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PROBIOTIC BEVERAGE FROM BLACK CARROT JUICE FERMENTED with *Lactobacillus casei* and *Lactobacillus paracasei*

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Agenda

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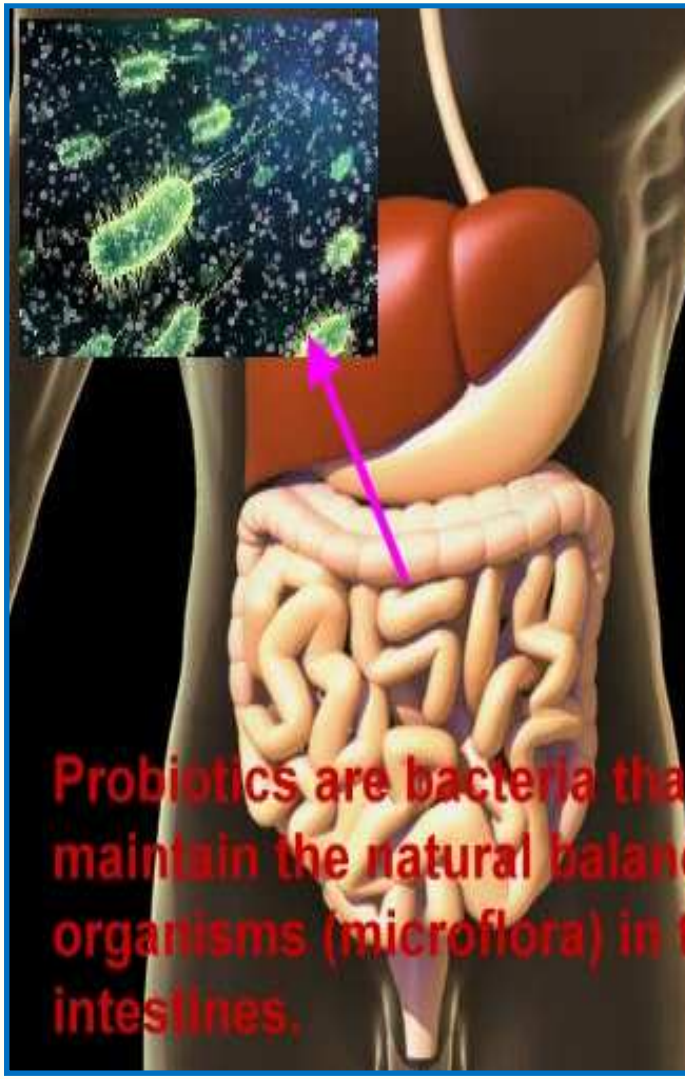
The Mevlâna Museum, located in Konya, Turkey

1. INTRODUCTION

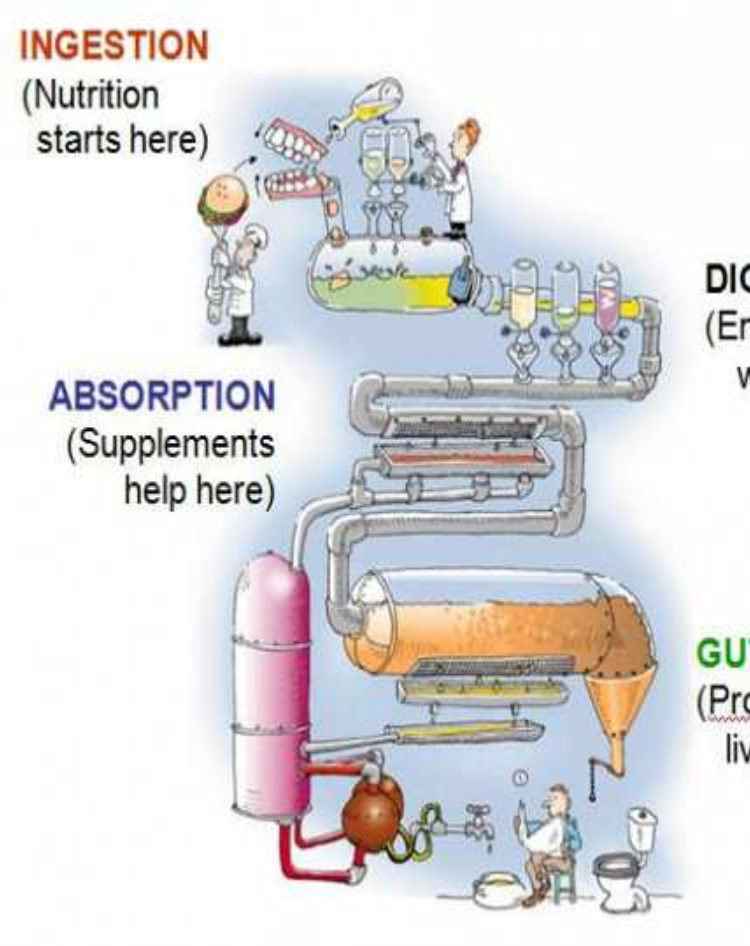
nctional foods are defined as the foods that provide trients and feed on people, in addition to contribute to re the diseases and provide several health benefits rado et al 2008).

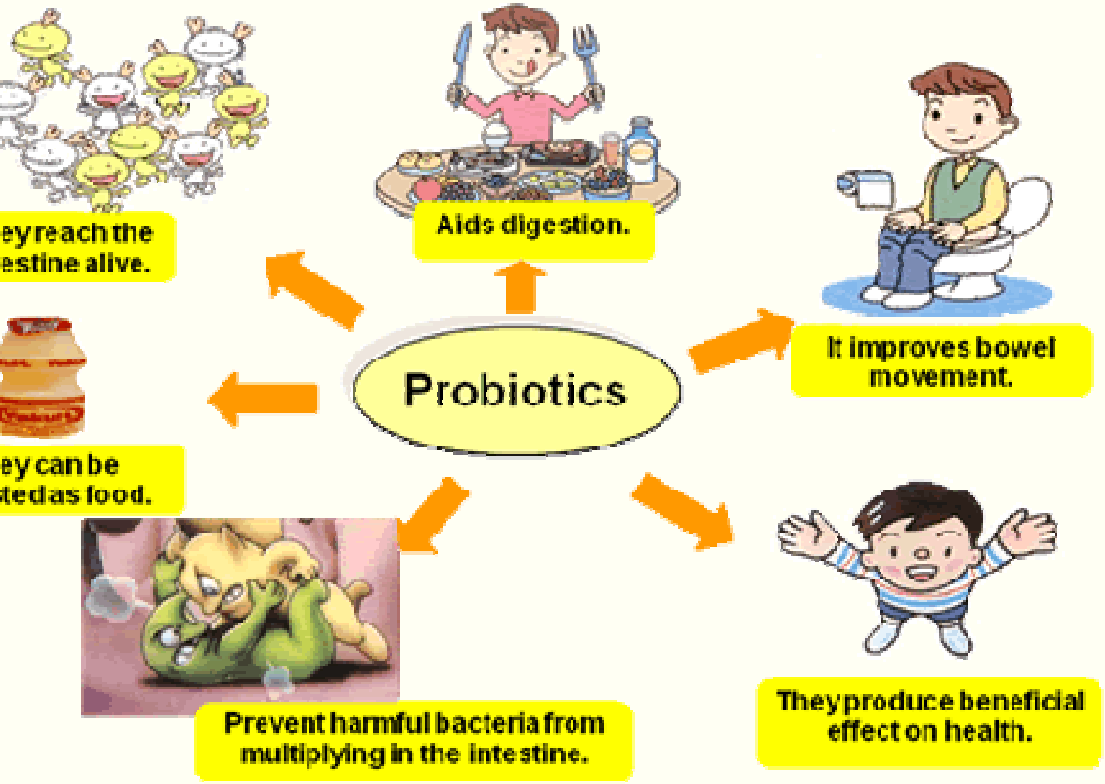
obiotics are one of the most popular functional foods. obiotics are defined as live microbial feed supplement at beneficially influence the host by enhancing its strointestinal balance(Fuller, 1989; Yoon, 2006).



- These microorganisms have many health-supporting effects such as easing of lactose intolerance, prevention of intestinal tract infections and colon cancer and good industrial characteristics such as resistance to acid and bile, attachment to the epithelial cells and colonization in the human intestine (Jack,Tagg and Ray, 1995; Prado et al. 2008).

- They show antagonistic effect to food-borne pathogens. One of the most commonly used bacteria for commercial probiotic applications are species of *Lactobacillus* (Sheehan et al., 2007).





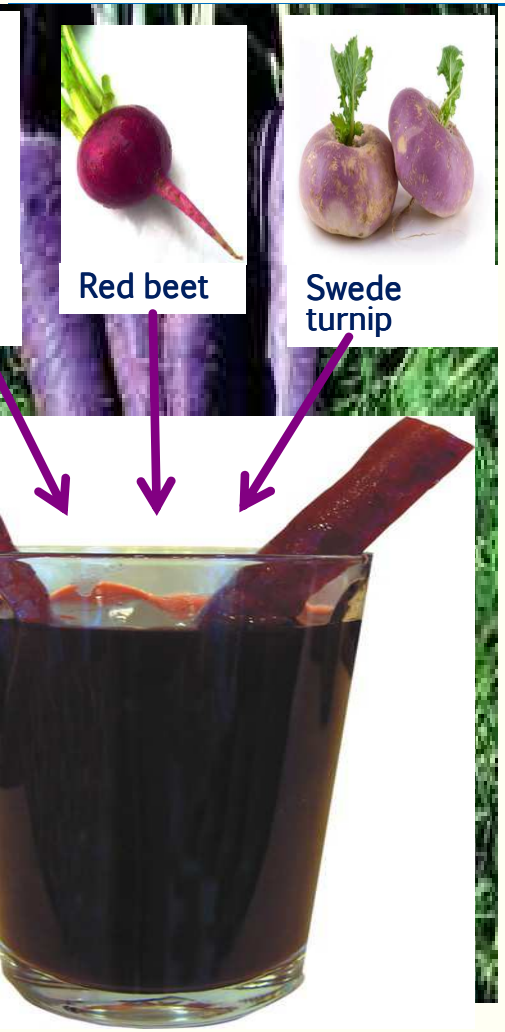
- Probiotics have ordinarily been add fermented milk products, espe yoghurts but there are se disadvantages related to their consum like lactose intolerance, the chole content and/or allergic to milk pro (Heenan, Adams, Hosken, & Fleet, Yoon, Woodams, & Hang, 2006; Ku al.2008).

- However, there is relative little publ information on the survival of probiot non-fermented food matrices, wherea stability of probiotics in yoghurts has widely studied (Kailasapathy Rybka, 1997).

recent years, consumer interest to non-dairy probiotic products such as fruit and es, cereal-based products has increased (Shah, 2001) and there is a little publish biotic stability in non-fermented foods and beverages.

There are a wide variety of traditional non-dairy fermented beverages produce around the world lately. Fruits and vegetables have been showed a appropriate for probiotic products and fruits and vegetables do not contain an dairy allergens that might prevent usage by part of the population (Luckow an Delahunty, 2004). Also they have several functional food components such a minerals, vitamins, dietary fibers, and antioxidants (phytochemicals).

In recent years studies about non-dairy probiotic beverages such as tomat cabbage, blackcurrant, orange, beetroot and carrot juices have bee performed in conjuction with different probiotic strains and obtained appealin results.



- Black carrots are a good source of anthocyanin pigments.
- The anthocyanin content of black carrots was reported to be 1750 mg kg^{-1} fresh weight (Mazza & Miniati, 1993).
- Black carrots also contain high amounts of acylated anthocyanins.
- Moreover, black carrot anthocyanins provide an excellent bright strawberry red shade at acidic pH values; therefore, black carrot juice can be a good choice for colouring fruit juices and nectars, soft drinks, cans, jellies and confectionery (Downham & Collins, 2000; Kırca et al.,2006).

- Black carrots (BCs) are grown in Turkey, Afghanistan Pakistan and India.
- Ereğli district in Konya is th production area for BCs. BC in Turkey were often proces juice, concentrate and shalg a traditional lactic acid fe beverage (Turkyilmaz ve ark.,

The aim of the present study was to determine the survivability of *L. casei* and *L. paracasei* on black carrot juices throughout refrigerated storage for 42 days and therefore to assess suitability of black carrot as a raw material for production probiotic beverages.

2. MATERIAL AND METHODS

Raw Material

Black carrots were provided by black carrot producers in Ereğli District, Konya as a concentrate, it was diluted 1:10.

A strain of *L. casei* NRRL B-442 and *L. paracasei* NRRL B-442 were obtained from ARS Culture Bacterial Collection (NRRL Culture Collection, United States Department of Agriculture, Peoria П,USA)

Microorganisms, inoculum preparation and juice fermentation

Pasteurization were applied to freshly prepared black carrot juices at 80°C for 20 min. for the purpose of decrease microbial population to below the detection limit.

A strain of *L. casei* NRRL B-442 and *L. paracasei* NRRL B-442 was statically activated for 12 h at 37°C in 25 ml erlenmayer flasks containing 100 ml of MRS Broth (de Man, Rogosa, & Sharpe, 1960).

- Stock cultures were prepared and stored frozen (-20

tion

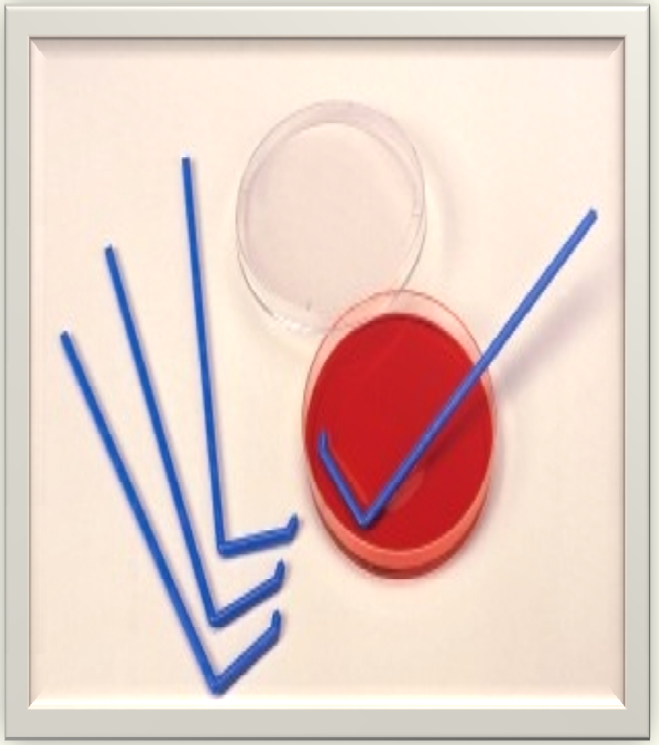
- The growth of *L. casei* and *L. paracasei* was quantified by measu the optical density at 590 nm. The absorbance was recorded for fresh juice inoculated with *L.casei* (initial absorbance) and after 24 fermentation (final absorbance).
- The procedure consisted of diluting with distilled water an aliquot of juice containing microbial cells and reading the absorbance at 590 against water.
- The difference between final and initial absorbance corresponde the growth of the microorganisms during the fermentation.
- Growth was expressed as dry mass concentration (g/L) calcula using the calibration curve given in Eq. (1), built using *L.casei* dry ce

L. casei (g/L) = ABS(590 nm) **Eq. 1**

erial dilutions were prepared for microbial counts. These iluted samples were inoculated on plates containing MRS gar, plating on the surface with the aid of a Handle rigalsky.

he plates were incubated at 37°C for 72 h. Typical colonies re round, white creamy with diameters ranging from 0,9 to ,3 mm (Vinderola and Reinheimer, 2000).

or stability assay, black carrot juice was fermented at 30°C r 48 h. After fermentation the bottles were stored under efrigeration temperature for 42 days. Each seven days, a ottle of each sample was analysed (pH, microbial viability nd color).



pH Analysis

pH values of the black carrot juice was determined by WTW pH meter (İnolab Ph720, Weilheim, Germany).

Color Analysis

Color analysis of samples was determined using CR400 chroma meter (Konica Minolta, Inc., Osaka, Japan). The chroma meter was standardized by using the illuminant D65 and measurements were made through an 8 mm viewing area (Minolta, 1998). The instrument measured lightness (L*), redness (a*) and yellowness (b*).

d. Sugar Determination

- The sugars were analysed by high performance liquid chromatography in a Shimadzu HPLC equipped with LC-10ADvp pump, RID 10A dedector, CTO-10ACvp column oven and DGU-14A degasser. Seperation was applied Aminex by HPX-87C carbohydrate column (300*7.8mm) at 80°C. Injection volume was 20 µL and flow velocity 0,6 ml/min. Sample preparation was carried out according to Veberic and Stampar (2005) with some modification.

d. Statistical Analysis

- JMP 5.0 (SAS Institute Inc., Cary, NC, USA) software was used to perform the statistical analysis according to one-way analysis of variance (ANOVA). Means that were statistically different from each other were compared by using Student's t comparison tests at %5 confidence interval.

3. RESULT AND DISCUSSION

lame	Fermentation and Storage Time (days)	pH values	L* values	a* values	b* val
obacillus casei	0	3,78^{a}	8,10^{a}	23,37^{ab}	-5,78^{a}
	1	3,74^{b}	8,98^{a}	22,18^{ab}	-4,81^{a}
	2	3,74^{b}	9,43^{a}	25,22^{ab}	-4,21^{a}
	7	3,67^{c}	8,33^{a}	18,77^{b}	-5,97^{a}
	14	3,64^{d}	9,19^{a}	27,53^{a}	-4,78^{a}
	21	3,63^{d}	9,16^{a}	19,60^{b}	-5,67^{a}
	28	3,64^{d}	9,44^{a}	22,45^{ab}	-5,13^{a}
	35	3,64^{d}	9,38^{a}	24,22^{ab}	-3,59^{a}
	42	3,65^{d}	8,70^{a}	20,89^{ab}	-4,50^{a}
	42	3,69^{d}	8,16^{c}	17,54^{c}	-5,85^{cd}

3. RESULT AND DISCUSSION

a. pH Analysis

- ✓ No pH adjustment was done at the beginning of the fermentation.
- ✓ At the beginning of the fermentation, pH values for *L. casei* and *L. paracasei* were measured as 3,78. It was measured as 3,65 and 3,69 for *L. casei* and *L. paracasei* , respectively, after the storage.
- ✓ Statistically significant reduction was not determined, after 21st day of storage for *L. paracasei* and 14th day of storage for *L. casei* .

pH reduction which would affect to viability of *L. casei* and *L. paracasei* too much was not observed.

In a study about fermented cashew apple juice, it was explained that initial pH value was approximately 4,3 and it decreased to about 3,8 at the end of the storage, and viable cell population of *L. casei* remained around 8,5 log CFU/ml in these pH conditions after the storage.

In our study, reduction of pH did not affect unfavourably viability as well, after 42 days viability remained approximately 7,5 log CFU/ml.

Cashew a

- In a study of Yoon et al. (2004), they expressed that probiotic cultures, including L. casei, maintained their viability in low pH such as tomato juice's pH after 72 hour of fermentation at 30 ^{O}C

b. Color analysis “L”, “a”, “b”

- ✓ “L” (lightness) value increased during fermentation, it hac fluctuated during storage after the fermentation, however, it was determined that this value declined by the end of the storage compared to the beginning of the storage. In a research about viability of *L. casei* in cashew apple juice, it was determined that “L” value decreased during the storage , authors associated this reduction with increase of biomass during the storage.

✓ **“a”** (redness) value had fluctuated, too. But a decrease was determined last of the storage compared to at the beginning of the storage. In the study about viability of *L. casei* in cashew apple juice, it was explained that **“a”** value decreased during the storage, as well.

✓Reduction of a value of *L. casei* were observed no statistically significant but reduction of **“a”** value of *L. paracasei* were observed statistically significant. However, in a study about storage of açai, acerola, pomegranate and apple, was reported that **“a”** values of samples decreased any without probiotic culture.

✓It was observed that **“b”** (yellowness) value for both *L. casei* and *L. paracasei*, more decreased than at the beginning of the storage.

✓ Negative values was observed in **“b”** value.This showed that Blue was more dominant than yellow in black carrot juice.

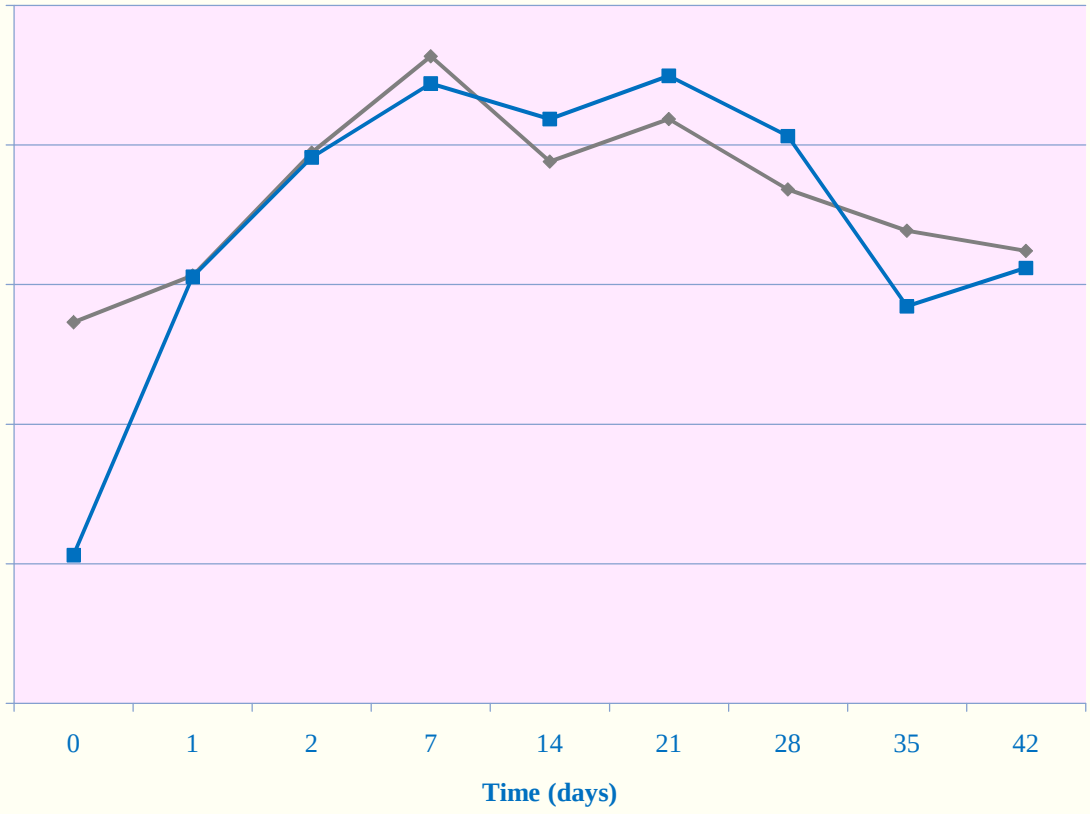
gain, it was found significant for *L.paracasei* whereas it was not stically important for *L.casei*.

it known to all, black carrot is one of the widely used natural colorant.

ven if black carrot juice colour diluted 1\10 rate , it was so intensive, reases of **“a”** and **“b”** values in black carrot juice were not olutely distinctive as visual during storage.

rom this point of view (during fermantation and storage) it could be comfortly that adding *L.casei* and *L.paracasei* did not change black ot juice characteristic colour.

c. Viability



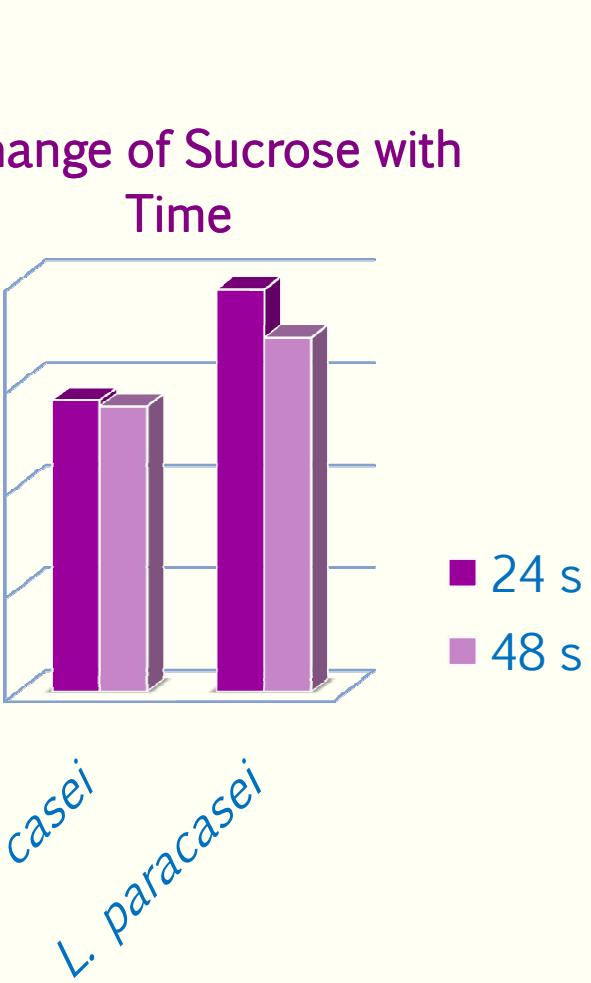
- ✓ Count of *L. casei* which was 6,5 CFU/ml, increased to about 8 CFU/ml after 48 hour fermentation. At the same time *paracasei* was 7.4 log CFU/ml at beginning of the fermentation whi was 8,4 log CFU/ml a fermentation.
- ✓ Raises of both *L. casei* and *paracasei*'s numbers which were 1 log CFU/ml after fermentation, w expected increases. After th raises, it was anticipated that num of probiotic bacteria which reached 8,5 log CFU/ml remained upper tha

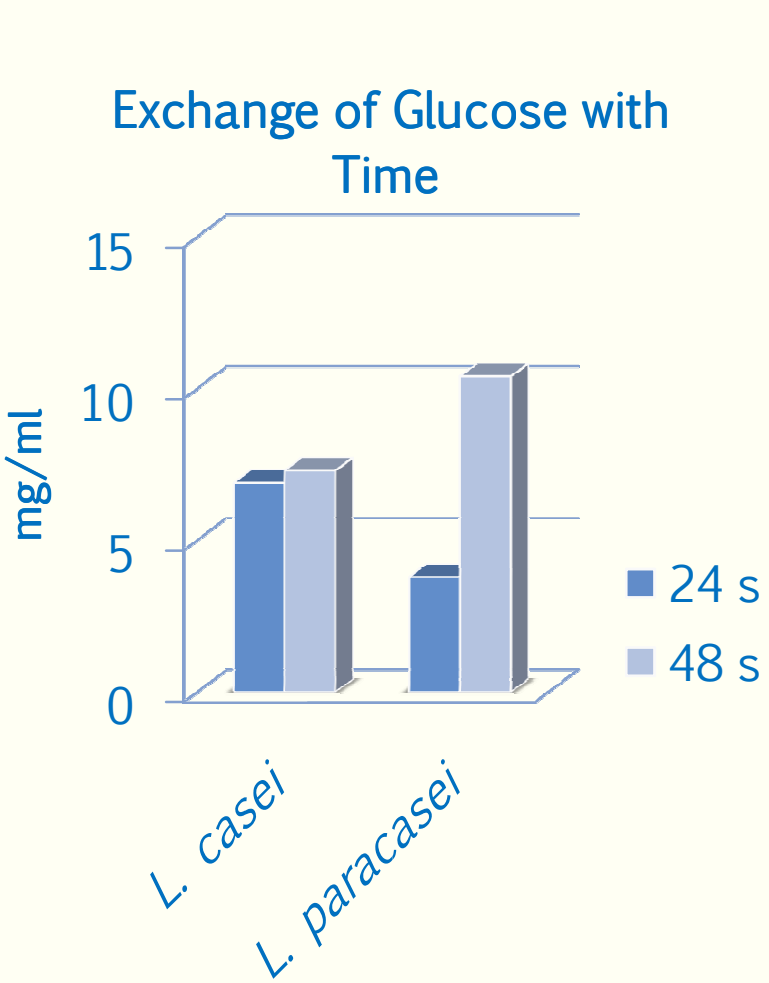
CFU/ml by the end of the end of the storage at 4^{O}C. And it occured as it expected to be. Fa rs belonging to both two bacteria were observed after 21st day, however, number of ba ed in the range of 7,5 log CFU/ml after 42^{sd} day.

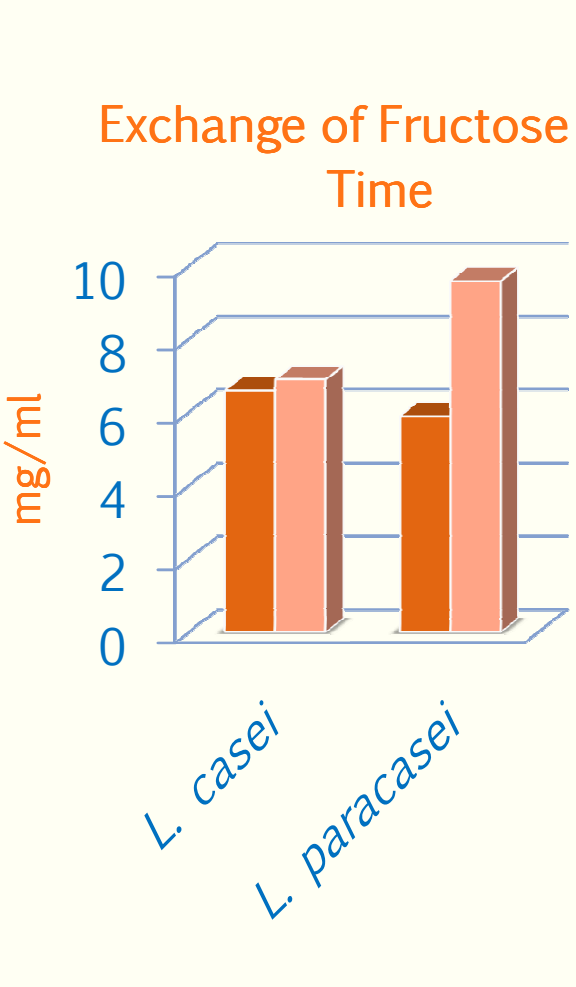
✓ In a study conducted by Yoon et al. (2005), 4 type of bacteria were inoculated into beet juice and their viabilities were examined during fermentation and storage time (28 days). While number of *L. acidophilus* was falling to $16x10^4$ CFU/ml, number of *L. delbrueckii* remained approximately $9x10^6$ CFU/ml. On the other hand, it was reported that *L. casei* and *L. paracasei* stayed alive such high rate as $7,2x10^7$ and $7,7x10^7$ CFU/ml, respectively.

✓ In another study about tomato juice performed by Yoon et al. (2004), it was notified that *L. casei* kept its viability about $1,7x10^8$ CFU/ml after the storage for 4 weeks. When it comes to another research about probiotic cashew, initial number of *L. casei* was 7,5 log CFU/ml in the fermentation and then it rose up around 8,5 log CFU/ml after the fermentation. It increased by the time 21st day and had declined after that day, and remained about 8,6 log CFU/ml on 42^{sd} day.

d. Sugar analysis

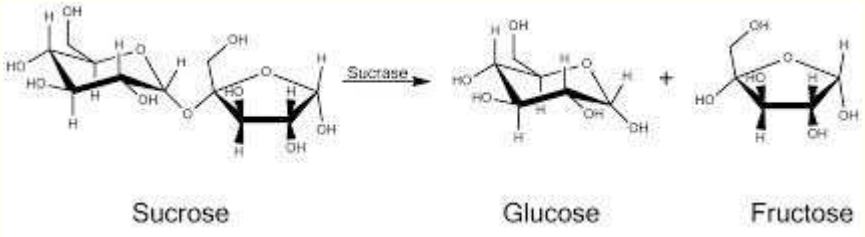






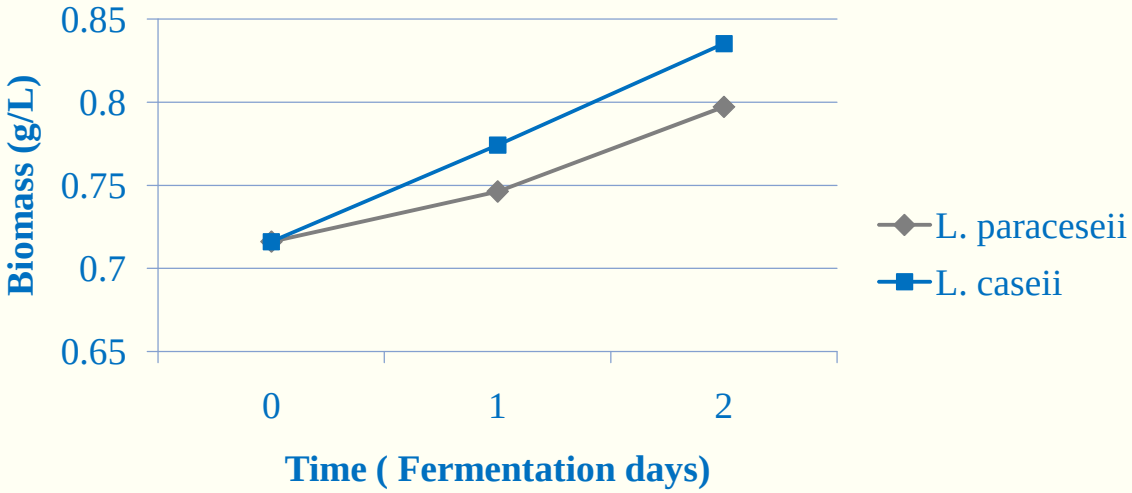
✓ In our study, it is seemed that the amount of sucrose decreased at the end of the fermentation and this falling was found to be more for *L. paracasei*. Glucose and fructose contents rose up due to decomposition of sucrose after fermentation, too. This increase was still more for *L. paracasei*. Reduction of sucrose and increment of glucose and fructose was an expected result. It indicated that probiotic bacteria used sugar resources to maintain their activity.

✓ Costa et all. (2013) performed sugar analysis in pineapple which was added with *L. casei*. Sucrose decreased during the fermentation while glucose and fructose were increasing. As a conclusion, they observed that sucrose reduced from 45 g/L to 32 g/L during the fermentation. In addition glucose and fructose contents which were approximately 4 g/L rose up to 6,5 g/L.



e. Biomass determination

✓Biomass analysis was performed only for fermentation.



✓It increased as expected during the fermentation. Biomass increase of black carrot ju enriched *L.casei* was higher than that of *L. paracasei.* This situation showed paralleli with increase of viable count and decrease of pH. Because augmentation of increase viable count in black carrot juice fermented with *L.casei* was high compared to that *L.paracasei.* Also decrease of pH was determined high in black carrot juice with *L.ca* than that of *L.paracasei.*

4. CONCLUSION

- *L. casei* and *L. paracasei* were found capable of utilizing black carrot juice for synthesis and lactic acid production without pH adjustment.
- Good viable cell counts were obtained in a fermentation time (48h.) and microbial viability was maintained within the acceptable range for 42 days and the characteristic color of the juice was preserved along fermentation and storage.
- Thus black carrot juice could be used as a raw material for fermentation of probiotic cultures and could consumed as a good healthy alternative functional beverage for consumers.

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