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The Effect of Coffee Extract on the Growth and Viability of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in Probiotic Milk and Yoghurt

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ABSTRACT: The aim of this study was to determine the suitability of different doses (0, 0.4, 0.8 and 1.2%) of coffee extract on fermentation and survival of Lactobacillus acidophilus and *Bifidobacterium bifidum* in milk and yoghurt. The produced samples were examined in terms of pH, acidity and microbial count during incubation period and permanence. The number of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in the coffee milk and yoghurts were significantly higher than those in the control milk and yoghurt. Increased concentrations of coffee extract create a favorable taste in milk and yoghurt in the samples containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. The investigation showed that the yoghurt containing 0.8% coffee extract had superior taste and color. The samples with 0.4% coffee extract in milk and yoghurt had increased viscosity as compared to other samples investigated. The bioability of probiotic bacteria was measured by direct counting method. In day seven, the organoleptic properties of milk and yoghurt were evaluated. Higher sensory scores were obtained by the addition of 0.8% coffee extract in milk and yoghurt. The results suggest that coffee extract promoted the metabolic activity of lactic acid bacteria in milk and yoghurt. According to the findings, addition of coffee extract to milk and yoghurt might be recommended to take the advantage of their beneficial properties on human health attributed to the antioxidant and antimicrobial activities. Coffee extract might also enhance the functional properties of milk and yoghurt with potential therapeutic values for treatments.

Keywords: *Bifidobacterium bifidum, Coffee Extract, Lactobacillus acidophilus, Probiotic.*

Introduction

Consumers across the world are becoming more interested in foods with health promoting features as they gain more awareness of the links between food and health. Among the functional foods, products containing probiotics are showing promising trends worldwide. Probiotics such as *Lactobacillus* and *Bifidobacterium spp.* are bacterial members of the normal human intestinal flora (Tamime *et al*., 2005) that exert several beneficial effects on human health and well-being through production of short-chain fatty acids and improve the intestinal microbial balance, resulting in the inhibition of bacterial pathogens, reduction of colon cancer, improving the immune system and lowering serum cholesterol levels (Saarela *et al*., 2002).

Probiotics are recognized for their applications in dairy products, particularly yoghurts and the market for these products is still rising. To achieve the claimed health benefits, one of the most important requirements for manufacturing and marketing of probiotic yoghurt is to maintain a high number of probiotic organisms P6 log CFU/g at the point of consumption (Lourens Hattingh & Viljoen, 2001).

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However, in commercial products various probiotic lactobacilli and bifidobacteria show a decline in their viability during product's shelf life (Hull *et al*., 1984; Medina & Jordano, 1994).

Recently, the food biotechnology industry has developed a number of commercial products containing a single probiotic strain or bacterial associations of various complexities. Yoghurt has been known for its nutraceutical, therapeutic, and probiotic effects (Guler Akin *et al*., 2007).

Lactic acid bacteria and its metabolites have shown important roles in improving microbiological quality and shelf-life of many fermented food products. Dairy products have long been consumed by consumers and provide a good example of bio- preservation (Zottola *et al*., 1994).

Today LAB is a focus of intensive international research for its pivotal role in most fermented foods. Basically, for its ability to produce various anti-microbial compounds promoting probiotic properties (Temmerman *et al*., 2002) that include antitumoral activity (De vuyst and Deggest, 1999), reduction of serum cholesterol (Desmazeaud, 1996; Jack-son *et al*., 2002), alleviation of lactose intolerance (De vrese *et al*., 2001), stimulation of the immune system (Isolauri *et al*., 2001), and stabilization of gut micro flora (Gibson *et al*., 1997).

Furthermore, LAB strains synthesize short chain fatty acids, vitamins, and exopolysaccharides (EPS) that are employed in the manufacturing of fermented milk to improve its texture and viscosity (Curk *et al*., 1996).

Several factors are responsible for the viability of probiotic organisms e.g. the strains used, culture conditions, antagonism among cultures present, storage time and temperature, initial counts, hydrogen peroxide and oxygen contents in the medium, and the amount of organic acids in the product (Medina *et al*., 1994).

Probiotic organisms especially bifidobacteria grow slowly in milk due, in part, to their lack of proteolytic activity, thus requiring the incorporation of essential growth factors such as peptides and amino acids to enhance their growth (Klaver *et al*., 1993).

Considerable studies have been conducted to stimulate the growth of probiotic bacteria during yoghurt fermentation and to improve their survival until the use-by-date, by supplementing yoghurt milk with growth factors such as vitamin enriched protein hydrolysate, amino nitrogen and whey protein concentrate (Akalin *et al*., 2007; Amatayakul *et al*., 2006).

Development of dairy products with new products and flavors has potential health benefits thereby increasing sales and consumers satisfaction. Traditional preparation of yoghurt may be beneficial by including other ingredients such as soya protein, vegetables, sweet potato, pumpkin and plum (Joo *et al*., 2001) to enhance the flavor as well as the nutritional quality (Shori and Baba, 2011).

Coffee is one of the most popular beverages at a global level, appreciated not only for its taste, but also for its stimulating properties.

Coffee is a product consumed daily in the world by all social classes. Brazil is the largest producer and second consumer market in the world. The coffee has about 1 to 2.5% caffeine and other substances in greater quantity. The coffee beans (green Coffee) feature a large variety of minerals, amino acids, lipids and sugars. Additionally, the coffee also has a vitamin B, niacin (vitamin B3 or vitamin PP), and chlorogenic acids, that after roasting**,** form several compounds with pharmacological effects. Coffee beverages contain significant amounts of soluble fibre (mainly galactomannans and arabinogalactan-proteins) and phenolic compounds

(chlorogenic acids), that are well utilized by the human faecal microbiota. Although traditionally considered as containing low nutritional value, regular coffee drinking has been shown to impact on several aspects of health. Most of this evidence was obtained either from in vitro studies using static batch fermentations with faecal slurries (Plumb *et al*., 1999; Couteau *et al*., 2001; Borrelli *et al*., 2004; Gniechwitz *et al*., 2007, 2008) or in human intervention studies with by-products of spent coffee grounds, an industrial waste (Umemura *et al*., 2004; Asano *et al*., 2004).

The purpose of this study is to evaluate the effect of different dosses of coffee extract (0, 0.4, 0.8 and 1.2 %) on growth and viability of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* and functional properties of milk and yoghurt during refrigerated storage.

Materials and Methods

Coffee powder was purchased from the market (Kazerun, Iran). Low-fat sterilized milk and yoghurt (1.5% fat content) were locally purchased. Commercially available probiotic cultures of *Lactobacillus acidophilus* LAFTI® L10 and *Bifidobacterium bifidum* LAFTI® B94 were obtained from DSM Food Specialities Australia Pty Ltd. (Moorebank, NSW, Australia). MRS Agar culture medium was used for carrying out the microbial test (MERCK, Germany).

- *Preparation of ethanol extract of coffee*

Coffee extract was prepared by mixing coffee powder with ethanol (96%) in the ratio of 20:400 by soxhlet system. The extraction lasted for three hours and ethanol was evaporated on rotary evaporator. The obtained extract was percolated through a bed of activated carbon (1 g of activated carbon for every 100 mL of extract). The filtered sample was transferred to the vacuum oven for four days to concentrate the coffee extract and the extract was kept for further use in a cold (4°C) and dry place. Rotary evaporator (Heidolph model no Laboro TA4000) was used to separate the coffee extract through the process of evaporation.

- *Preparation of probiotic Bifidobacterium Bifidum milk containing coffee extract at the first passage*

In order to produce milk containing the probiotic bacterium *Bifidobacterium bifidum*, four containers each containing 1 liter of low-fat sterilized milk (1.5% fat) were considered as our four groups. The starter (*Bifidobacterium bifidum*) was added directly to all the containers, followed by adding coffee extract of 0 (Control sample), 0.4, 0.8, and 1.2% to all the containers and they were finally placed in the incubator at 38°C. The acidity test was performed approximately every 2 hours until reaching 42° Dornic.

The samples were then taken out of incubator and transferred to a refrigerator and stored at 2°C. The produced probiotic milk was evaluated once every 7 days by counting the microorganisms using direct counting method.

- *Preparation of probiotic Bifidobacterium bifidum yoghurt containing coffee extract at the second passage*

In order to produce *Bifidobacterium bifidum* yoghurt, 4 containers were provided and 1 liter of the low - fat sterilized probiotic milk (1.5 % fat) from the control group at the first passage and the starter of low-fat yoghurt (1.5%) were added to each container.

Different concentrations of coffee extract (0, 0.4, 0.8 and 1.2%) were added respectively to the containers and mixed properly, therefore the coffee extract was uniformly dissolved. All the containers were placed in the incubator at 38°C. Approximately every 2 hours, the acidity

and the pH determinations were carried out until the acidity reached 90° Dornic. The samples were taken out of the incubator and transferred to a refrigerator and stored at 2°C. The produced probiotic coffee yoghurt was evaluated every 7 days by counting the microorganisms using direct counting method and after 7 days the yoghurt was evaluated for sensory properties, using questionnaires filled by 15 participants. The respondents were asked to rate the factors of scent, taste and permanence on a scale ranging from very good, good, medium, to weak. The results were analyzed in a statistical descriptive test by SPSS version 17software.

- *Preparation of probiotic Lactobacillus acidophilus milk containing coffee at the first passage*

All the procedures were followed as mentioned earlier with the exception of using *Lactobacillus acidophilus* instead of *Bifidobacterium bifidum.*

- *Preparation of probiotic Lactobacillus acidophilus yoghurt containing coffee at the second passage*

All the procedures were followed as mentioned earlier with the exception of using *Lactobacillus acidophilus* instead of *Bifidobacterium bifidum.*

Having produced the above-mentioned products, each product was stored in a disposable container placed in a refrigerator for 21 days. During this period, each sample was tested after 1, 7, 14 and 21 days for acidity, pH, and sensory properties.

- *Statistical analysis*

All the above experiments were repeated three times with each test carried out in triplicate order. SPSS17 was used for one-way analysis of variance for all data, and significant differences ($p < 0.05$) among the means were determined by the least significant difference test.

Results and Discussion

Table 1 shows the degrees of acidity for coffee *Lactobacillus acidophilus* milk and yoghurt during storage period in the refrigerator. Table 2 shows the growth rates of microorganisms for coffee *Lactobacillus acidophilus* milk and yoghurt during storage in the refrigerator. Table 3 shows the microbial growth on MRS-A cultivation environment for coffee *Lactobacillus acidophilus* milk and yoghurt during storage in the refrigerator.

Table 4 shows the acidity degrees for coffee *Bifidobacterium bifidum* milk and yoghurt during storage in the refrigerator. Table 5 shows the growth rates of microorganisms for coffee *Bifidobacterium bifidum* milk and yoghurt during storage in the refrigerator.

The microbial growth on MRS-A cultivation environment of *Bifidobacterium bifidum* coffee milk and yoghurt during refrigeration for 21 days was poor since *Bifidobacterium bifidum* has good growth on MRS broth. The microbial growth of *Bifidobacterium bifidum* on MRS broth was high. It was observed that *Bifidobacterium bifidum* has high inhibitory activity on MRS agar during 21 days of storage. These results indicated that coffee was a suitable ingredient for this microorganisms that kept it viable up to the end of fermentation (21days). All the tested *Bifidobacterium bifidum* was capable of growing well on coffee milk and yoghurt without nutrient supplementation.

Figures 1 – 4 present variations in acidity in *Lactobacillus acidophilus* coffee milk and yoghurt and *Bifidobacterium bifidum* coffee milk and yoghurt.

Table 1. The acidity level based on Dornic degree for coffee *Lactobacillus acidophilus* milk and yoghurt during storage in the refrigerator

Acidity level in Dornic degree									
Coffee Milk	**1th day**	**7th day**	**14th day**	**21th Day**	**Coffee Yoghurt**	**1th day**	**7th Day**	**14th day**	**21th day**
0%	48	52	52	55	0%	92	97	98	98
0.4%	57	67	73	89	0.4%	103	111	121	139
0.8%	58	60	69	78	0.8%	108	124	132	150
1.2%	53	58	65	73	1.2%	111	119	129	141

Table 2. Growth rates of microorganisms for coffee *Lactobacillus acidophilus* milk and yoghurt during storage in the refrigerator

Coffee Milk	**1th day**	**7th day**	**14th day**	**21th Day**	**Coffee Yoghurt**	**1th day**	**7th Day**	**14th day**	**21th day**
0%	18.5×10^{10}	14×10^{10}	12.5×10^{10}	4.75×10^{10}	0%	13.25×10^{10}	11.75×10^{10}	8.5×10^{10}	5×10^{10}
0.4%	53.5×10^{10}	65.5×10^{10}	78.5×10^{10}	49×10^{10}	0.4%	45×10^{10}	43×10^{10}	49.5×10^{10}	39.75×10^{10}
0.8%	58.25×10^{10}	56.25×10^{10}	45×10^{10}	40.75×10^{10}	0.8%	51.5×10^{10}	38.5×10^{10}	13.75×10^{10}	37.5×10^{10}
1.2%	29.5×10^{10}	43.75×10^{10}	29.75×10^{10}	25.5×10^{10}	1.2%	60.25×10^{10}	26.25×10^{10}	25.75×10^{10}	19.5×10^{10}

Table 3. Microbial growth on MRS-A cultivation environment for coffee *Lactobacillus acidophilus* milk and yoghurt at refrigerator during storage in the refrigerator

Coffee Milk	**1th day**	**7th day**	**14th day**	**21th Day**	**Coffee Yoghurt**	**1th day**	**7th Day**	**14th day**	**21th day**
0%	95×10^{10}	395×10^{9}	285×10^{9}	205×10^{9}	0%	105×10^{10}	80×10^{10}	175×10^{9}	130×10^{9}
0.4%	125×10^{10}	185×10^{10}	160×10^{10}	95×10^{10}	0.4 %	130×10^{10}	210×10^{10}	145×10^{10}	110×10^{10}
0.8%	365×10^{10}	105×10^{10}	90×10^{10}	235×10^{9}	0.8 %	175×10^{10}	105×10^{10}	90×10^{10}	375×10^{9}
1.2%	225×10^{9}	365×10^{9}	325×10^{9}	215×10^{9}	1.2 %	105×10^{10}	85×10^{10}	235×10^{9}	160×10^{9}

Table 4. The acidity level based on Dornic degree for coffee *Bifidobacterium bifidum* milk and yoghurt during storage in the refrigerator

Acidity level in Dornic degree									
Coffee Milk	**1th day**	**7th day**	**14th day**	**21th Day**	**Coffee Yoghurt**	**1th day**	**7th Day**	**14th day**	**21th day**
0%	49	51	53	56	0%	94	96	98	107
0.4%	56	60	71	78	0.4%	104	121	124	143
0.8%	59	69	75	91	0.8%	114	129	145	168
1.2%	60	65	69	72	1.2%	117	122	125	132

Table 5. Growth rates of microorganisms for coffee *Bifidobacterium bifidum* milk and yoghurt during storage in the refrigerator

Coffee Milk	**1th day**	**7th day**	**14th day**	**21th Day**	**Coffee Yoghurt**	**1th day**	**7th Day**	**14th day**	**21th day**
0%	13.5×10^{10}	25.25×10^{10}	12.25×10^{10}	16.75×10^{10}	0%	18.5×10^{10}	25.5×10^{10}	3.75×10^{10}	9.75×10^{10}
0.4%	27.5×10^{10}	41.75×10^{10}	65.75×10^{10}	35.5×10^{10}	0.4 %	21.5×10^{10}	39.75×10^{10}	12.25×10^{10}	22.5×10^{10}
0.8%	42.25×10^{10}	71.5×10^{10}	89.5×10^{10}	51.5×10^{10}	0.8 %	58.5×10^{10}	56.25×10^{10}	78.75×10^{10}	30×10^{10}
1.2%	40.5×10^{10}	35.25×10^{10}	22.75×10^{10}	46.5×10^{10}	1.2 %	71.5×10^{10}	69.75×10^{10}	22.5×10^{10}	17.5×10^{10}

In the present study, the effects of coffee extract on the growth and viability of the bacteria *Bifidobacterium bifidum* and *Lactobacillus acidophilus* in probiotic milk and yoghurt were investigated.

The acidity, pH and survival of the bacteria in coffee probiotic milk and yoghurt were evaluated at two hours intervals till reaching the acidity of 42°Dornic for milk and 90°Dornic for yoghurt in the incubator at 38°C.

Lactobacillus acidophilus milk containing 0.4 and 0.8% coffee extract reached the acidity of 42°Dornic followed by 1.2, and 0% concentration. Once they reached this acidity level, they were transferred to a refrigerator at 2°C. The storage time in the refrigerator was determined to be 21 days.

In direct microbial count for the first day, the highest counts were in the following order for 0.4, 0.8 and 1.2 percent coffee extract and control samples.

The *Lactobacillus acidophilus* yoghurt with 0.8% coffee extract reached the acidity of 90°Dornic followed by the samples with 1.2 and 0.4 percent coffee extract and the control and once this acidity level was reached, they were transferred to a refrigerator at 2°C .The storage time in the refrigerator was found to be 21days.

Although the basic feature of the probiotic products consumption is their medicinal effects (bio value), their associated sensory properties are also important. In other words, sensory properties rather than medicinal effects play the most important role in their daily consumptions. Among the probiotic products, fermented ones especially the probiotic yoghurt is popular worldwide for its unique sensory properties (Mortazavian and Sohrabvandi, 2006).

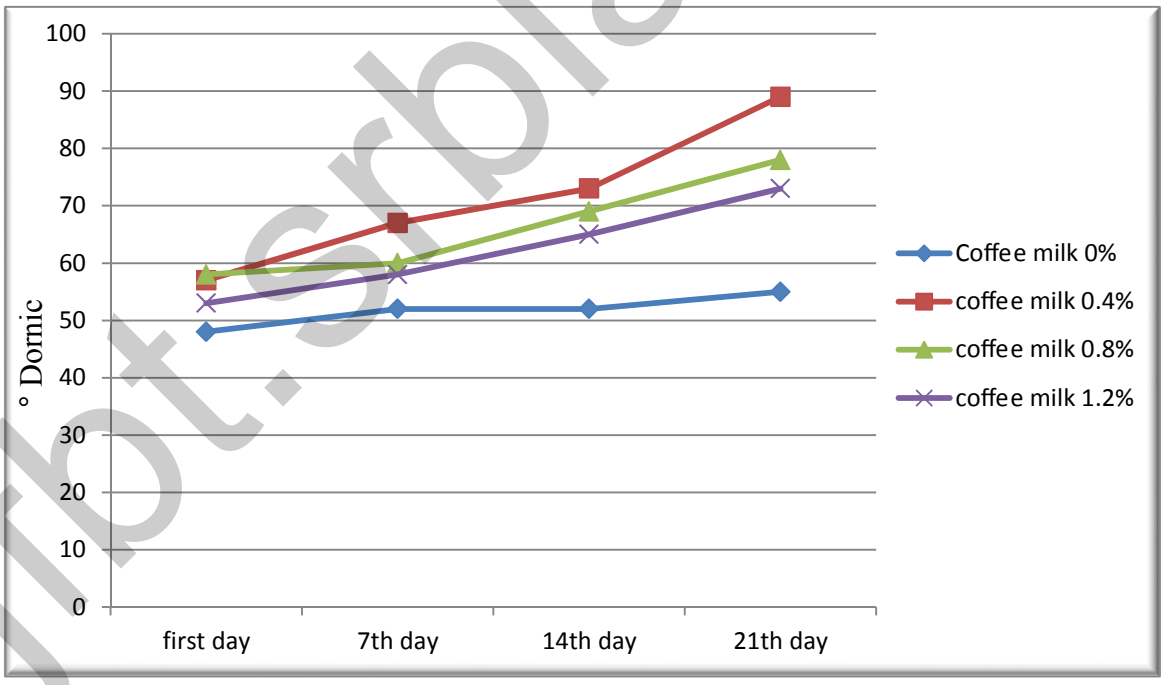

Fig. 1. Variation curve of acidity value in coffee *Lactobacillus acidophilus* milk during refrigeration

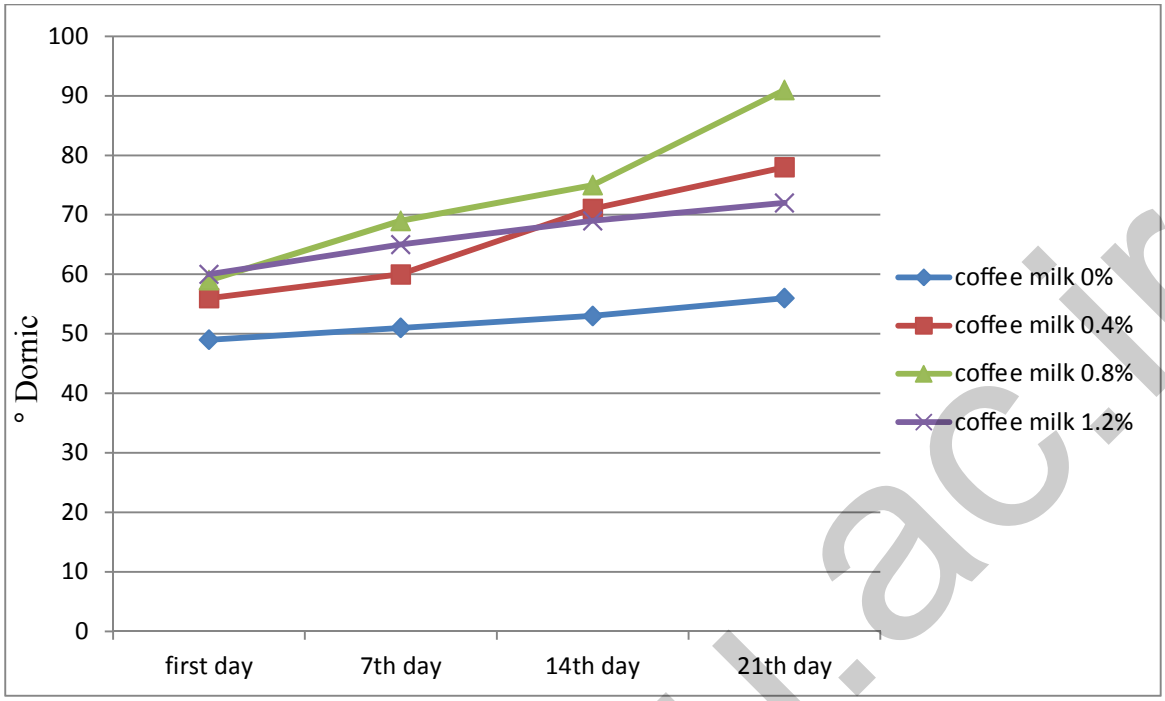

Fig. 2. Variation curve of acidity value in coffee *Bifidobacterium bifidum* milk during refrigeration

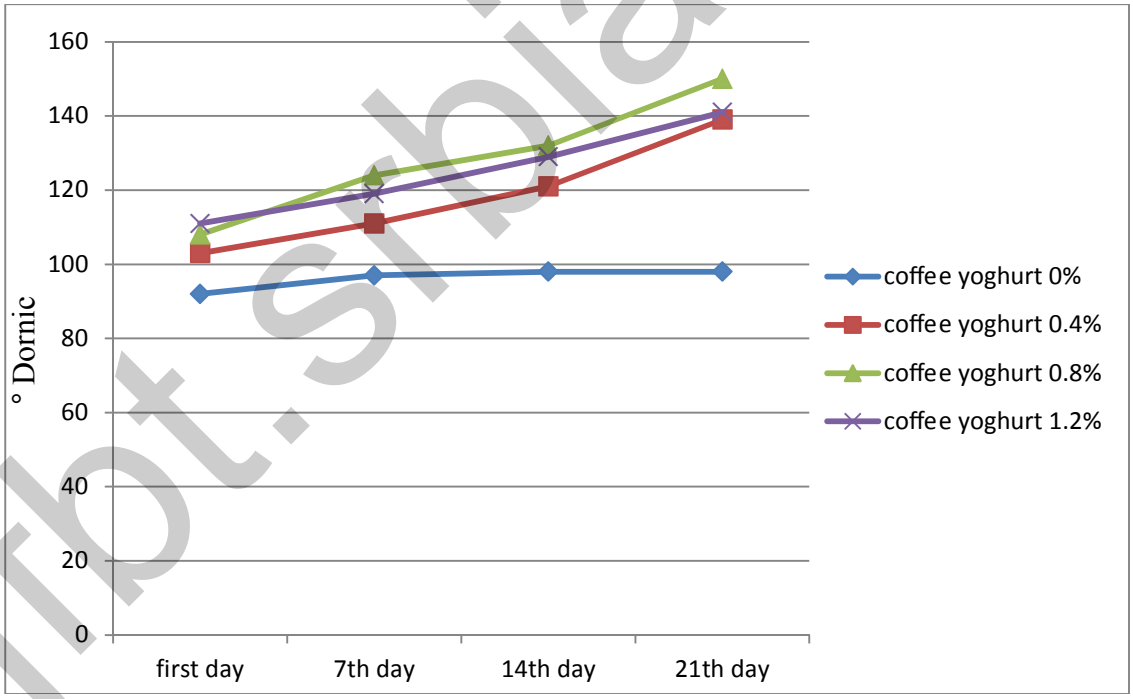

Fig. 3. Variation curve of acidity value in coffee *Lactobacillus acidophilus* yoghurt during refrigeration

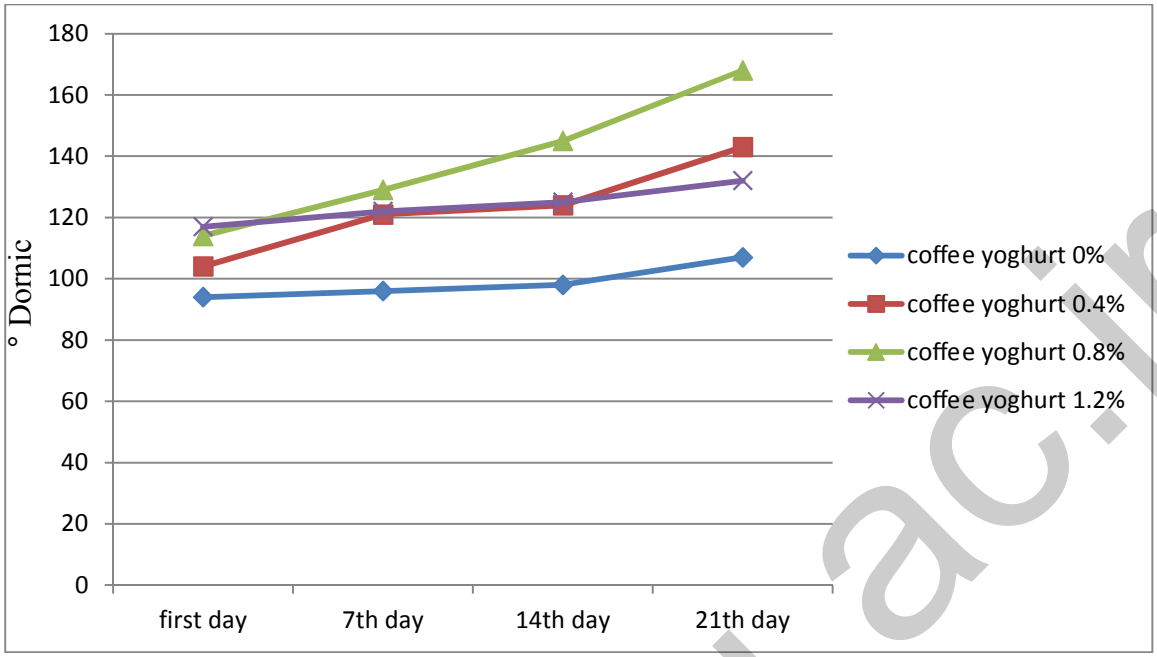

Fig. 4. Variation curve of acidity value in coffee *Bifidobacterium bifidum* yoghurt during refrigeration

The sensory evaluation was performed by 15 participants for the probiotic *Lactobacillus acidophilus* yoghurt with varying concentrations of coffee extract after seven days. There were significant differences between the samples ($p < 0.05$) and it was shown that the increase of coffee extract gives rise to favorable taste, color, scent and thickness.

The minimum required level of probiotic bacteria to be useful for the consumer's body is 10^7CFU.ml^{-1} of living bacteria. The level in the present study was found to be 10^{10}, that is regarded beneficial for the consumers (Marhamatizadeh *et al*., 2009).

Evaluation of the samples on MRS agar indicated that *Lactobacillus acidophilus* with coffee extract had counts equal to logarithmic 10^9 in day 7, and the sample product with 0.4% and 0.8% coffee extract possessed the highest counts.

Bifidobacterium bifidum milk containing 0.8 and 0.4% coffee extract reached the acidity of 42°Dornic faster than others, followed by the milk with 1.2% extract and finally the control. Once reached the acidity of 42°Dornic, the samples were transferred to a refrigerator at 2°C. The permanence of the product in the refrigerator was determined to be 21 days during which the acidity of control sample was lower than other samples.

As revealed in direct microbial counting, the counts after 7 days were higher, as compared to day 1, for all the coffee extract concentrations, but possessed the logarithmic coefficient of 10^9. The bactericidal and inhibitory effect of low pH was stronger for *Bifidobacterium bifidum* than *Lactobacillus acidophilus* and it seems that during the storage time and enhanced fermentation process, decreased pH caused decreased growth of *Bifidobacterium bifidum*.

The first hours of production, the *Bifidobacterium bifidum* yoghurt with 0.8 and 0.4% coffee extract reached the acidity of 90°Dornic, followed by the yoghurt sample with 1.2% coffee extract and the control. They were transferred to a refrigerator at 2°C, once the desired acidity (90°Dornic) was reached.

The product permanence in the refrigerator was found to be 21 days. No

significant difference was observed in the *Bifidobacterium bifidum* yoghurt with coffee extract in terms of color, thickness, taste and scent. The sample with 0.8% had the highest bacterial counts, as the samples were evaluated by direct counting method.

The results of the studies concerned with the probiotic bacteria have demonstrated that the increased concentration of malt and soya caused increases in the microorganism growth and acidity that in turn resulted in a shorter incubation time for the desired acidity. In a study concerned with the effects of soya powder on the growth of the bacteria, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, in probiotic products, it was demonstrated that the shelf life for the acidity reaching the desired level during incubation decreased for milk with both bacteria and combined soya and malt, as compared to the milk with only soya. As for the yoghurt with both bacteria, the same results were obtained and incubation time for the yoghurt with malt and soya was decreased (Marhamatizadeh *et al*., 2009; 2011).

The effect of honey on the growth of the above-mentioned bacteria was investigated and the results indicated that yoghurt with only Lactobacillus acidophilus tasted more sour than the yoghurt with both bacteria. The products containing *Bifidobacterium bifidum* were compared to those with *Lactobacillus acidophilus* and indicated that the former had slower growth rate and also tasted less sour and were of longer permanence. The taste was not favorable when the concentration of honey was increased (Marhamatizadeh *et al*., 2010).

In a separate work the effect of cinnamon on bacterial growth was studied and was concluded that the increased cinnamon concentration promoted the growth of the bacteria in probiotic milk and yoghurt (Yaghtin, 2010).

Further works concerned with spearmint garlic, dill extract and juices were carried out on the bacterial growth and concluded that these products promoted the growth of bacteria in probiotic milk (Marhamatizadeh *et al*., 2011).

The effect of permeate on the growth and survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* was investigated and indicated that permeate was a suitable support for intestinal bacteria (Marhamatizadeh *et al*., 2012).

Conclusion

The present work demonstrates that the presence of coffee extract at 0.4%, 0.8% and 1.2 %(w/v) have positive effect on fermentation and indicates the survival of probiotic bacteria in milk and yoghurt during three-weeks storage period at 2°C. All tested strains showed a good growth rate in coffee milk and yoghurt without added nutrients. It seemed that the nutrients are available in acceptable forms and in optimal concentrations in the tested coffee milk and yoghurt. The viability of the probiotics is essential for the quality of the fermented dairy products. The decrease in the number of probiotic bacteria in coffee milk and yoghurt during 21 days could be avoided by strain selection and the use of greater initial inoculum levels. It is important to emphasize that all the products possessed excellent stability during 21 days of storage. It can be concluded that the addition of coffee extract might cause an increase in viscosity and consistency of the final product by promoting a major protective effect on the gel-factors that have great importance on the product acceptability. When compared with commercial yoghurt, the coffee-flavored milk and yoghurt presented satisfactory rheological properties. The results might suggest that coffee extract can be successfully used in formulation of dairy products.

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