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Effects of Inulin and Oligofructose levels on survival of probiotic bacteria and sensory properties in synbiotic Doogh

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ABSTRACT

Background: The use of probiotic Doogh as a functional dairy product has gained popularity in Iran. Incorporating probiotic bacteria and maintaining their viability in Doogh can contribute to improved consumer health.
Aims: The purpose of this research was to investigate the effects of adding inulin (1%, 2% and 3%w/w), oligofructose (1%, 2% and 3%w/w) and inulin- oligofructose blends (1-1%, 2-2% and 3-3% w/w) as prebiotic components, compared to a control sample (without prebiotics). These were combined with *Bifidobacterium lactis* and *Lactobacillus acidophilus* as starter cultures (1%) to manufacture synbiotic Doogh.
Methods: The study evaluated the physicochemical and sensory properties of Doogh, along with the viable counts of probiotic bacteria, throughout the storage period.
Results: Significant differences ($P<0.05$) were observed across the treatments for SNF(Solids-Not-Fat), *Bif. lactis* and *L. acidophilus* bacterial counts and sensory characteristics (taste and flavor attributes). Furthermore, Doogh containing 1% inulin was associated with the maximal viability of *L. acidophilus* on 21st storage day, while Doogh containing 1% oligofructose showed minimal viability. Similarly, Doogh containing 2% inulin showed maximal viability for *Bif. lactis*, whereas the control sample showed minimal viability.
Conclusion: Overall, the results indicated that incorporating inulin and oligofructose can produce synbiotic Doogh containing probiotic bacteria with enhanced survival rates, positioning it as a functional dairy drink.

Introduction

In recent years, global consumption of prebiotics and probiotics has flourished due to their association with improved health and a reduced risk of disease. Within the preceding decade, more than 500 new products have entered the market (Ashraf & Shah, 2011). Bifidobacteria, as probiotic bacteria are primarily utilized in dairy products, especially beverages, yoghurts and fermented milks (Castro et al.,2013). Many diverse types of fermented dairy products exist worldwide under various names. examples include yoghurt beverages in Europe, Kumiss and Kefir in the Middle East, Ayran in Turkey and Doogh in Iran (Ghorbani Gorji et al., 2011). Traditionally, Doogh is made by blending yoghurt, water, a small amount of salt and certain aqueous extracts of local herbs (Joudaki et al., 2013). Recently, Doogh consumption has become prevalent in Iran and other Asian regions. Due to the presence of probiotic bacteria and prebiotics, it offers many health benefits that enhance the nutraceutical value of the final product (Azarikia & Abbasi, 2010). The primary site of activity for probiotics is the gastrointestinal tract, particularly the colon. Key functions of probiotics include preventing gastrointestinal disorders, boosting the immune system, exhibiting anti-cancer properties, reducing cholesterol, improving joint disease management, producing various enzymes, demonstrating antimicrobial activity and enhancing lactose metabolism. Foods containing probiotics are now recognized as leading functional food products, their health benefits are amplified by prebiotics. Prebiotics are food ingredients that confer beneficial effects on host health through modulation of intestinal flora, achieved by stimulating the activity and/or growth of probiotics. One strategy to maintain high viability of probiotic bacteria in both the intestine and fermented dairy products until consumption is through prebiotic supplementation. These components can also modify the sensory profile, rheological properties and physicochemical characteristics of probiotic fermented dairy beverages (Oelschlaeger, 2010). Consumption of synbiotic products-combining of inulin (a prebiotic) with fermented milk produced by *Bifidobacterium bifidum* can increase bifidobacteria populations in the large intestine (Chouraqui et al., 2004). Prebiotics are indigestible carbohydrate dietary fibers that stimulate the growth of bacteria like *bifidobacterium* and *lactobacillus* in the colon, thereby improving host health (Roberfroid, 2005). In vivo and in vitro studies indicate that fermentation of inulin and fructans selectively stimulates *bifidobacteria* growth in humans (Karimi et al., 2015). The recommended daily dose of inulin for enhancing healthy bacterial microflora is 2.5-10 g. As effects occur dose-dependently, 2.5-5 g daily may be insufficient for significant bifidogenic effects (Kelly, 2009). Research shows that *Bifidobacterium lactis* and *Lactobacillus casei* can grow in basal media supplemented with inulin (Karimi et al., 2015). Nikmaram et al. (2016) investigated the effects of pH, inulin and storage duration on viable *Lactobacillus* counts in a probiotic fruity yoghurt beverage using Monte Carlo simulation. Their results indicated that *Lactobacillus casei* growth was significantly affected by inulin concentration and pH, whereas *Lactobacillus acidophilus* growth was less influenced and fell below 106 CFU/mL by the end of storage. adding prebiotics like oligosaccharides and inulin which possess bifidogenic properties and minimal flavour impact can promote high viability of *Bifidobacterium lactis* in dairy products (Roberfroida, 2007; Roberfroidb, 2007). Microencapsulation (e.g., alginate beads) combined with 1–2% inulin further improved probiotic viability by protecting cells from acidic and oxidative stress in Doogh (Qaziyani, et al., 2019). While most studies focus on inulin, oligo-fructose (a short-chain fructo-oligosaccharide, FOS) is similarly effective. For example, 0.5% FOS combined with *Bacillus subtilis* synergistically enhanced disease resistance in other food systems, suggesting potential for Doogh applications (Pawal et al., 2023). De Castro et al. (2009)

investigated the influence of oligofructose addition on the characteristics of fermented probiotic lactic drinks. Their results showed that prebiotic supplementation (oligofructose) resulted in beverages with higher total carbohydrates and total solids content, without altering other physicochemical characteristics, including color. Moreover, sensory evaluation selected oligofructose containing drinks over the control, indicating good overall acceptance, most judges also indicated willingness to purchase the product. Da Silveira et al. (2015) investigated the effects of a combination of inulin and oligofructose with goat cheese whey on the physicochemical characteristics and sensory acceptability of a probiotic chocolate goat dairy beverage. *Bifidobacterium lactis* counts ranged between 6 and 8 log CFU/ mL. Formulation F4 (6 g. 100 mL-1 prebiotics and 45 mL. 100 mL-1 whey) displayed the highest median sensory scores for aroma and flavor, likely associated with its higher whey and prebiotic content. Thus, F4 was identified as the optimal formulation for the beverage. Therefore, this study aimed to evaluate the effects of inulin (1%, 2% and 3% w/w), oligofructose (1%, 2% and 3%w/w) and inulin- oligofructose blends (1-1%, 2-2% and 3-3% w/w) as prebiotic components (versus a no-prebiotic control) in synbiotic Doogh produced with *Bif. lactis* and *L. acidophilus* starter cultures (1%). Physicochemical properties, sensory characteristics, and viable probiotic counts were assessed throughout storage.

Materials and Methods

Materials

Probiotic starter cultures (ABY-2, Christine Hansen, Denmark) containing Lactobacillus acidophilus and Bifidobacterium bifidum were prepared. Also, prebiotic compounds including inulin and oligofructose were prepared from Beneo Orafti, Belgium. Microbial culture media MRS-bile Agar was provided from Merck (Darmstadt, Germany).

Preparation of synbiotic Doogh

Doogh was prepared by first inoculating milk with a standard yogurt starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*, Chr. Hansen, Denmark; 2% w/w) to produce yogurt. After fermentation (42°C, 4–6 h; pH 4.6), this yogurt was then blended with water, salt (1% w/w), and other ingredients using a magnetic stirrer (500 rpm, 10 min). In this study, prebiotic compounds - inulin (1%, 2%, 3% w/w), oligofructose (1%, 2%, 3% w/w), and inulin-oligofructose blends (1:1%, 2:2%, 3:3% w/w) were incorporated into Doogh alongside probiotic bacteria (*Bifidobacterium lactis* and *Lactobacillus acidophilus*; 1% inoculation) to create synbiotic formulations. These were compared to a control (no prebiotic) to assess physicochemical properties, sensory characteristics, and viable probiotic counts during refrigerated storage.

Chemical analysis of produced Doogh

Physicochemical characterization of Doogh samples was performed according to Iranian National Standard No. 11324 (equivalent to ISO 1211 | IDF 1 for milk fat; ISO 2446 | IDF 226 for solids-not-fat). Analyses were conducted at four storage intervals (Days 1, 7, 14, 21) in triplicate: Fat content: Gerber acid hydrolysis method (Standard 5.3), Solids-not-fat (SNF): Gravimetric method after drying at 102°C ± 2°C (Standard 9.1), Titratable acidity: Expressed as % lactic acid via NaOH titration (0.1N) to phenolphthalein endpoint (pH 8.3; Standard 11.4).

Microbiological analyses

MRS-bile agar medium (MRS agar: Merck, Darmstadt, Germany and bile: Sigma-Aldrich, Inc., Reyde, USA) was used for the selective enumeration of *L. acidophilus* and *B. lactis* in the ABY culture composition according to Mortazavian et al. (2006), by applying the subtractive enumeration method (SEM). The plates were incubated at 37°C for 3 days under aerobiosis and anaerobiosis. Anaerobiotic conditions were produced using the GasPac system (Merck, Darmstadt, Germany). Viable probiotic cell populations were enumerated throughout the refrigerated storage period, at 4-day intervals.

Sensory assessment of probiotic Doogh

Sensory evaluation was conducted using a 5-point hedonic scale (1 = dislike extremely; 5 = like extremely) to assess consumer acceptance of probiotic Doogh samples. Nine semi-trained panelists (aged 25–45 years, familiar with fermented dairy products) evaluated organoleptic attributes, Flavor (sourness, sweetness, herbal notes), Taste and aroma (fermented, acidic). Samples (30 mL) were served at room temperature (20°C ± 1) in clear glasses coded with random 3-digit numbers. Panelists performed evaluations in individual sensory booths under white lighting. Between samples, panelists cleansed palates with unsalted crackers and filtered water.

Statistical analysis

For statistical analysis, repeated-measures analysis of variance (ANOVA) was used to evaluate the effects of treatments; Control (no prebiotic), inulin (I1%, I2%, I3%), oligofructose (O1%, O2%, O3%), and inulin-oligofructose blends (IO1:1%, IO2:2%, IO3:3%) and storage times (1, 7, 14 and 21days). Based on significant ANOVA results ($P<0.05$), Duncan's test was applied to identify pairwise differences between treatment groups. All analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC). A general linear model (GLM) procedure was employed, with least square means difference tests determining statistical significance ($P<0.05$). Data are reported as mean ± standard error of the mean (SEM).

Results and discussion

General properties of produced Dooghs

The Analysis of Variance(ANOVA) results for the effects of storage time and treatments on qualitative and physicochemical properties of synbiotic Doogh with 1% starter addition are presented in Table 1.

Table 1. ANOVA analyses of the influences of various times and treatments on the several parameters in the microbial starter 1%

CV	df	SNF	Acidity	L. acidophilus	*Bif. lactis*	Taste and Flavor
Time	3	0.73^{ns}	142.05^{**}	315.10^{**}	733.44^{**}	0.04^{ns}
Treatment	9	4.05^{**}	6.26^{**}	10.86^{**}	0.2^{ns}	2472.38^{**}
Time* Treatment	27	1.66^{*}	2.38^{**}	7.41^{**}	0.2^{ns}	0.4^{ns}

ns: non-significant, *: Significant at $P<0.05$, **: Significant at $P<0.01$

Protein and fat content showed no significant diferences ($P>0.05$). ANOVA revealed that storage time significantly affected acidity and probiotic viability ($P<0.05$), but not solids non-fat (SNF) content or taste/flavor attributes ($P>0.05$). Conversely, treatment type had no significant effect on acidity ($P>0.05$) but significantly influenced SNF content, probiotic viability, and taste/flavor attributes ($P<0.05$). Figure 1 shows significant acidity changes in synbiotic Doogh during storage. Acidity increased progressively with storage time due to organic acid production by growing probiotic bacteria. Figure 2 demonstrates that the highest and lowest SNF contents occurred in the 3% inulin and control treatments, respectively.

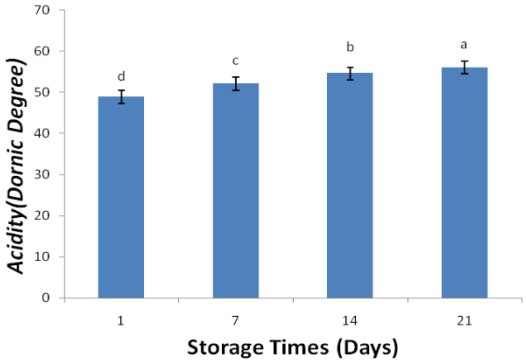

Fig. 1. Acidity of Dooghs at various storage days in the microbial starter inoculated with1%.

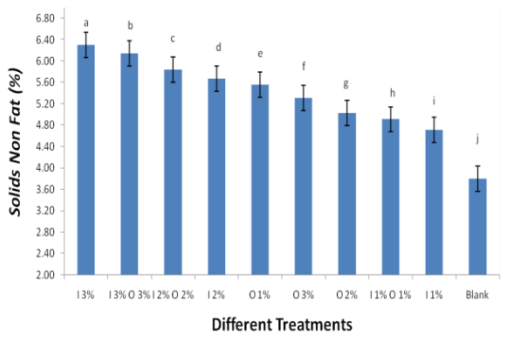

Fig. 2. Solids Non Fat of Dooghs in the various treatments of microbial starter inoculated with 1%. I%1= Inulin 1%, I%2= Inulin 2%, I%3= Inulin 3%, O%1= Oligofructose 1%, O%2= Oligofructose 2%, O%3=Oligofructose 3%, I%1O%1= Inulin1% and Oligofructose1%, I%2O%2= Inulin2% and Oligofructose2%, I%3O%3= Inulin3% and Oligofructose3%.

A decrease in inulin/oligofructose concentrations reduced SNF content. Starter addition (1%) modified Doogh characteristics through acidity changes that influenced bacterial survival. These findings align with Taheri et al. (2009), who reported higher acidity in probiotic Doogh versus controls due to increased acidification by *Lactobacillus acidophilus* during fermentation and storage. Consistent with Fathi Achachlouei & Mahmoudi Moghas (2018), xanthan and inulin treatments showed no significant acidity differences across starter levels ($P>0.05$). Our results also correspond with Hashemi et al. (2015), who observed decreasing pH in synbiotic Doogh containing *Lactobacillus plantarum* and inulin during storage. Their study confirmed that inulin enhances *L. plantarum* viability. The observed SNF increase is attributable to inulin and oligofructose supplementation, as both compounds contribute directly to SNF content.

Microbiological properties of produced Doogh

Figures 3–4 illustrate the effects of storage duration and treatments (various inulin, oligofructose, and inulin-oligofructose combinations) on *L. acidophilus* viability in synbiotic Doogh. As shown in Figure 3, *L. acidophilus* counts decreased time-dependently during storage, yet remained above the probiotic viability threshold (10^5 CFU/g) throughout the 21-day period. Viability was significantly higher in prebiotic-supplemented samples than in controls ($P<0.05$), with oligofructose and inulin enhancing survival. On day 21, the highest and lowest viability occurred in samples containing 1% inulin and the 1% inulin–1% oligofructose blend (I1%-O1%), respectively (Figure 4). These findings align with Rahmati Roudsari et al. (2013). Similarly, Figures 5–6 depict treatment and storage time effects on *Bifidobacterium lactis*. Though counts declined during storage (Figure 5), viability exceeded the standard probiotic threshold (10^5 CFU/g) on day 21. The highest and lowest *B. lactis* viability at this endpoint occurred in the 2% inulin treatment and control, respectively (Figure 6). In all samples with different percentages of inulin and oligofructose, *L. acidophilus* survival complied with Iran's national standard (>10^5 CFU/g until expiration, typically one month). In sheep milk ice cream, 4% inulin enhanced survival of *Bifidobacterium animalis BB-12* and *L. acidophilus* during simulated digestion, reducing bacterial loss by 16.7% in gastric conditions.

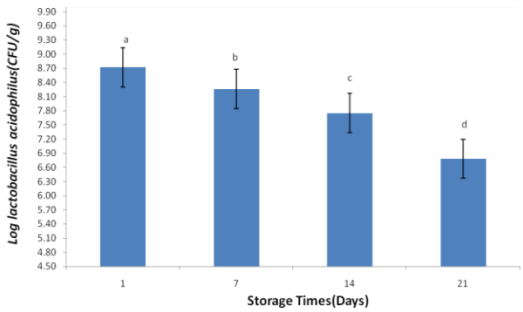

Fig. 3. *Lactobacillus acidophilus* (CFU/g) of Dooghs at various storage days in the microbial starter inoculated with 1%.

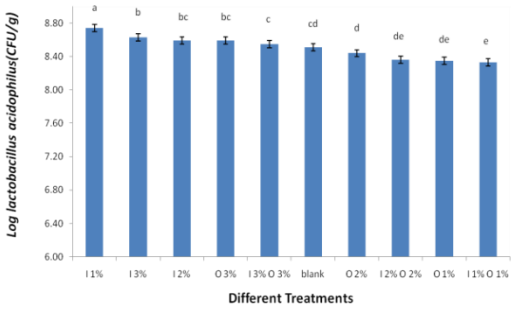

Fig. 4. *L. acidophilus* (CFU/g) of Dooghs in the various treatments of microbial starter inoculated with 1%. I%1= Inulin 1%, I%2= Inulin 2%, I%3= Inulin 3%, O%1= Oligofructose 1%, O%2= Oligofructose 2%, O%3=Oligofructose 3%, I%1O%1= Inulin1% and Oligofructose1%, I%2O%2= Inulin2% and Oligofructose2%, I%3O%3= Inulin3% and Oligofructose3%.

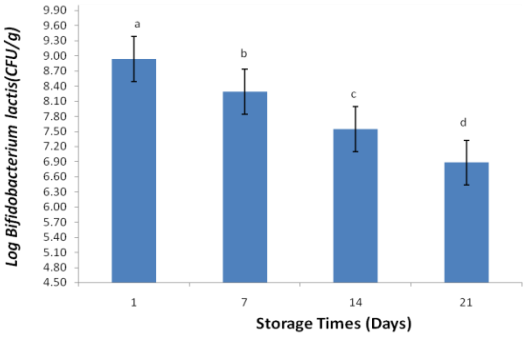

Fig. 5. *Bif. lactis* (CFU/g) of Dooghs at various storage days in the microbial starter inoculated with 1%.

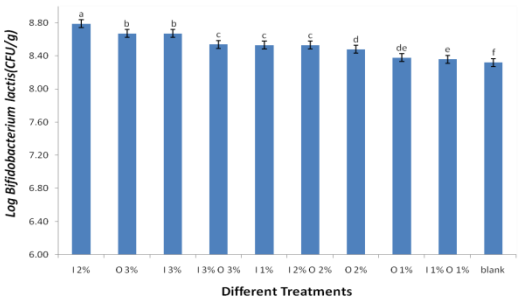

Fig. 6. *Bif. lactis* count (CFU/g) of Dooghs in the various treatments of microbial starter inoculated with1%. I%1= Inulin 1%, I%2= Inulin 2%, I%3= Inulin 3%, O%1= Oligofructose 1%, O%2= Oligofructose 2%, O%3=Oligofructose 3%, I%1O%1= Inulin1% and Oligofructose1%, I%2O%2= Inulin2% and Oligofructose2%, I%3O%3= Inulin3% and Oligofructose3%.

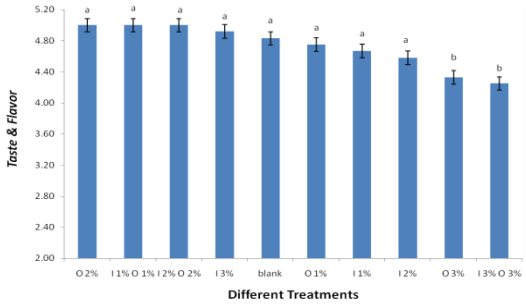

Fig. 7. Sensory properties of Dooghs in the various treatments of microbial starter inoculated with 1%. I%1= Inulin 1%, I%2= Inulin 2%, I%3= Inulin 3%, O%1= Oligofructose 1%, O%2= Oligofructose 2%, O%3=Oligofructose 3%, I%1O%1= Inulin1% and Oligofructose1%, I%2O%2= Inulin2% and Oligofructose2%, I%3O%3= Inulin3% and Oligofructose3%.

Inulin (4%) increased *Bifidobacterium BB-12* survival in intestinal bile by 22%, while oligofructose improved acid resistance. Replacing 1.5% inulin with apple fiber in ice cream improved probiotic survival (15.6% reduction) and texture (Kowalczyk et al., 2021, 2022). ABY-type cultures reduce probiotics (e.g., *L. acidophilus*) through hydrogen peroxide production by *L. delbrueckii subsp. bulgaricus* (Shah, 1997). Inulin (2-4%) improved survival of *L. acidophilus* and *Bifidobacterium spp.* in fermented foods like yogurt and Doogh, maintaining >10^6-10^7 CFU/g during storage (Pereira et al., 2023; Taşkoparan et al., 2025). Oligofructose (0.5-1%) outperformed inulin in some matrices (e.g., ice cream), supporting *B. animalis* viability for 90 days due to faster fermentation by probiotics (Taşkoparan et al., 2025). Addition of 2% inulin increased *L.*

acidophilus viability by 20% in yogurt during 21-day storage at 4°C (Taşkoparan et al., 2025). These results agree with Dini et al. (2013), who reported reduced *B. lactis* survival during storage. Viability loss may result from environmental stress, overgrowth of yogurt bacteria, and insufficient nutrients (Shah, 1997). *Bifidobacteria* produce acetic acid during fermentation; growth is inhibited below pH 5.5 (optimal pH 6.5-7.0) (Mortazavian et al., 2007; Shah, 1997). *L. acidophilus* (microaerophilic) and *Bifidobacterium spp.* (anaerobic) suffer oxygen-induced cell death (Kieronczyk et al., 2006), though oxygen sensitivity varies by strain (Tamime et al., 2005). The result of this research indicated suitable survival of *bifidobacteria* compared to *L. acidophilus* (Tamime et al., 2005).

Sensory properties of probiotic Doogh

Figure 7 shows the impact of treatments on taste and flavor of Doogh. Significant differences were observed ($P<0.05$). Inulin and oligofructose (except O3% and I3%-O3%) had no remarkable effect on flavor. Oligofructose at 1–2% also showed no significant taste/flavor impact ($P>0.05$), but concentrations >2% (O3%, I3%-O3%) significantly reduced flavor scores ($P<0.05$). The greatest reduction occurred at 3% oligofructose and I3%-O3%. These results align with Mazloumi et al. (2011), who reported 2% inulin did not affect probiotic yogurt flavor. Similarly, Cardarelli et al. (2008) found inulin had no significant effect on synbiotic cheese flavor. Prebiotic supplementation minimally impacted flavor, color, or texture, with 2% inulin being most preferred (Kowalczyk et al., 2021). The incorporation of oligofructose (1%) and *B. pseudocatenulatum* in whey drinks improved shelf life but required flavor masking due to excessive sweetness (Taşkoparan et al., 2025).

Furthermore, Voosogh et al. (2009) reported a statistically significant difference in taste among Doogh variants, with ziziphora extract-containing Doogh achieving higher taste scores than conventional Doogh. Ebrahimzadegan et al. (2014) examined the survival of free and encapsulated *Bif. lactis* and its influence on the physical, chemical, and sensorial characteristics of Iranian Doogh. They stated that probiotics not only had no adverse effects on these properties but also improved rheological properties, stability, and taste. Oligofructose supplementation functions to modulate taste, reduce aftertaste, modify sweetness profiles (Kaur & Gupta, 2002), and enhance fruit flavors (Roberfroid, 2005), underscoring the sensory priority observed in dairy products.

Indeed, taste is critically important for functional foods; relying on consumer willingness to accept compromised taste for health benefits constitutes a high-risk strategic approach (Verbeke, 2006). Consequently, after health considerations, most research identifies taste as the primary factor in food selection (Tepper & Trail, 1998; Tuorila & Cardello, 2002). Consumers associate sensory experiences during consumption (e.g., texture, appearance, taste, aroma) with pleasure, positioning them as essential drivers of eating behavior (Westenhoefer & Pudel, 1993).

Conclusion

ANOVA results indicated significant differences ($P<0.05$) among treatments for SNF, sensory characteristics (taste/flavor), and viable counts of *Bif. lactis* and *L. acidophilus*. Time significantly affected acidity, *Bif. lactis*, and *L. acidophilus* counts in 1%-inoculated starters ($P<0.05$), but not SNF or taste/flavor ($P>0.05$). On day 21, maximal and minimal *L. acidophilus* viability occurred in 1% inulin Doogh and 1% oligofructose Doogh, respectively. Maximal *Bif. lactis* viability occurred in 2% inulin Doogh, minimal in control. Overall, inulin and oligofructose yielded synbiotic Doogh with improved physicochemical/sensory properties and enhanced probiotic viability.

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تاثیرسطوح اینولین و الیگوفروکتوز بر زنده‌مانی باکتری‌های پروبیوتیک و ویژگی‌های حسی در دوغ سینبیوتیک

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چکیده

زمینه مطالعاتی: استفاده از دوغ پروبیوتیک به عنوان یکی از محصولات لبنی پروبیوتیک در ایران رواج یافته است. علاوه بر این، افزودن باکتری‌های پروبیوتیک و حفظ زنده‌مانی آن‌ها در دوغ می‌تواند در ارتقا سلامت مصرف کنندگان نقش داشته باشد.

هدف: در مطالعه حاضر، تأثیرات افزودن اینولین (۱ درصد، ۲ درصد و ۳ درصد وزنی/وزنی)، الیگوفروکتوز (۱ درصد، ۲ درصد و ۳ درصد وزنی/وزنی) و مخلوط اینولین-الیگوفروکتوز (در سطوح ۱-۱ درصد، ۲-۲ درصد و ۳-۳ درصد وزنی/وزنی) به عنوان ترکیبات پری‌بیوتیک در مقایسه با نمونه شاهد (بدون پری‌بیوتیک) همراه با باکتری‌های ترکیب شده با *بیفیدوباکتریوم لاکتیس* و *لاکتوباسیلوس اسیدوفیلوس* به عنوان استارتر (۱ درصد) برای تولید دوغ سینبیوتیک مورد بررسی قرار گرفت.

روش کار: برخی خصوصیات فیزیکی- شیمیایی، حسی وتعداد باکتری‌های پروبیوتیک زنده مانده در دوغ در طی زمان نگهداری ارزیابی شد.

نتایج: در بین تیمارها از نظر ماده جامد بدون چربی (SNF)، شمارش *بیفیدوباکتریوم لاکتیس* و *لاکتوباسیلوس اسیدوفیلوس* و خصوصیات حسی (ویژگی‌های طعم و مزه) تفاوت معنی داری ($P < 0.05$) مشاهده شد. علاوه بر این، دوغ حاوی ۱ درصد اینولین و ۱ درصد الیگوفروکتوز، به ترتیب حداکثر و حداقل زنده مانی *لاکتوباسیلوس اسیدوفیلوس* را در روز ۲۱ نگهداری دوغ داشتند. همچنین دوغ حاوی ۲ درصد اینولین و شاهد نیز به ترتیب حداکثر و حداقل زنده مانی *بیفیدوباکتریوم لاکتیس* را در روز ۲۱ نگهداری دوغ داشتند.

نتیجه‌گیری کلی: به طور کلی، نتایج این مطالعه نشان داد که می‌توان با استفاده از افزودن اینولین و اولیگوفروکتوز یک دوغ سینبیوتیک حاوی باکتری‌های پروبیوتیک با بهبود زنده مانی آنها، به عنوان یک نوشیدنی لبنی عملگرا تولید کرد.

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