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Evaluation of cacao-pudding as a probiotic food carrier and sensory acceptability properties

Reyhan IRKIN[1*] and Metin GULDAS[2]

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ABSTRACT

A number of health benefits have been claimed for probiotic bacteria such as *Lactobacillus acidophilus*, *Bifidobacterium* spp. and *Lactobacillus casei*. These benefits include anti-mutagenic effects, anti-carcinogenic properties, improvement in lactose metabolism, reduction in serum cholesterol and immune system stimulation. Because of the potential health benefits, these microorganisms are increasingly being incorporated into dairy foods. Several studies in recent years have shown the benefits deriving from the ingestion of probiotics and a large number of products containing lactobacilli and bifidobacteria formulated. The purpose of this study was to develop a pudding with cacao to which probiotic microorganisms were added and investigate the viability of probiotic microorganisms during the shelf-life along 25 days at 4 °C. Organoleptic properties of the puddings were also evaluated during the storage. *Bifidobacterium animalis* ssp. *lactis* LAFTI B94, *Lactobacillus acidophilus* LAFTI L10 and *Lactobacillus casei* LAFTI L26 cultures were activated and incorporated into the product. Pudding with cacao was shown to be a good vehicle for the delivery of *Bifidobacterium animalis* ssp. *lactis*, *Lactobacillus acidophilus* during 15 and 25 days respectively and these microorganisms did not interfere in the sensorial preferences of the product except *Lactobacillus casei.* The pH values and organoleptic scores of the pudding samples, except *Lactobacillus casei* LAFTI L26 containing ones, did not change for 20 days during the storage period, statistically ($P<0.01$). The pudding containing *Lactobacillus casei* LAFTI L26 was taken the lowest sensorial scores. The all samples were lost their organoleptic properties at the 25 days of storage period.

Key words: Probiotic cacao-pudding, *Lactobacillus acidophilus, Bifidobacterium animalis* ssp. *lactis* , *Lactobacillus casei.*

IZVLEČEK

SPREJEMLJIVOST KAKAVOVEGA PUDINGA KOT OSNOVE ZA PROBIOTIČNE PREHRANSKE IZDELKE

Probiotične bakterije kot so *Lactobacillus acidophilus*, *Bifidobacterium* spp. in *Lactobacillus casei* so pomembne za pripravo živil, primernih za prehrano za varovanje zdravja. Varujejo lahko pred mutagenimi in rakotvornimi učinki, izboljšajo presnovo laktoze, prispevajo k zniževanju holesterola in stimulirajo imunski sistem. Zaradi potencialnega pomena za zdravje te mikroorganizme čedalje bolj uporabljajo pri pripravi mlečnih izdelkov. Več raziskav je v zadnjih letih pokazalo prehransko prednost uživanja probiotikov; razvili so vrsto izdelkov, zasnovanih na uporabi laktobacilov in bifidobakterij. Namen te raziskave je bil uvesti kakavov puding z dodatkom probiotskih mikroorganizmov in ugotoviti viabilnost probiotskih mikroorganizmov tekom roka trajanja 25 dni pri 4 °C. V času skladiščenja so bile preverjene tudi organoleptične lastnosti pudingov. Pri pripravi izdelkov smo uporabili kulture *Bifidobacterium animalis* ssp. *lactis* LAFTI B94, *Lactobacillus acidophilus* LAFTI L10 in *Lactobacillus casei* LAFTI L26. Kakavov puding se je izkazal kot primerna osnova za živila z *Bifidobacterium animalis* ssp. *lactis*, *Lactobacillus acidophilus* tekom 15 in 25 dni in razen mikroorganizma *Lactobacillus casei* niso neugodno vplivali na senzorične lastnosti izdelkov. Vrednosti pH in organoleptične lastnosti vzorcev pudingov se, razen enega z *Lactobacillus casei* LAFTI L26, se tekom 20 dnevnega skladiščenja niso statistično značilno spreminjale ($P<0,01$). Puding z *Lactobacillus casei* LAFTI L26 je imel najnižjo senzorično vrednost. Vsi vzorci so po preteku 25 dni skladiščenja izgubili značilne senzorične lastnosti.

Ključne besede: Probiotični kakavov puding, *Lactobacillus acidophilus, Bifidobacterium animalis* ssp. *lactis*, *Lactobacillus casei.*

[1] Balikesir University, Susurluk Vocational School, Susurluk, Balikesir, Turkey

[2] Uludag University, Karacabey Vocational School, Karacabey, Bursa, Turkey

* Corresponding Author: Dr. Reyhan Irkin, Balikesir University, Susurluk College TR10600, Susurluk, Balikesir, Turkey. Tel: +90266 865 71 53; Fax: +90266 865 71 55. E-mail address: rirkin@hotmail.com, reyhan@balikesir.edu.tr

1 INTRODUCTION

The definition of probiotics has been started during the past decade. In general, it means that a food product which contains live organisms and very beneficial for the consumer's health (Rössle et al., 2010; Lamsal and Faubion, 2009; Jay et al., 2005). The improvement of nutritional value of foods, inhibition of enteric pathogens, and alleviation of diarrhea/constipation, hypocholesterolaemic action, anticancer activity and stimulation of immune systems are beneficial effects of lactic acid bacteria which are determined mainly (Ibrahim et al., 2010; Adams and Moss, 2008; Viena et al., 2008). The most commonly used bacteria in probiotic additives are *Lactobacillus* and *Bifidobacterium*. In *Lactobacillus*, probiotic properties are present only in a restricted number of species (Elahi et al., 2008). Probiotic bacteria like bifidobacteria and lactobacilli are natural inhabitants of the human gut. They affect human health by improving the gut microbiota balance beneficially and the defenses against pathogens. The health benefits derived by the consumption of foods containing *Lactobacillus acidophilus*, *Bifidobacterium* and *Lactobacillus casei* are well documented (Shah, 2007).

One of the well-researched probiotic bacteria is *Lactobacillus acidophilus* and it is able to inhibit enteropathogens, such as *Salmonella, Listeria* and *Campylobacter*. The other most known probiotic is *Bifidobacterium lactis* Bb-12. This bacterium was originally isolated from the feces of a healthy adult and has been marketed for more than 15 years in a wide range of fermented dairy and non-dairy products. *Bf. lactis* Bb-12 has been reported to reduce the incidence of rotavirus diarrhea antibiotic-associated diarrhea and travelers' diarrhea (Espinoza and Navarro, 2010; Kun et al., 2008; Ouwehand et al., 2004). *Lactobacillus casei* has good viability in the matrices and it has probiotic features, e.g. survival in the human gastrointestinal tract and an antimicrobial effect against pathogens (Shah, 2007).

Because of the potential health benefits these microorganisms are increasingly being incorporated into dairy foods. Functional foods are defined as 'foods that contain some health-promoting component(s) beyond traditional nutrients'. Foods can be modified to become functional is by addition of probiotics (Shah, 2007). These products should have acceptable taste and suitable for the production of commercial products, too (Kearney et al., 2008). In 2001, a joint committee Food and Agriculture Organization of the United Nations/ World Health Organization (FAO/WHO) redefined probiotics as "live microorganisms in adequate amounts and confer health benefits on the host. Probiotic microorganism viability and efficacy in food products are also important during the storage. In general, the food industry has applied the recommended level of 10^6 cfu/g at the time of consumption for *Lactobacillus acidophilus*, bifidobacteria and other probiotic bacteria (Aragon-Alegro, 2007; Ross et al., 2005; Helland et al. 2004).

The dairy industry has found probiotic cultures to be a tool for the development of new functional products. Yoghurts and fermented milks are the main vehicles for probiotic cultures. In some studies have shown that some commercial dairy products such as yoghurts, do not contain adequate populations of viable probiotic bacteria during their shelf-life (Heenan et al., 2004). But nowadays, some new products such as milk-based desserts, powdered milk for newborn infants, ice-creams, butter, mayonnaise, various types of cheese, products in the form of capsules and fermented foods of vegetable origin are being found place in the international markets. There is an increased variety of products available in the market and consumers are getting more used to the probiotic concept (Ranadheera et al., 2010; Ozcan et al., 2010; Cruz et al., 2009).

The purpose of this study was to develop a pudding with cacao to which probiotic microorganisms were added and investigate the viability of probiotic microorganisms during the shelf-life along 25 days at 4 °C. Organoleptic properties of the puddings were also evaluated during the storage. *Bifidobacterium animalis* ssp. *lactis* LAFTI B94, *Lactobacillus acidophilus* LAFTI L10 and *Lactobacillus casei* LAFTI L26 cultures were activated and incorporated into the product.

2 MATERIALS AND METHODS

2.1 Probiotic Cultures, Media and Growth Conditions

Probiotic cultures of *Lb. acidophilus* LAFTIs L10 and *Bf. animalis ssp. lactis* LAFTI B94 and *Lb. casei* LAFTI L26 were obtained from DSM Food Specialties, Istanbul, Turkey. Ten grams from the each culture was weighed and shaked into 20 mL sterilized water for obtaining a homogenized culture and then immediately added and mixed for 1 kg of of cacao-pudding, separately. The cacao-puddings contained about 10^8 – 10^9 cfu probiotic microorganism/g.

MRS (deMann, Rogosa and Sharpe) D-sorbitol (10 g/100 mL) media (Tharmaraj and Shah, 2003) was used for the selective

enumeration of *Lb. acidophilus* at 37 °C for 72 h. For selective enumeration of *Bf. animalis ssp. lactis*, RCA (reinforced clostridial agar) with 0.03 g/100mL aniline blue and dicloxacillin (2 mg/mL, Sigma) were used. Plates were incubated under the anaerobic conditions at 37 °C for 48 h (Kailasapathy et al., 2008). MRS-Vancomycine agar was used for enumeration of *Lb. casei*, for preparing MRS-Vancomycine agar, 2 ml of 0.05 g vancomycine (Sigma)/100 ml solution was added to 1 L of MRS broth to obtain 1 mg/L final concentration. Agar powder was added to broth and the media were autoclaved. Inoculated plates in duplicates were incubated under anaerobic conditions at 43 °C for 72 h. The average count of the duplicate plates was used for statistical analysis.

2.2 Production of Cacao-Pudding

Full-fat (4.5 % fat) standardized and pasteurized milk, cacao powder, sucrose, corn starch and wheat-flour were used for preparing of cacao-pudding. Four pilot-scale cacao-pudding trials denoted; (C) control, (PA) probiotic cacao-pudding with *Lb. acidophilus* culture, (PB) probiotic cacao-pudding with *Bf. lactis* culture and (PC) cacao-pudding with *Lb. casei* culture, were produced in triplicate. The ingredients and quantities employed for the trials of puddings are seen in Table 1.

Table 1. Ingredients and quantities (%) employed for the production the cacao- pudding trials.

Ingredients	**Trials**			
	C	**PA**	**PB**	**PC**
Full fat milk (4.5 % fat)	75.10%	75.09%	75.09%	75.09%
Sucrose	15.03 %	15.03 %	15.03 %	15.03 %
Cacao powder	2.36 %	2.36 %	2.36 %	2.36 %
Flour	4.51 %	4.51 %	4.51 %	4.51 %
Corn starch	3.00 %	3.00 %	3.00 %	3.00 %
***Lb. acidophilus* LAFTI L10 DSL**	-	0.01%	-	-
***Bf. lactis* LAFTI B94 DSL**	-	-	0.01 %	-
***Lb. casei* LAFTI L26 DSL**	-	-	-	0.01 %
TOTAL	100	100	100	100

Each batch of cacao-pudding was produced in amounts to obtain 4 kg of the final product. For this purpose, after weighing all ingredients individually, they were all mixed together, and heated to 80–85 °C in a stainless-steel boiler about 20 min, after it was cooled to 40 °C in a water bath being continuously stirred. As soon as the mixture reached the desired temperature, cacao-pudding was separated as 4 batches. One batch was remained as control group without any probiotic culture. The probiotic cultures were added into other three batches and stirred homogenously with a sterile mixer. After the stirring of cacao-puddings, they were packaged in individual plastic cups, each one containing approximately 150 g of cacao- pudding, cooled and then stored at 4±1 °C for up to 25 days.

2.3 Microbiological analysis

Viabilities of *Lb. acidophilus*, *Bf. lactis* and *Lb. casei* were monitored during the storage period (1., 3., 5., 10., 15., 20., 25. days) for cacao-puddings PA, PB and PC. Populations of the contaminants yeasts and moulds, coliforms were also monitored for all trials of cacao-puddings studied (C, PA, PB and PC). At each sampling day, portions of 25 g were collected aseptically and blended with 225 mL of 0.1 % peptone water in sterilized blender and submitted to serial dilutions with the same diluents. For the enumeration of probiotic bacteria media and conditions which were stated at section 2.1 were applied. Potato Dextrose Agar (PDA) was used for the enumeration of yeast and moulds in samples at 25 °C for 3-5 days (Ozer et al., 2002). Total coliform counts were determined on Violet Red Bile Agar (VRBA, pH 7.4) incubated at 37 °C for 24 h under anaerobic conditions according to Martinez-Villaluenga et al. (2008). All data belonging to counts were calculated as logarithms (log cfu/g) prior to the statistical analyses.

2.4 Physico-chemical analysis

The pH value was recorded using a pH meter (Hanna HI221 Microprocessor, Hanna Instruments Inc., Woonsocket, Rhode-Island), at each sampling day. The moisture content was determined according to AOAC (1995) procedure.

2.5 Sensory Analysis

The sensory characteristics were carried out according to Metin (2006) on each day of sampling. A panel composed of 10 experienced members from our university was used to evaluate the puddings for external appearance (color), flavor, taste and texture with a point scale from 0 to 5 (0 spoiled sample and unfit for human consumption; 5, very good). PA, PB and PC samples were compared with control group puddings. The results were analyzed statistically as described in the next section.

2.6 Statistical Analysis

SPSS 15.0 software for windows (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analyses. One-way analysis of variance (ANOVA) test was done for determining mean differences. The level of significance between means was determined by the Tukey HSD test (Ozdamar, 2004).

3 RESULTS AND DISCUSSION

3.1 Physico-chemical analysis results

Average moisture of puddings was varied between 38.9 ±0.2 – 39.2±0.3 % during the storage period.

The pH values of puddings were given in Figure 1. There were no significant differences among the PA, PB and C trial groups during the storage ($p > 0.05$). But, pH value of PC group was different significantly ($p < 0.01$) after the 15th days, because of the high growth and acid production ability of *Lb. casei* than the other bacteria throughout the storage period. The optimum pH for growth of *Bifidobacterium* is 6–7 and they could not growth at pH 4.0–5.0 or below. In similarly, survival of *Lb. acidophilus* is affected by the low pH of the environment, too. But, we suppose that the milk and other protein rich ingredients such as wheat flour increase the buffering capacity of the puddings and all the bacteria's viability didn't affect from pH (Ranadheera et al., 2010; Helland et al., 2004) in the study.

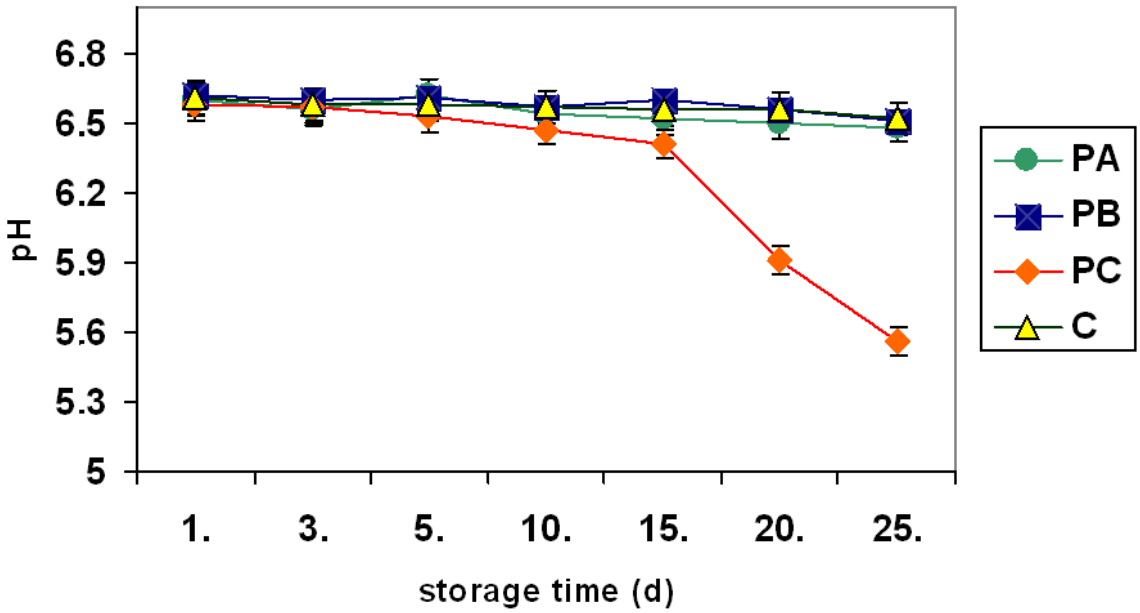

Figure 1. The pH values of cacao-puddings during the storage at 4°C. Data represent mean values of triplicate measurements, and error bars are indicated.
PA; Cacao-puddings are incorporated with *Lb. acidophilus*
PB; Cacao-puddings are incorporated with *Bf. lactis*
PC; Cacao-puddings are incorporated with *Lb. casei*

3.2 Microbial analysis results

The population of the contaminants total coliforms, yeast and moulds during storage of the different trials of cacao-puddings are shown in Table 2. Total coliforms were not detected in all puddings. Yeast and mould counts were at a level of < 1-3.9 log cfu g^{-1} during the storage.

Table 2. Population of total coliforms and yeast-mould counts during storage of the trials of cacao puddings studied.

Trials	Days	Total Coliforms	Yeast and Moulds [1]
C	1.	ND	ND
	3.	ND	ND
	5.	ND	<1-3.2
	10.	ND	<1-3.0
	15.	ND	<1-3.5
	20.	ND	<1-3.5
	25.	ND	<1-3.6
PA	1.	ND	ND
	3.	ND	ND
	5.	ND	ND
	10.	ND	ND
	15.	ND	<1-3.6
	20.	ND	<1-3.7
	25.	ND	<1-3.8
PB	1.	ND	ND
	3.	ND	ND
	5.	ND	ND
	10.	ND	ND
	15.	ND	<1-3.3
	20.	ND	<1-3.1
	25.	ND	<1-3.9
PC	1.	ND	ND
	3.	ND	ND
	5.	ND	<1-3.4
	10.	ND	<1-3.1
	15.	ND	<1-3.3
	20.	ND	<1-3.5
	25.	ND	<1-3.8

ND; Not detected

[1] ; Minimum - maximum counts obtained for all samples analyzed.

The viability of *Lb. acidophilus*, *Bf. lactis* and *Lb. casei* during storage of different trials of cacao-puddings studied is shown in Fig.2.

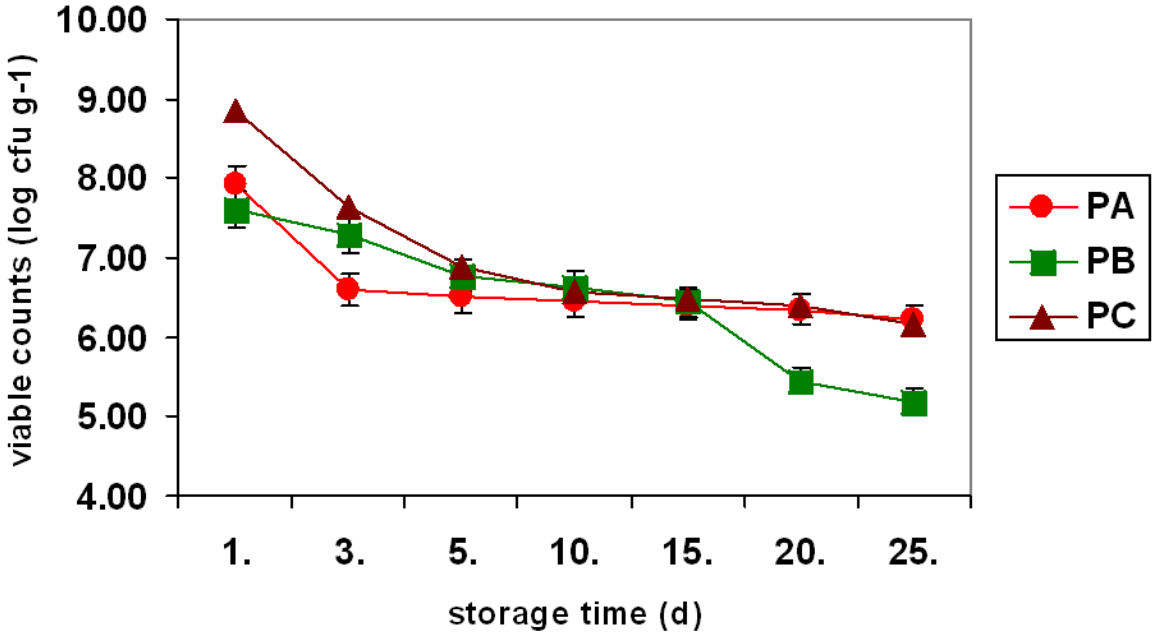

Figure 2. Survival of probiotic microorganisms in cacao-puddings stored at 4°C for 25 days. Data represent mean values of triplicate measurements, and error bars are indicated.
PA; Cacao-puddings are incorporated with *Lb. acidophilus*
PB; Cacao-puddings are incorporated with *Bf. lactis*
PC; Cacao-puddings are incorporated with *Lb. casei*

Initial counts of all probiotic bacteria were about 8-9 log cfu mL^{-1} before incorporated into the puddings in our research. *Lb. acidophilus* maintained nearly constant populations after the 3 rd day during the whole refrigerated storage in cacao-puddings and above the 6 log cfu g^{-1}. These results indicate good and the highest viability of *Lb. acidophilus* in cacao-puddings. According to Lamsal and Faubion (2009) recommended level of probiotic microorganisms in food should be available in level of 10^6 cfu g^{-1} at the time of consumption, to provide beneficial effects for consumers. The viability of *Bf. lactis* were decreased from 7.61 to 5.19 log cfu g^{-1} at the end of the storage period. The puddings with *Bf. lactis* didn't maintained probiotic property after the 15th days. There were no significant differences ($p>0.05$) for populations of *Bf. lactis* between the days 5 and 15 in the puddings. *Lb. casei* showed the good viability during the storage period in the pudding trials, too. This samples' population were very close to the counts of *Lb. acidophilus* after the 10 days.

Acid pH tolerance in probiotic bacteria is strain dependent and *Bifidobacteria* strains are more sensitive than *Lactobacillus* strain (Cruz et al., 2009). The viability of probiotics in a food matrix depends, among many factors, on the strain selected interactions between the microbial species present, production of hydrogen peroxide due to the metabolism of bacteria and acidity of the product (Cruz et al., 2009). It was reported that the most important factor affecting loss of cell viability is decreasing pH during storage (Kailasapathy et al., 2008; Shah, 2000). Probiotic bacteria populations are also influenced from the environmental temperatures. In addition to this, agitation caused from mechanical stress might result incorporation of air in a smaller population of viable cells. Most of the *Lactobacillus* and *Bifidobacterium* spp. are gut derived microorganisms. They are microaerophilic and anaerobic, respectively (Cruz et al., 2009). *B. animalis ssp. lactis* is more anaerobic than *Lb. acidophilus* and *Lb. casei*. The decrease observed in *Bifidobacteria* counts depended on incorporation of air in cacao-puddings.

In addition, lactobacilli and bifidobacteria need some complex requirements (carbonhydrates, amino acids, peptides, fatty esters and salts, etc.) to survive and that vary widely from species to species (Lamsal and Faubion, 2009).

Survival of probiotic bacteria added into dairy products were found in the researches Heenan et al. (2004) determined the suitability of non-fermented frozen vegetarian dessert as a food carrier for probiotic cultures. Their research demonstrated that this product may be used as a vehicle for probiotic bacteria

especially for *Bifidobacteria* and *Lb. acidophilus*. Possemiers et al. (2010) showed that chocolate was a good carrier for oral delivery of probiotic mixture of *L. helveticus* and *B. longum* in their study. In Helland et al. (2004) study *Lb. acidophilus* La5 and 1748, *Bf. animalis* Bb12 and *Lb. rhamnosus* showed good growth and survival in milk-based puddings at 4-6 ° C for 21 days. In similarly, Ozcan et al. (2010) study showed that high levels of viable *Lb. acidophilus* LA-5 and *B. bifidum* BB-12 in rice pudding is a good source for probiotic bacteria deliver with high sensory quality.

Ranadheera et al. (2010) stated that *Lb. paracasei subsp. paracasei* LBC 82 viability in Minas fresh cheeses increased from 6.61 up to 8.22 at 5 °C during 21 days storage and the same strain of bacteria in chocolate mousse increased slightly from 7.36 up to 7.66 log cfu g^{-1} at the same conditions demonstrating influences of different types of food product on probiotic growth and viability.

3.3 Sensory Analysis Results

As seen from sensorial scores in Table 3, the best pudding samples were the puddings with *Bifidobacteria*. The puddings containing *Lb. acidophilus* were scored with slightly lower points than bifidobacteria containing ones. The sensorial scores of control group were higher than the puddings with *Lb. casei* and lower than the puddings with *Lb. acidophilus*.

Generally, the significant changes were observed after 20 days of storage in the all pudding samples ($P<0.01$). The watery texture (syneresis) was designated by the panelists in the puddings with *Lb. casei* after 15 days of storage. Probably, high proteolytic activity of *Lb. casei* can be related with the texture defects in dairy products. Therefore, the lowest points were obtained from these samples with *Lb. casei.* In terms of flavor and taste, the sensorial scores of the samples with *Bifidobacteria* were even higher than the control samples.

Similarly, Akin et al. (2007) determined high sensorial scores for probiotic ice-cream trials produced with *Lb. acidophilus* and *Bf. lactis*. Cruz et al. (2009) reported that *Lb. acidophilus* showed good sensory properties in artisan strawberry ice-cream, too. But, also they stated that sensory property of the probiotic products respect to its acidity, pH at level 5.6 lead to better flavor and taste.

On the other hand, no significant differences between probiotic chocolate mousse and sweet whey cheese which were produced by addition of *Lb. paracasei* on sensory properties were determined (Aragon-Alegro et al., 2007; Madureira et al., 2008). Contrary, Majchrzak et al. (2010) reported some sensory differences between probiotic and conventional yoghurts. Cardarelli et al. (2008) emphasized that the chocolate mousse dessert is a suitable food for the delivery of bacterial probiotic strains with excellent viability and sensorial attributes. They also stated that, addition of *Lb. paracasei* did not make any effect on taste and aroma at 7 days of storage in chocolate mousses.

Table 3. Sensory characteristics of the cacao-pudding trials.

DAYS	Control				PB				PA				PC			
	C[1]	F[1]	TA[1]	TE[1]	C	F	TA	TE	C	F	TA	TE	C	F	TA	TE
1	5.0±0.2^{a}	4.9±0.3^{a}	4.5±0.2^{a}	4.5±0.5^{a}	5.0±0.6^{a}	4.8±0.2^{a}	4.8±0.4^{a}	4.7±0.4^{a}	4.9±0.6^{a}	4.6±0.2^{a}	4.6±0.5^{a}	4.5±0.6^{a}	4.7±0.3^{a}	4.4±0.3^{a}	4.4±0.5^{a}	4.2±0.1^{a}
3	4.9±0.3^{a}	4.9±0.2^{a}	4.5±0.6^{a}	4.3±0.2^{b}	5.0±0.4^{a}	4.8±0.5^{a}	4.8±0.2^{a}	4.7±0.3^{a}	4.9±0.3^{a}	4.5±0.5^{a}	4.4±0.3^{b}	4.4±0.2^{a}	4.7±0.4^{a}	4.3±0.3^{a}	4.3±0.2^{a}	4.2±0.3^{a}
5	4.9±0.4^{a}	4.5±0.5^{b}	4.4^{a}±0.3	4.3±0.2^{b}	4.9±0.3^{a}	4.8±0.3^{a}	4.6±0.6^{b}	4.7±0.6^{a}	4.6±0.4^{b}	4.3±0.4^{b}	4.4±0.6^{b}	4.4±0.2^{a}	4.6±0.6^{ab}	4.2±0.6^{a}	4.3±0.4^{a}	4.1±0.4^{ba}
10	4.9±0.6^{a}	4.4±0.6^{b}	4.3±0.2^{ba}	4.2±0.3^{b}	4.8±0.4^{b}	4.6±0.6^{b}	4.4±0.2^{c}	4.5±0.5^{b}	4.6±0.3^{b}	4.3±0.5^{b}	4.3±0.4^{b}	4.2±0.6^{b}	4.5±0.2^{b}	4.2±0.2^{a}	4.1±0.3^{b}	4.0±0.2^{c}
15	4.7±0.2^{b}	4.3±0.2^{b}	4.1±0.5^{c}	4.0±0.5^{c}	4.8±0.3^{b}	4.3±0.2^{c}	4.4±0.5^{c}	4.5±0.2^{b}	4.3±0.5^{c}	4.3±0.2^{b}	4.1±0.3^{c}	4.1±0.2^{b}	4.2±0.3^{c}	4.0±0.5^{b}	4.0±0.2^{b}	4.0±0.5^{c}
20	4.5±0.4^{c}	4.0±0.4^{c}	4.0±0.6^{c}	4.0±0.4^{c}	4.8±0.5^{b}	4.1±0.4^{d}	4.2±0.2^{d}	4.0±0.4^{c}	4.3±0.1^{c}	4.1±0.5^{c}	4.1±0.4^{c}	4.0±0.1^{b}	4.0±0.5^{d}	4.0±0.2^{b}	3.7±0.5^{c}	3.3±0.3^{d}
25	4.4±0.5^{c}	3.4±0.4^{d}	3.1±0.2^{d}	3.1±0.4^{d}	4.6±0.4^{c}	3.8±0.5^{e}	3.5±0.3^{e}	3.3±0.5^{d}	4.2±0.2^{c}	3.5±0.4^{d}	3.3±0.6^{d}	3.2±0.5^{c}	3.0±0.5^{e}	3.1±0.4^{c}	2.9±0.2^{d}	2.7±0.4^{e}

PB; Cacao-puddings are incorporated with *Bf. lactis*
PA; Cacao-puddings are incorporated with *Lb. acidophilus*
PC; Cacao-puddings are incorporated with *Lb. casei*

Values (± standard deviation) in a column with same superscripts are not significantly different (P<0.01).
*n: 5
[1] C: Color, F: Flavor, TA: Taste, TE: Texture
Scoring scale: very good=5, good=4, fair=3, poor=2, very poor=1, unfit for human consumption=0.

4 CONCLUSIONS

Cacao-pudding is an advantageously food can be consumed by all age groups and especially by children. In the present study cacao-pudding was shown to be a good vehicle for the incorporation of *Lb. acidophilus* during 25 days storage. The same organoleptical properties in cacao-puddings with *Bf. lactis* was only preserved for 15 days. This problem may be removed by increasing of initial counts of *Bf. lactis* into the puddings or addition of some prebiotics supplements. Although, *Lb. casei* showed good viability in the puddings, they were taken low sensorial scores during the storage. Further researches are needed to develop probiotic-cacao pudding by addition some prebiotics to improve viability of *Bf. lactis*.

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