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# FERMENTATION OF YELLOW CARROT JUICE (Daucus carota L.) VIA PROBIOTIC LACTIC ACID BACTERIA DURING STORAGE

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# ABSTRACT

There is a great demand for the vegetarian probiotic products, as an alternative to fermented dairy products. Probiotication may improve flavour, taste and extends the shelf-life of vegetarian juices with great regards to its high nutritional value. Therefore, the aim of this study was to evaluate the effect of using probiotic Lactic Acid Bacteria (LAB) namely, (Lactobacillus plantarum ssp. plantarum EMCC 1027, Lactobacillus delbrueckii ssp.bulgaricus EMCC 1102) and their mixture for the fermentation of carrot juice during cold storage  $(4\pm1^{\circ}C)$  for 12 days. Fresh carrot juice was inculcated with selected LAB (approximately  $5-6 \times 10^9$  CFU/ml of juice). The juice was initially fermented at 37°C for 3 days. Bacteria survival in intervals at 3, 6, 9, and 12 days were counted. The number of LAB bacteria after 12 days of cold storage  $(4\pm1^{\circ}C)$  was more than  $6.5\times10^{8}$  CFU/ml of juice. After 3 days of fermentation, the pH values was decreased from 5.7 to 4.2, while, the acidity (%) was increased from 0.15 % (as lactic acid) to 0.65%, the number of L. bulgaricus was higher than L. plantarum, or mixed culture (1:1) in consumption of reducing sugars, 2.11, 2.45, and 2.51, after 3 days, and 1.45, 1.97, and 1.77 g/100 ml, after 12 days of cold storage, respectively. The viable cell counts of L. plantarum decreased slightly during cold storage, but the cell viability remained at a considerably high level (>10<sup>6</sup> CFU/ml) after 12 days of cold storage (4±1°C).TSS (%) decreased from 7.80 to 6.90 in the fermented sample with mixed culture (1:1), L. bulgaricus, was 7.80 to 6.90, and L. plantarum decreased from 7.8 to 7.10, from initial time to 12 days of storage, respectively. ( $\Delta E$ ) values were highest for the sample with mixed culture =73.8, L. plantarum =72.2, and L. bulgaricus =73.5, after 12 days of cold storage. This suggests that fermented carrot juice, which containing LAB, could serve as a healthy beverage for vegetarians and lactose-allergic consumers.

Key words: Probiotication, lactic acid bacteria, carrot juice, fermentation.

# **INTRODUCTION**

The concept of functional foods has gained universal acceptance as a preventive and therapeutic approach to combat many diseases that decrease the work productivity due to poor health (Chonan, 2011). Lactic acid bacteria (LAB) are commercially used as starter cultures for the manufacture of dairy-based probiotic foods (Heenan *et al.*, 2002).

Traditionally, probiotics have been added to yoghurt and other fermented dairy products, but lactose intolerance and the cholesterol contents are two drawbacks related to their consumption (Penna et al., 2007). In recent years, consumer demand for non-dairy-based probiotic products have increased, and probiotics have been incorporated into drinks as well as marketed as supplements in the form of tablets, capsules, and freeze-dried preparations (Shah, 2001). Furthermore, fruits and vegetables do not contain any dairy allergens that might prevent usage by certain segments of the population (Luckow and Delahunty, 2004). Adding probiotic cultures to fresh juice is a novel concept and provides a research opportunity for food professionals.

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Carrot (*Daucus carota* L.) is a widely used vegetable that is rich in functional food components, such as vitamins (A, D, B, E, C, and K) and minerals (calcium, potassium, phosphorus, sodium, and iron) (Sheela *et al.*, 2011). Beta carotene content of carrot is 2–10 mg per 100 g of carrot (Sharma *et al.*, 2012). Carotenoids and other antioxidants are present in carrot juice, and it is useful in the inhibition of oxidation processes, as well as in counterbalancing free radical activities (Li *et al.*, 2006; Quek *et al.*, 2007; Kun *et al.*, 2008).

Lactic acid starters such as *L. plantarum* can produce pectolytic enzymes; such as polygalacturonase, pectinlyase and pectinesterase (Sakellaris *et al.*, 1988; Karam and Belarbi 1995; Wong, 1995), which can degradate ester bonds, and decrease pectin content. During the lactofermentation, high and low esterified pectins of vegetable mash are depolymerized which can increase the yield to a greater extent, and  $\beta$ -carotene content of the vegetable juice also increases (Demir, 2000).

Desirable properties of fermented vegetable juices can be achieved by choosing lactobacilli strains suitable for the lactic acid fermentation of individual raw materials. The criteria used to gange a strain's suitability are as follows: the rate and total production of acids, change of pH, decrease of nitrate concentration and production of biogenic amines (Karovicova *et al.*, 1999).

Lavinia *et al.* (2009) performed the lactic acid fermentative processes using different probiotic bacteria species unspecific to epiphytic microbiota of vegetables *e.g.* the carrots and the red beet, all the tested strains were found to be capable of rapidly utilizing vegetables for cell synthesis and lactic acid production. They produce a greater amount of lactic acid and reduce the pH of fermented juices from an initial value of 6.4 to below 4.4 after 48 hr., of fermentation. The lactic acid cultures in fermented juices gradually lost their viability during cold storage.

Nosrati *et al.* (2014) investigated lactic acid bacteria, including *Lactobacillus casei* and *Lactobacillus plantarum*, for production fermentative functional drinks based on vegetable juice. The results indicated that, mixed vegetable juice without any nutrient supplementation ; could be considered as a proper matrix for growth of lactic acid bacteria and functional beverage production.

The aim of this study was to produce probiotic carrot juice as a healthy beverage for vegetarians and lactose-allergic consumers, *via* selected LAB, as an alternative to fermented dairy products. In addition, the changes on physicochemical properties of fermented carrot juice were examined during shelf-life.

# **MATERIALS AND METHODS**

# Materials

## Preparation of yellow carrot juice

Carrot roots were purchased from hyper market in Zagazig, Egypt. They were washed thoroughly; both ends were removed, then peeled by sharp knife and cut longitudinally into halves. The halves were blanched in water at 85°C for 5 minute for tenderization the carrot tissues and inactivate pectinase and peroxidase enzymes. Blender (Moulinex blender-LM241, France) was used to extract the juice. The extracted juice was filtrated throw 4-fold cheese cloth. Then; the filtrated juice was heated up to 85°C for 10 min. then was cooled to 37°C.

#### Source of experimental starter cultures

Lactobacillus plantarum ssp. plantarum (EMCC 1027) and L.delbrueckii ssp. bulgaricus (EMCC 1102) were obtained from the Egyptian Microbial Culture Collection of Cairo MIRCEN (EMCC), Faculty of Agriculture, Ain Shams University, Egypt.

# Preparation of experimental fermented carrot juice

Forty-eight hours prior to the start of each experiment; cultures were revived by a series of two inoculations into 10 ml of MRS broth and incubation at 37°C for 24 hours.

The used cultures were grown at  $37^{\circ}$ C separately for 24 hr., in de Man, Rogosa and Sharpe MRS broth (Difco Laboratories, Detroit, MI,USA) in order to attain approximately  $10^{6}$  cfu/ml as inocula before inoculation into carrot juice as 0.5% (*V/V*). Enumeration of the cells was performed by plating serial dilutions of bacterial suspensions on MRS agar plates, and

incubating at 37°C, and counting the colonies after 48 hr. The inoculation by *L. plantarum* and *L.bulgaricus* was done in the range of  $10^5-10^7$  cfu/ml, and lactofermented juices were processed.

#### Methods

#### **Determination of pH-value**

pH-value was measured in all samples with a glass electrode of a digital pH meter (Model Mettler Toledo, Switzerland) (AOAC, 2005).

#### **Determination of titratable acidity**

Acidity of samples was determined according to the general, titration method based on lactic acid percentage (AOAC, 2005).

#### **Determination of total soluble solids (TSS)**

Total soluble solids (TSS) and the refractive index were assayed using the refractometric method, with an Abbe refractometer and corrected to the equivalent reading at 20°C (AOAC, 2005).

#### **Determination of reducing sugars content**

Reducing sugars contents were assayed according to Miller *et al.* (1959), colorimetrically. The colour intensities were measured in a UV spectrophotometer (Jenway-UV–VIS Spectrophotometer) at 575 nm.

#### **Determination of vitamin (C) content**

Vitamin C (ascorbic acid) content was determined using 2,6-dichlorophenol indophenol reagent (Fluka, Deisehofen, Germany) according to the method described by AOAC (2005).

#### **Determination of viscosity**

The viscosity of each sample was determined at room temperature by using a Brookfield digital viscometer (NDJ-85, Niryn Intelligent Company limited, Shanghai). A suitable spindle (spindle 2) and rotational speed (60 rpm) was selected for this study (Ying *et al.*, 2006).

#### **Determination of colour**

Carrot juice colour was measured using the Hunter-Lab (Hunter Lab Colour Flex EZ, USA). Colour parameter (L) indicates degree of lightness to darkness, (a) indicates degree of redness to greenness, and (b) indicates degree of yellowness to blueness (Hunter, 1958).

#### Microbiological analysis

Viable cell counts were determined by serial dilutions and standard plate method after incubation. Dilutions of  $10^{-7}$  and  $10^{-8}$  cfu/ ml were prepared of the fermented samples and plated in double plates. Then, sterilized MRS agar (Merck, Germany) medium was poured on them (standard plate count method). The plates was incubated at  $30^{\circ}$ C for 48 hr. Plates containing 30-300 colonies were counted and recorded as colony forming units (CFU) per ml of solution (Vinderola *et al.*, 2000). Also the viability of lactic acid cultures was determined during the cold storage period by using the mentioned method and expressed as cfu/ml (AOAC, 2005).

#### **Sensory evaluation**

Sensory evaluation was done according to Min *et al.* (2003).Ten panelists were selected (Staff of Food Science Department, Faculty of Agriculture, Zagazig University, Egypt) without care of age or sex. The panelists were asked to indicate their preference on a 9-point Hedonic scale with a degree of liking : 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely. In each session, five different samples were given to rate the Colour, Flavour, Texture, and Overall acceptability of the samples.

#### **Statistical Analysis**

All fermentation experiments were conducted in triplicate and the results in duplicate, and expressed as Mean± SD (standard deviation).

#### **RESULTS AND DISCUSSION**

# Effect of Cold Storage on Physicochemical Properties of Carrot Juice

#### pH-value

The changes in pH during carrot juice fermentation by *L. plantarum*, *L. bulgaricus*, and mixed culture (1:1), are given in Table 1. The carrot juice had an initial pH value of 5.7. Carrot juice containing *L. plantarum* (T2) showed a more rapid drop in pH (3.98) than *L. bulgaricus* juice (T3) (pH=4.20), and mixed

# culture juice (1:1) (T4) pH=4.10. After 12 days of cold storage, pH values were 3.90, 4.13 and 4.24, respectively.

## Acidity (%)

As shown in Table 1, L. plantarum juice (T2) had significantly more acid (0.67%) after 3 days during refrigerated storage than the other juice samples examined (0.64%). It was reported that acid production ability by lactic acid bacteria, especially post-incubation (postacidification), affected the cell viability of probiotic bacteria including L. plantarum and L.bulgaricus (Ishibashi and Shimamura, 1993; Shah et al., 1995). As results, the majority of the changes observed in microbial population, pH. acidity, sugar consumption, and lactic acid metabolism, happened in the period between 30 to 48 hr., of the fermentation. Further extension of the fermentation process (from 48 to 72 hr.) did not result in significant changes, and similar results were reported by Yoon et al. (2004, 2006) and Mousavi et al. (2011).

#### Reducing sugars content (g/100 ml)

The results of sugars consumption (Table 1) show that amount of reducing sugars of all samples were dropped after 3 days. Sugar dropping is because of bacterial growth and organic acids production. L. bulgaricus was higher than mixed culture (1:1) and L. plantarum in consumption of reducing sugars, which valued as much as 2.11, 2.45 and 2.51, after 3 days, and 1.45, 1.97 and 1.77g/100 ml, after 12 days of cold storage, respectively. These results are in agreement with the study of Tsen et al. (2003). They investigated lactic acid production in medium based on mashed banana by L. acidophilus. They used k-carrageenan gum for more efficiency of fermentation. The selected strain metabolized low molecular weight sugars *i.e.*, fructose and glucose as a carbon source for acid production. They reported that the amount of carbon sources with high molecular weight, i.e., Fructo-oligo-Saccharide didn't change during fermentation.

The concentration of reducing sugars was reduced in fermented carrot juice compared with fresh carrot juice due to varied carbohydrate utilization by *Lactobacillus* spp. This enhanced the viable count and increased acidity, with an increase in fermentation time from 24 to 72 hr. An earlier study with fruit juices indicated the growth of *L. plantarum*, which resulted in a viable count of  $8.0 \times 10^8$  CFU/ml after 72 hr., of fermentation (Mousavi *et al.*, 2011). Similar results were reported from other authors on carbohydrate fermentation that varied depending upon the type of substrate consumption and even on fermentation time (Hou *et al.*, 2000).It was also reported that glucose is the primary energy source for *Lactobacillus* spp. Therefore, glucose has been introduced as the most important carbohydrate source for probiotic lactobacilli (Wang *et al.*, 2003).

#### Total soluble solids (TSS%)

The results show decrease in (TSS%) for probiotic carrot juice, especially mixed culture (1:1), *L.plantarum* and *L. bulgaricus* which were 7.60, 7.60, 7.50 and 7.00, 7.20, 7.10 and 6.90,7.10, 6.90, after 3, 9 and 12 days of cold storage, respectively. As result for cell synthesis and growth rate of starter cultures.

#### Viscosity (centipoise)

The rheological behaviour of carrot juice was influenced by its quantitative and qualitative composition and therefore, it will depend on the treatment at which it is subjected during processing. Table 1 illustrates the viscosity of carrot juice; it is obvious that all samples had increased in the viscosity. *L. bulgaricus* had the highest degree in viscosity= 16.15 (cps) and 18.12 (cps), after 9 and 12 days of cold storage, respectively. While, samples with mixed culture (1:1) were 12.8 (cps), and 14.14 (cps), *L. plantarum*, were 12.15 (cps) and 14.16 (cps), after 9 and 12 days of cold storage, respectively.

The increase in the viscosity of the fresh carrot juice upon storage might be due to the increase in the microbial load particularly molds and yeasts, which led to spoilage of the juice and increase its viscosity. Similarly, Huisint (1996) reported that spoilage of food may also bring about physical changes such as increase the viscosity, gelation, sedimentation or colour change of the food.

#### Vitamin (C) content (mg/100 ml)

Vitamin (C) contents were decreased rapidly in *L. bulgaricus* samples being, 2.9, and 2.5 mg/

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| Parameter                             | Storage<br>(day) | Control           | <b>T1</b>         | T2                | Т3                | T4                |
|---------------------------------------|------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                                       | 0                | $5.70\pm0.0047$   | $5.74{\pm}0.0329$ | $5.71 \pm 0.0081$ | 5.75±0.0294       | 5.74±0.0339       |
|                                       | 3                | $5.33 \pm 0.0205$ | $5.73 \pm 0.0402$ | $3.98 \pm 0.0294$ | $4.25 \pm 0.0244$ | 4.14±0.0374       |
| pH                                    | 6                | 4.95±0.0326       | $5.64 \pm 0.0368$ | 3.96±0.0163       | 4.15±0.0286       | 4.10±0.0294       |
|                                       | 9                | 4.64±0.0169       | $5.53 \pm 0.0339$ | 3.94±0.0169       | 4.15±0.0326       | 4.13±0.0329       |
|                                       | 12               | $4.45 \pm 0.0286$ | $5.33 \pm 0.0309$ | 3.90±0.0326       | 4.13±0.0402       | $4.24 \pm 0.0262$ |
|                                       | 0                | $0.15 \pm 0.0057$ | $0.15 \pm 0.0094$ | $0.15 \pm 0.0029$ | $0.15 \pm 0.0024$ | $0.15 \pm 0.0020$ |
|                                       | 3                | 0.34±0.0339       | $0.15 \pm 0.0188$ | $0.67 \pm 0.0012$ | $0.64 \pm 0.0053$ | 0.65±0.0029       |
| Acidity (%)                           | 6                | 0.44±0.0329       | $0.16 \pm 0.0032$ | $0.67 \pm 0.0009$ | $0.65 \pm 0.0061$ | 0.65±0.0026       |
|                                       | 9                | $0.48 \pm 0.0309$ | 0.16±0.0036       | $0.68 \pm 0.0037$ | $0.66 \pm 0.0014$ | 0.65±0.0030       |
|                                       | 12               | $0.54 \pm 0.0374$ | $0.18 \pm 0.0032$ | $0.69 \pm 0.0028$ | $0.66 \pm 0.0024$ | 0.68±0.0035       |
|                                       | 0                | $2.95 \pm 0.0203$ | $2.95 \pm 0.0206$ | 2.97±0.0181       | 2.95±0.0186       | $2.97 \pm 0.0197$ |
|                                       | 3                | 2.77±0.0029       | 2.95±0.0185       | 2.51±0.0216       | 2.11±0.0020       | 2.45±0.0028       |
| Reducing sugars<br>content (g/100 ml) | 6                | $2.35 \pm 0.0026$ | 2.91±0.0075       | 2.33±0.0205       | $1.95 \pm 0.0030$ | 2.33±0.0024       |
| content (g/100 nn)                    | 9                | 2.23±0.0030       | 2.88±0.0032       | 1.98±0.0035       | 1.73±0.0016       | 2.15±0.0033       |
|                                       | 12               | 2.01±0.0028       | 2.76±0.0094       | 1.77±0.0003       | 1.45±0.0016       | 1.97±0.0029       |
|                                       | 0                | 7.80±0.0262       | 7.80±0.0205       | 7.80±0.0286       | 7.80±0.0169       | 7.80±0.0294       |
|                                       | 3                | 7.70±0.0188       | 7.80±0.0216       | 7.60±0.0368       | 7.50±0.0235       | 7.60±0.0205       |
| Total soluble solids<br>(°Brix)       | 6                | 7.60±0.0235       | 7.80±0.0309       | 7.40±0.0163       | 7.30±0.0262       | 7.20±0.0216       |
| ( DIIX)                               | 9                | 7.60±0.0216       | 7.70±0.0294       | 7.20±0.0262       | 7.10±0.0355       | 7.00±0.0262       |
|                                       | 12               | 7.50±0.0094       | 7.70±0.0216       | 7.10±0.0081       | 6.90±0.0249       | 6.90±0.0163       |
|                                       | 0                | 5.6±0.0047        | 5.6±0.0377        | 5.6±0.0329        | 5.6±0.0282        | 5.6±0.0235        |
|                                       | 3                | 5.2±0.0188        | 5.1±0.0141        | 4.2±0.0081        | 3.7±0.0047        | 3.8±0.0402        |
| Vitamin (C) content                   | 6                | 4.8±0.0047        | 5.1±0.0094        | 3.9±0.0262        | 3.2±0.0124        | 3.4±0.0169        |
| (mg/100 ml)                           | 9                | 4.3±0.0169        | 4.9±0.0124        | 3.8±0.0355        | $2.9 \pm 0.0402$  | 3.2±0.0081        |
|                                       | 12               | 3.9±0.0309        | 4.5±0.0262        | 3.5±0.0216        | 2.5±0.0355        | 3.0±0.0309        |
|                                       | 0                | 52.00±0.0040      | 52.00±0.0035      | 52.00±0.0030      | 52.00±0.0026      | 52.00±0.0021      |
|                                       | 3                | 23.33±0.0028      | 52.00±0.0016      | 11.34±0.0033      | 11.71±0.0029      | 11.69±0.0024      |
| Brix- acid ratio                      | 6                | 16.88±0.0026      | 48.75±0.0012      | 11.04±0.0016      | 11.23±0.0020      | 11.07±0.0004      |
|                                       | 9                | 15.83±0.0012      | 48.12±0.0008      | 10.58±0.0037      | 10.75±0.0032      | 10.76±0.0030      |
|                                       | 12               | 14.70±0.0088      | 42.77±0.0022      | 10.28±0.0023      | 10.45±0.0020      | 10.14±0.0018      |
|                                       | 0                | 5.02±0.0124       | 5.03±0.0141       | 5.03±0.0188       | 5.03±0.0235       | 5.05±0.0262       |
|                                       | 3                | 7.06±0.0294       | 6.05±0.0235       | 8.04±0.0205       | 12.13±0.0262      | 8.07±0.0555       |
| Viscosity (cps)                       | 6                | 9.15±0.0286       | 8.04±0.0141       | 11.12±0.0205      | 15.14±0.0339      | 9.14±0.0141       |
| • • • • •                             | 9                | 13.17±0.0163      | 8.03±0.0124       | 12.15±0.0374      | 16.15±0.0339      | 12.18±0.0141      |
|                                       | 12               | 15.16±0.0188      | 9.10±0.0612       | 14.16±0.0124      | 18.12±0.0852      | 14.14±0.0205      |

Table 1. Physicochemical properties of probiotic carrot juice during cold storage (4±1°C)

Control: Fresh carrot juice, T1: Carrot juice pasteurized at  $85^{\circ}$ C for 10 min., T2: Carrot juice containing *L. plantarum*, T3: Carrot juice containing *L. bulgaricus*; T4: Carrot juice containing *L. plantarum* + *L. bulgaricus*; (1:1).

100 ml juice, after 9 and 12 days of cold storage, respectively. Then, samples with mixed culture (1:1) were 3.2, and 3.0 mg/100 ml juice, respectively. While, *L. plantarum*, valued 3.8 and 3.5 mg/100 ml juice, after 9 and 12 days of cold storage, respectively. The decreasing in contents of Vitamin (C) during storage may be due to metabolism of lactic acid bacteria, cell synthesis and growth.

#### Effect of Acidification on Carrot Juice Cloud Stability

That may be due to acidification of the medium by LAB metabolites (organic acids *i.e.* lactic acid). These results agree with Alison *et al.* (2014) who examined the effects of acidity on cloud stability in pasteurized carrot juice over the pH range of 3.5 - 6.2. Cloud sedimentation, particle diameter were measured at each pH condition to quantify juice cloud stability and clarification during 3 days of storage. Acidification below pH 4.9 resulted in an increased particle size, and unstable cloud, leading to juice clarification.

Also Reiter *et al.* (2003) studied carrot juice cloud stability on pilot-plant scale using decanter technology. Production steps investigated were mode of acidification, different enzymatic mash treatments. Acidification showed the strongest effect on cloud stability. Whereas acidifying the coarse mash resulted in cloud stability, acidification following juice extraction even enhanced cloud sedimentation.

# Effect of Cold Storage (4±1°C) on Cell viability of Lactic Acid Bacteria

The effect of cold storage on the cell viability of two lactic acid cultures in fermented carrot juice was presented in Fig. 1.

The viable cell counts of *L. plantarum*, and *L. bulgaricus* were higher than  $1.0 \times 10^6$  CFU/ml even after 12 days of cold storage at  $4\pm1^\circ$ C. Especially, the viable cell counts of *L. bulgaricus* decreased gradually during cold storage and remained at  $1.5 \times 10^6$  CFU/ml, after 12 days of cold storage. The viable cell counts of *L. plantarum* decreased slightly during cold storage, but the cell viability remained at a considerably high level (>10<sup>6</sup> CFU/ml) after 12 days of cold storage ( $4\pm1^\circ$ C). It is important to have a significant number of viable lactic acid bacteria present in the probiotic products for maximum health benefits (Shah *et al.*, 2001; Mousavi *et al.*, 2011).

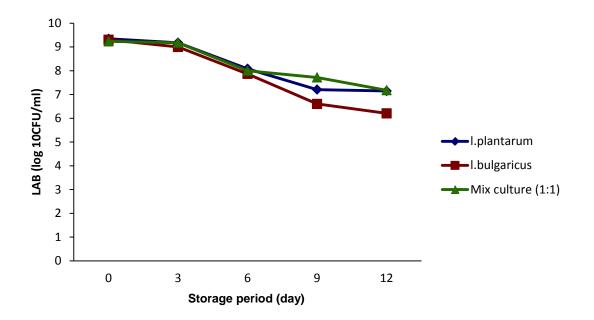


Fig.1. Effect of cold storage (4±1°C) on the viability of lactic acid bacteria cultures in fermented carrot juice

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Several factors could affect the cell viability of lactic acid cultures in probiotic food products. Probiotic cultures are commonly used in the dairy industry, and some products produced during lactic acid fermentation such as lactic acid, diacetyl, and acetaldehyde could be associated with the loss of viability of added probiotic bacteria (Post, 1996). Probiotic lactic acid starters have been suggested to produce bacteriocin against probiotic bacteria and *vice versa* (Prado *et al.*, 2008; Kumar *et al.*, 2015).

In general, the cell viability depends on the strains used, interactions between species present, culture condition, oxygen content, final acidity of the product, and the concentration of lactic acid and acetic acid. The main factors for loss of viability of probiotic organisms have been attributed to the decrease in the pH of the medium and accumulation of organic acid as a result of growth and fermentation (Hood and Zottola 1988; Saarela *et al.*, 2002).

# Effect of Cold Storage (4±1°C) on Colour Attributes

Fig. 2 shows that colour values  $(l^*, a^*, b^*)$  decreased during storage period.  $(L^*)$  values were the highest in samples with mixed culture (1:1) then followed by *L. plantarum*, and *L. bulgaricus*, at 9 days of storage, their colour values were 41.49, 38.63 and 33.36, respectively. While at 12 days, they were 30.12, 28.74 and 29.21, respectively.

(a\*) values were the highest in samples with mixed culture (1:1) then followed by L. *plantarum*, and L. *bulgaricus*, at 9 days of storage, and valued 24.07, 16.83 and 8.93, respectively. While at 12 days, they amounted 11.71, 7.67 and 10.56, respectively.

(b\*) values were the highest in samples with mixed culture (1:1) followed by *L. plantarum*, and *L. bulgaricus*, at 9 days of storage, and amounted 22.95, 21.78, and 15.2, respectively. While at 12 days, the values were 14.74, 11.73 and 13.87, respectively.

Degradation in carotenoids could be due in part to the metabolism of bacteria; while on the other hand, it could be affected by fermentation conditions such as temperature and pH. These results agree with Kun *et al.* (2008) they determined that storage caused a decrease in b-carotene. It is thought that oxygen in head space caused the degradation of b-carotene. After the sixth day of cold storage, an increasing in colour attributes was noticed, till the ninth day. That increasing, could be due to decrease in total count of LAB; or the medium conditions of pH and Acidity.

Figs. 3, 4 and 5 show Colour intensity (C), Hue angel (h) and Total Colour Difference ( $\Delta E$ ), (C) values were highest for the sample with mixed culture = 33.2 ,*L. plantarum* =27.4, and *L. bulgaricus* = 17.6 , in the ninth day of storage. ( $\Delta E$ ) values were highest for the sample with mixed culture =78.1, *L. plantarum* =72.7, and *L. bulgaricus* =69.8

These Results refer to higher increasing in these values than the beginning in the ninth day of cold storage.

## Effect of Cold Storage (4±1°C) on Sensory Evaluation

Table 2 shows that fermented carrot juice with the culture *L. plantarum* was the favorable product, when compared with the *L. bulgaricus* culture. The mixed culture (1:1) comes to be the second favorable product depending on colour, flavour, texture, and overall acceptability. Generally, panelists did not prefer the flavour of the products. The reason may be that, higher acidity and contents of lactic acid bacteria by-products such as Diacetyl, acetaldehyde and lactic acid, which could be drawbacks in flavour of products.

#### Conclusion

Carrot juice could serve as a raw material for the production of probiotic carrot juice by lactic acid fermentation with *L. plantarum*, *L. bulgaricus*, and mixed culture (1:1). The fermented juice has a pH-value of less than 4.5 (high acid) and contains a significant number of beneficial lactic acid bacteria ( $10^7$  CFU/ml) during cold storage ( $4\pm1^{\circ}$ C).

The fermented sample with the culture of *L*. *plantarum* was the favorable product, when compared with the *L*. *bulgaricus* culture, and mixed culture (1:1). The mixed culture (1:1) comes to be the second favorable product depending

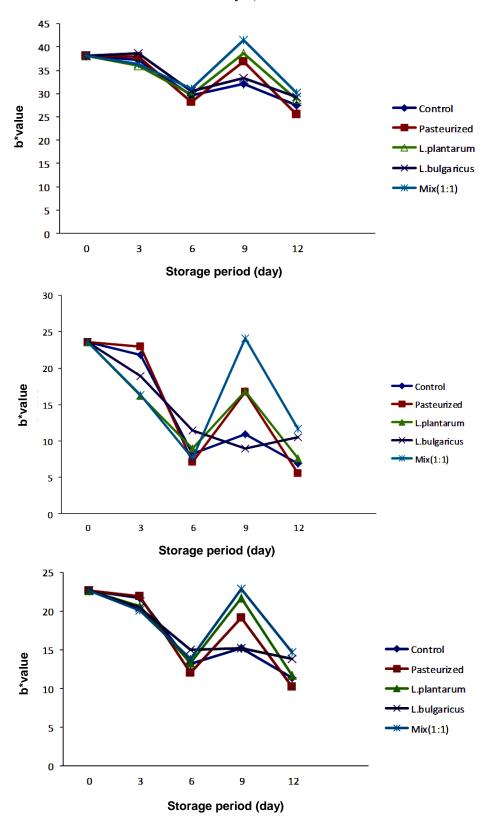


Fig. 2. a, b, and c (a=L\* value, b=a\* value, and c=b\* value) shows the effect of cold storage (4±1°C) on colour attributes in fermented carrot juice

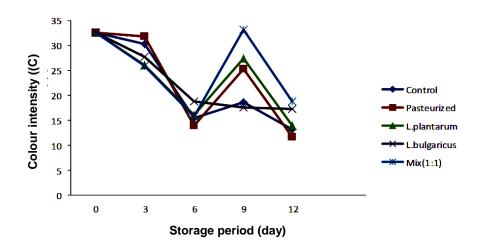


Fig. 3. Effect of cold storage (4±1°C) on colour intensity(C) in fermented carrot juice

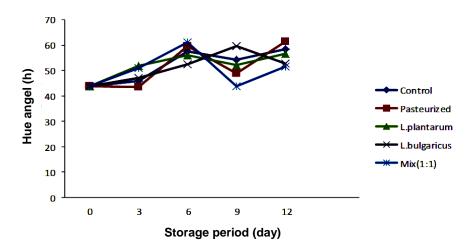


Fig. 4. Effect of cold storage (4±1°C) on colour Hue angel (h) in fermented carrot juice

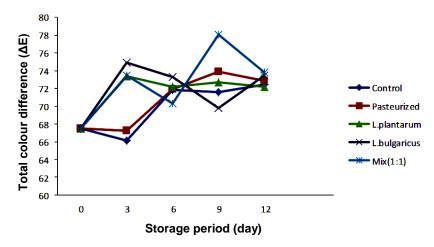


Fig. 5. Effect of cold storage  $(4\pm 1^{\circ}C)$  on total colour difference  $(\Delta E)$  in fermented carrot juice

| Sample | Sensory evaluation        |                  |             |             |                  |             |  |  |
|--------|---------------------------|------------------|-------------|-------------|------------------|-------------|--|--|
| day    | Parameter                 | Control          | <b>T1</b>   | T2          | Т3               | T4          |  |  |
| 0      | Colour (9)                | 8.4±0.4898       | 8.2±0.4000  | 8.3±0.4582  | 8.1±0.3000       | 8.5±0.5000  |  |  |
|        | Flavour<br>(9)            | 8.2±0.4000       | 8.00±0.0000 | 8.3±0.4582  | $7.7 \pm 0.4582$ | 7.6±0.4898  |  |  |
|        | Texture (9)               | 8.00±0.6324      | 8.4±0.4898  | 8.5±0.5000  | 8.4±0.4898       | 8.3±0.4582  |  |  |
|        | Overall acceptability (9) | 8.3±0.4582       | 8.1±0.3000  | 8.2±0.4000  | 8.1±0.3000       | 8.00±0.0000 |  |  |
| 3      | Colour (9)                | 7.7±0.4582       | 8.1±0.3000  | 8.2±0.4000  | 8.3±0.4582       | 8.4±0.4898  |  |  |
|        | Flavour (9)               | 7.00±0.0000      | 8.5±0.5000  | 8.4±0.4898  | 7.4±0.4898       | 7.3±0.4582  |  |  |
|        | Texture (9)               | $7.4 \pm 0.4898$ | 7.2±0.4000  | 7.00±0.0000 | 7.1±0.3000       | 7.3±0.6403  |  |  |

Table

|    | Flavour (9)              | $7.00 \pm 0.0000$ | 8.5±0.5000       | 8.4±0.4898        | 7.4±0.4898       | 7.3±0.4582  |
|----|--------------------------|-------------------|------------------|-------------------|------------------|-------------|
|    | Texture (9)              | $7.4 \pm 0.4898$  | 7.2±0.4000       | $7.00 \pm 0.0000$ | 7.1±0.3000       | 7.3±0.6403  |
|    | Overall acceptability(9) | 7.3±0.6403        | 8.3±0.4582       | 7.3±0.4582        | $7.7{\pm}0.4582$ | 7.4±0.6633  |
| 6  | Colour (9)               | 6.7±0.4582        | 7.00±0.0000      | 8.3±0.4582        | 6.5±0.5000       | 8.5±0.5000  |
|    | Flavour (9)              | 6.3±0.4582        | 8.4±0.4898       | 6.5±0.5000        | 7.2±0.4000       | 7.1±0.3000  |
|    | Texture (9)              | 7.6±0.4898        | 7.4±0.6633       | 7.4±0.4898        | 7.3±0.6403       | 7.00±0.0000 |
|    | Overall acceptability(9) | 6.6±0.4898        | 7.3±0.4582       | 7.2±0.4000        | 6.5±0.5000       | 7.6±0.4898  |
| 9  | Colour (9)               | 5.4±0.6633        | $7.7{\pm}0.4582$ | 8.5±0.5000        | 7.2±0.4000       | 7.3±0.6403  |
|    | Flavour (9)              | 5.1±0.3000        | 8.2±0.4000       | 6.5±0.5000        | 7.00±0.0000      | 7.3±0.4582  |
|    | Texture (9)              | 6.5±0.5000        | 7.1±0.3000       | 7.3±0.4582        | 6.7±0.4582       | 6.7±0.4582  |
|    | Overall acceptability(9) | 5.3±0.4582        | 8.4±0.4898       | 7.4±0.4898        | 7.2±0.4000       | 6.6±0.4898  |
| 12 | Colour (9)               | 5.2±0.4000        | 7.3±0.6403       | 8.5±0.5000        | 6.7±0.4582       | 7.1±0.3000  |
|    | Flavour (9)              | 5.3±0.6403        | 7.6±0.4898       | 7.00±0.0000       | 6.6±0.4898       | 7.3±0.4582  |
|    | Texture (9)              | 5.8±0.8717        | 7.1±0.3000       | 7.2±0.4000        | 7.4±0.4898       | 7.6±0.4898  |
|    | Overall acceptability(9) | 5.6±0.8000        | $7.7{\pm}0.4582$ | 7.3±0.4582        | 6.7±0.4582       | 7.2±0.4000  |

Control: Fresh carrot juice, T1: Carrot juice pasteurized at 85°C for 10 min., T2: Carrot juice containing L. plantarum, T3: Carrot juice containing L. bulgaricus; T4: Carrot juice containing L. plantarum + L. bulgaricus (1:1).

on physiochemical properties. The last one was sample with *L. bulgaricus*, because of high acidity and LAB by-products such as Diacetyl, Acetaldehyde and lactic acid, which could be drawbacks in flavour of carrot juice samples. Probiotics could be another inexpensive substitute to the antibiotics that many bacteria are now becoming resistant to, because it is biosafe and easy in application.

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تخميس عصيسر الجسزر الأصفر بواسطة بكتيريا حمض اللاكتيك الحيوية أثناء التخزين

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المنتجات الحيوية المتاحة في الأسواق اليوم، تكون في العادة ألبان متخمرة والزبادي، إلا أن هناك طلب للمنتجات الحيوية النباتية، نتيجة زيادة أعداد المستهلكين النباتيين وارتفاع محتوي الكوليسترول في منتجات الألبان، وبناءاً على ذلك، فان الخضروات والفاكهة، ربما تكون ماده خام بديله للألبان والتي يمكن للبكتيريا الحيوية ان تنمو فيها وتؤدي دورها الحيوي، أجريت هذه الدراسة بهدف تقييم حيوية بكتيريا حمض اللآكتيك في عصير الجزر المتخمر، خلال فترة التخزين لمدة ١٢ يوما تحت تبريد (٤±١°م)، تم إنتاج عصير جزر متخمر بواسطة بكتيريا حمض اللاكتيك باستخدام سلالات فياسية هي Lactobacillus plantarum ssp. plantarum EMCC 1027, and Lactobacillus delbrueckii فياسية هي .(ssp.bulgaricus EMCC 1102 ،حيث انتج عصير جزر طازج ومعاملته حراريا في حمام مائي على درجة حرارة م لمدة ١٠ دقائق ، ثم التلقيح بالبادئ (تقريبا ٥-٦ ×١٠ خلية/مل) ، والتحضين علي درجة حرارة ٣٧° م لمدة ٣ أيام، ثم التخزين علي درجة حرارة (٤±١°م) لمدة ١٢ يوماً، وسحبت العينات في أيام ٣، ٦ ، ٩ و١٢يوم من التخزين،لتقدير العدد الكلي للبكتيريا، والحموضة ورقم ال pH والسكريات المختزلة واللون والتحكيم الحسى، أوضحت النتائج أن العدد الكلي لبكتيريا حمض اللاكتيك الحية كان أكثر من ٢.٥ × ١٠<sup>٧</sup> خلية حية/مل من العصير، وارتفعت قيمة الحموضة من ١٥.٠% إلى ٢٠.٠% وانخفض رقم الاس الهيدروجيني(pH) من ٢.٧ إلى ٤.٢ بعد ٣ أيام من التخمير. كانت bulgaricus الأعلى في استهلاك السكريات المختزلة ٢.١١ جم لكل ١٠٠ مل عصير، تلتها السلالات المختلطة ٢.٤٥، ثم السلالة L. plantarum الترتيب، انخفضت أعداد البكتيريا الحية بصورة طفيفة للسلالة L. plantarum وظلت أعدادها مرتفعة نسبيا (> ١٠ خليه حية لكل مل عصير) أثناء التخزين تحت تبريد ٤± ٥.٠٠م، انخفضت قيمة الجوامد الصلبة الذائبة (%) TSS من ٧.٨ الى ٦.٧ لعينات السلالات المختلطة (١:١) وكذلك السلالة L. bulgaricusمن ٨.٧ الي ٢.٧، يليهم السلالة L. plantarum ، انخفضت من ٨.٧ إلى ٢.٩، كان مقدار التغير في اللون اعلى للسلالات الخليطة (١:١)= ١٨.١ والسلالة VT. ٧L. plantarum والسلالة L. bulgaricus كانت ٨.٩٨ على الترتّيب، تشير النتائج إلى أنّ عصير الجزر المتخمر المحتوي على بكتيريا حمض اللاكتيك، يمكن أن يكون مشروب صحى رخيص الثمن للاشخاص اللذين لديهم حساسية للاكتوز اللبن والأشخاص النباتيين ، في الحصول على فوائد بكتيريا حمض اللاكتيك الصحية من خلال عصائر الخضر والفاكهة المتخمرة كبديل لمنتجات الالبان المتخمرة.

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