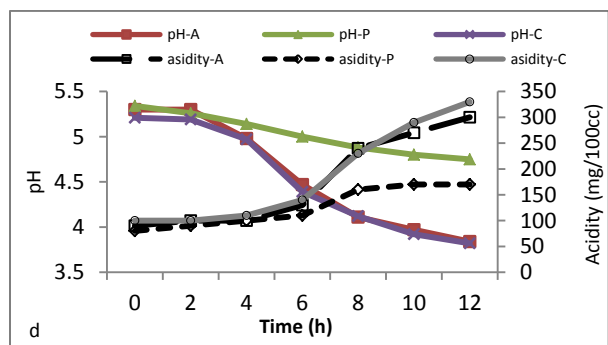
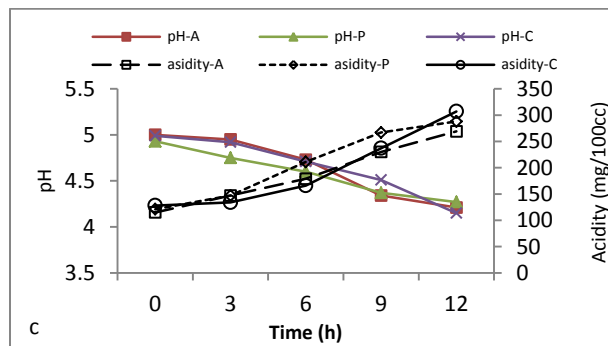


Fig2. Changes in pH and acidity of red (c) and yellow watermelon juice (d) at 12 hour of fermentation by *L. acidophilus*, *L. casei* and *L. plantarum*.