Preliminary communication

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Effect of acacia exudate gum on milk gel fermentation of flavoured synbiotic yoghurt enriched *with Daucus carota L. ssp. sativus var. atrorubens* Alef fibre

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Abstract

Fortifying yoghurt or dairy products with fibres and probiotic bacteria is an increasing interest to create functional foods with health benefits and improve their functionality. In this study milk fermented with acacia exudate gum and black carrot (*Daucus carota L. ssp. sativus var. atrorubens alef)* pulp components had a significantly shorter fermentation time than the others in milk fermented with *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis*. Potential prebiotic addition increased the cohesiveness, index of viscosity and decreased the index of syneresis in synbiotic yoghurt samples. Sensory evaluation showed that incorporation of functional components increased satisfactory texture, and had sensory characteristics with high scores during cold storage.

Key words: yoghurt; probiotic; prebiotic; Acacia exudate gum;
***Daucus carota* L. ssp. *sativus* var. *atrorubens* alef**

Introduction

Current global trends show that synbiotic products are claimed to have therapeutic and health-promoting properties in novel technology. Recent approaches are based on probiotics and fortifying fruits/vegetables and their extracts with bioactive ingredients, as they have received increasing attention for their beneficial role against cancers, cardiovascular disease, type 2 diabetes, osteoporosis, obesity, and age-related macular degeneration (Sun-Waterhouse, 2011; Dhingra et al., 2012; Mark et al., 2019).

However, in recent years, researchers have focused on the important effects of the increase in the usual consumption of naturally coloured functional products and beverages, such as black carrots that are rich in polyphenols and natural colour components (Carle et al., 2016). Black carrots represent a functional food with important health properties with a high content of water-soluble substances, fibre, colour pigments, phenols, carotens, minerals, vitamins, volatile compounds, and are widely studied for their physiological functions ranging from anti-oxidant to anti-mutagenic and anti-cancer activity (Arscott et al., 2010; Zadernowski et al., 2010; Akhtar et al., 2017; Iorizzo et al., 2020). Various studies on fermented milk and probiotic yoghurts with black carrot as flavouring and colouring agents have reached conclusions regarding the increase in biotherapeutic properties and the development of bacterial activity (Abou El Samh et al., 2013; Abdel-Hamid et al., 2020; Bari et al., 2020).

Gums and hydrocolloids are additives with specific technological and functional properties that are added to foods with their various carbohydrate contents. These properties mostly affect the rheological properties of food and modulation of the gut microbiota, energy metabolism and besides focuses on calorie reduction and prebiotic activity. Particularly, non-digestible carbohydrates or prebiotics can highly modify the composition and function of microbial balance such as Bifidobacteria and other lactic acid producing bacteria (Clemens and Pressman, 2017; García-Burgos et al., 2020; Ozcan and Akpinar-Bayizit, 2020)

In this study, the probiotic fermentation and gel properties of synbiotic yoghurt with exudate acacia gum and black carrot (*Daucus carota L. ssp. sativus var. atrorubens Alef)* pulp bioactive components was investigated.

Materials and method

Materials

Black carrots (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef variety) from the local market were used to carry out yoghurt making experiments. Strains of pure starter freeze-dried yoghurt cultures mix of *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis*, YO-MIX 205 were obtained from Danisco (Sassenage, France). Exudate acacia gum was purchased from Nexira (Rouen, France).

Preparation of flavoured synbiotic yoghurt with exudate acacia fibre and black carrot pulp

For the inoculum preparation, the freeze-dried cultures of yoghurt bacteria were prepared by dissolving in 50 mL of milk 11% (w/v) of total solids and autoclaved at 121 °C for 20 min. The culture was activated at 37 °C (approximately ~9 $\log_{10}$ cfu $mL^{-1)}$ and 3% (v/v) of the pre-culture was inoculated into milk. After inoculation, batch treatment fermentations were performed at 40 °C up to pH 4.70. Each fermentation was performed in triplicate (Barat and Ozcan, 2018).

Control (T1, consisting of a non-supplemented yoghurt) synbiotic yoghurts encoded as T2 (with black carrot pulp), prebiotic yoghurt as T3 (with exudate acacia gum) and yoghurt T4 (with exudate acacia gum and black carrot pulp) were produced from heated milk (90±1 °C/10 min.) standardized to <0.30% fat and 11.5% dry matter, with the addition 0.02% exudate acacia gum (wt/v) and 8% (wt/v) pasteurized black carrot pulp (85-90°C/10 min.) and probiotic mix culture containing *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium animalis* subsp. *lactis* (3% wt/v). Inoculated milks were incubated at 40±0.5 °C until pH 4.70. After cooling (20±1 °C/30 min.) yoghurts were stored in a cold room for 21 days.

Methods

Viable cell strains of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus* and *B. animalis* subsp. *lactis* were enumerated by method Barat and Ozcan (2018). All enumerations were carried out in triplicate as mean values at the beginning and at the end of the fermentation. Plates containing 30-300 colonies were recorded as a log of colony-forming units per g of yoghurt samples ($\log_{10}$ cfu g^{-1}). Growth proportion index (GPI) was calculated as follows (Ozcan et al., 2021):

GPI= Final cell population at the end of fermentation ($\log_{10}$ cfu g^{-1})/Initial cell population ($\log_{10}$ cfu g^{-1}).

The pH was determined using a pH meter (315i/SET, WTW, Germany) at 25°C. Instrumental texture profile parameters (cohesiveness and index of viscosity) were determined using TA-XT plus®, Stable Micro Systems analyser with mechanical compression of samples with the back extrusion test. The amount of drained whey was expressed as the index of syneresis as mL 25 g^{-1}) (Nguyen et al., 2015).

Sensory descriptive analysis was performed by a trained sensory panel consisting of seven female and four male (ages 22-45 years) members by Dimitrellou's et al. (2019) method of using a 9-point hedonic scale (9 = extremely like, 8 = very much like, 7 = moderately like, 6 = slightly like, 5 = neither like nor dislike, 4 = slightly dislike, 3 = moderately dislike, 2 = very much dislike, and 1 = extremely dislike). The preference index (PI) of consumer acceptability was calculated according to the equation AI (%) =Y*100/Z (Y= the average score obtained for the product, and Z= maximum score given to the product) (Silva et al., 2010).

Statistical analysis was carried out in triplicate and the results were presented as mean by using analysis of variance (ANOVA) using Statistica 9.2 software (StatSoft, Inc., Tulsa, OK, USA) and the differences of averages were compared by Tukey test at 5 % and 1 % of significance. The hierarchical cluster analysis (HCA) was performed using JMP 7 software (Li Vigni et al., 2013).

Results and discussion

Fermentation profile and bacterial growth

The acidification kinetic was primarily important for the fermentation. The lactose in milk is fermented by lactic acid bacteria (LAB) as the main energy and carbon source, and the metabolites formed are used in the production of fermented milk products (Fernández et al., 2015; Chen et al., 2017). Incubation profile and pH values of flavoured synbiotic yoghurt with exudate gum during the lactic acid fermentation was presented in Figure 1. When the pH change is examined, it was seen that the pH of the yoghurt samples containing black carrot pulp decreases faster and the formation of the yoghurt gel occurs earlier also with gel with exudate acacia gum (Figure 1).

It is thought that black carrot and gum increases acidity in yoghurts and provides growth to microorganisms with the sugar it contains, and it causes a faster decrease in pH as a result of increasing microbial activity. Specific growth rate and lactic acid concentration increase in mixed culture as compared with single strain cultures. In this synbiotic relationship, each of the bacteria produces substances favourable to metabolites in the food system. Meanwhile, in this matrix, it is stated that *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* in mixed culture can also stimulate the growth of other probiotic bacteria by the synbiotic metabolism-related interactions between these two species, which is called proto-cooperation (Angelov et al., 2009; Aghababaie et al., 2015).

Microbiological analysis was carried out at the beginning and at the end of the fermentation in order to investigate the potential prebiotic effect of natural flavour and colour components of black carrot and exudate gum on probiotic bacteria growth in synbiotic yoghurt gels. *Lactobacillus* spp. and *Bifidobacterium* spp. have been used and studied extensively as fermentation starter cultures (Barat and Ozcan, 2018; Aryana and Olson, 2017). *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus* and *B. animalis* subsp. *lactis* number was determined in T1, T2, T3 and T4 samples (p<0,01) (Figure 2). Multiple factors such as pH, oxygen concentration, and presence of anti-microbial components affect the survival and viability of bacterial cultures in the final product (Ozcan and Akpinar Bayizit, 2020). When the viability levels of these bacteria were examined, it was determined that exudate gum had a positive effect on yoghurt bacteria and encouraged bacterial growth. In addition, studies on the use of vegetable extracts in yoghurt indicated that phenolic compounds and dietary fibres increase the activity of lactic acid bacteria (Espírito-Santo et al., 2011; Holck et al., 2014; Barat and Ozcan, 2018; Fazilah et al., 2018; Dimitrellou et al., 2020; Ozdemir and Ozcan, 2020). Furthermore, black carrots are important with their high content of anthocyanins, phenolic compounds and carbohydrates (Smeriglio et al., 2018).

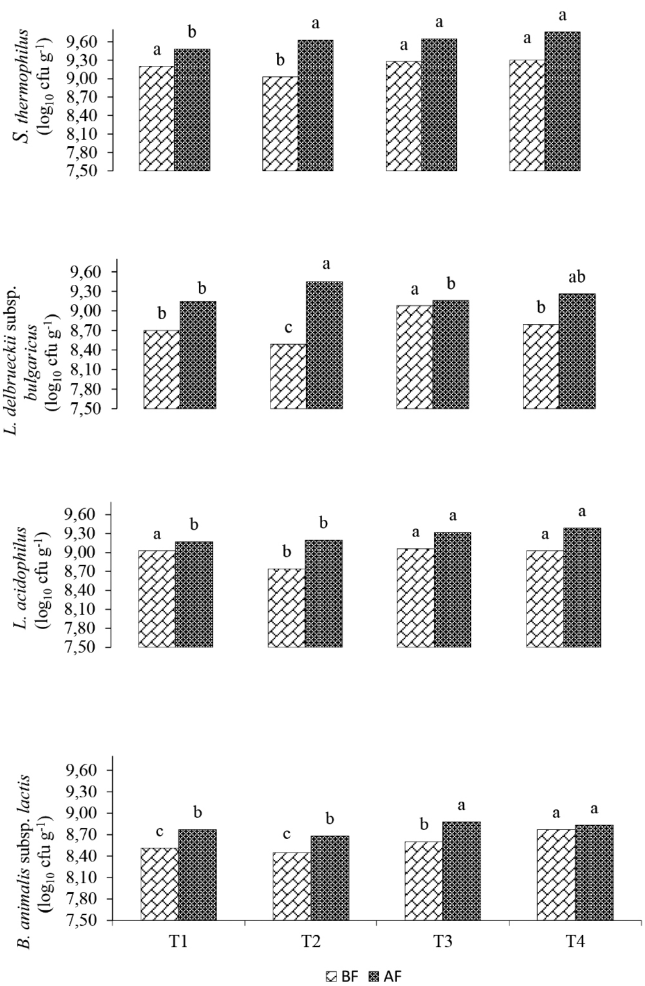

Figure 2. Microbial growth of lactic acid bacteria (log_{10} cfu g^{-1}) during fermentation in flavored synbiotic yoghurt with acacia exudate gum

†Values are means of triplicates. Significance level: significant at P<0.01 (**) and P<0.05 (*), ns (non-significant) different superscript letters on the same column indicate significant differences.
BF, before fermentation; AF, after fermentation
***Legend:** T1: control; T2: synbiotic yoghurt with black carrot fibre; T3: synbiotic yoghurt with acacia exudate gum; and T4: synbiotic yoghurt with acacia exudate gum and black carrot fibre*

Sah et al. (2016), Zhang et al. (2018) and Abdel-Hamid et al. (2020) reported that when functional dairy prod-

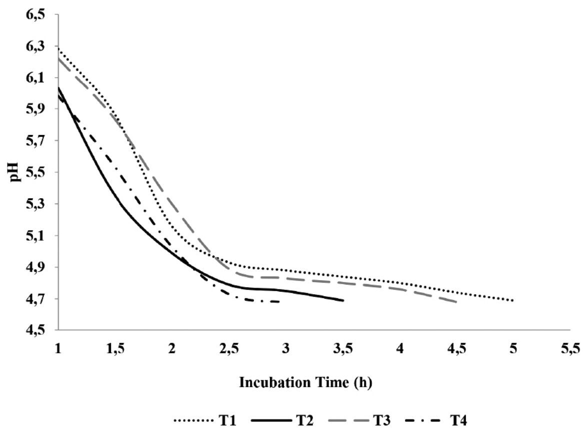

Figure 1. Acidification kinetics during fermentation of flavoured synbiotic yoghurt with acacia exudate gum

Legend: *T1: control; T2: synbiotic yoghurt with black carrot fibre; T3: synbiotic yoghurt with acacia exudate gum; and T4: synbiotic yoghurt with acacia exudate gum and black carrot fibre*

ucts with high nutritional content and bioactivity fortified with fruit and plant extracts were produced, there was an increase in anti-oxidant, anti-cancer and anti-bacterial activity along with the stimulated the growth of probiotic bacteria. In this study black carrot phenolic compounds have also been effective on the acidification kinetics of LAB bacteria. It has been stated that the galactose in the structure of exudate acacia gum is fermented by *S. thermophilus* and increases the number of bacteria (Niamah et al., 2016). Some results showed that acacia gum increased the growth of yoghurt bacteria (Pak et al., 2013; Niamah et al., 2016).

Terpend et al. (2013) stated that *Bifidobacterium* and *Lactobacillus* species are effective in breaking down arabinogalactan - the main polysaccharide forming the acacia exudate gum, ß-galactosidase and α-arabinofuranosidase; andcan use arabinogalactan as a prebiotic source by secreting its enzymes. In the probiotic product, probiotic microorganisms should be at least 10^6 cfu g^{-1} and acceptable levels 10^7-10^8 cfu g^{-1} (Lourens-Hattingh and Viljoen, 2011). However, it was found that the viability level of probiotic bacteria in all samples was above the therapeutically effective value ≥10^6 cfu g^{-1} (FAO/WHO, 2002; Tian et al., 2015).

GPI rates of bacterial counts were indicated in Table 2. The highest growth rate of *S. thermophilus* and *L. delbrueckii subsp. bulgaricus* and *L. acidophilus* was found in sample with black carrot (T2). *B. animalis subsp. lactis* showed a lower growth (GPI) in the sample (T4) containing black carrot fibre and the acacia exudate gum but still high in yoghurt with black carrot fibre. *Bifidobacterium* and *Lactobacillus* species, when used with *S. thermophilus* and *L. delbrueckii subsp. bulgaricus* and *L. acidophilus*, increase the growth rate, shorten the fermentation period and also improve the textural/sensory properties (Duffy et al., 2005; Barat and Ozcan, 2018).

Gel firmness and sensory properties

Textural properties and syneresis index of samples were given in Figure 3. The water binding, milk protein hydrolysis and gelation characteristics affect the desired rheological, textural and sensory properties of fermented milk products. Cohesiveness is defined as the forces of inner bond links, an important textural parameter of yoghurt (Delikanli and Ozcan, 2014; Mudgil et al., 2017). As can be seen from Figure 3, the highest cohesiveness and index of viscosity were observed in the sample with exudate acacia gum and exudate gum/black carrot pulp addition. The index of viscosity of all analysed synbiotic yoghurt samples significantly increased depending on stronger casein network during the cold storage period. The index of syneresis decreased in all samples during storage (p<0.01) (Figure 3).

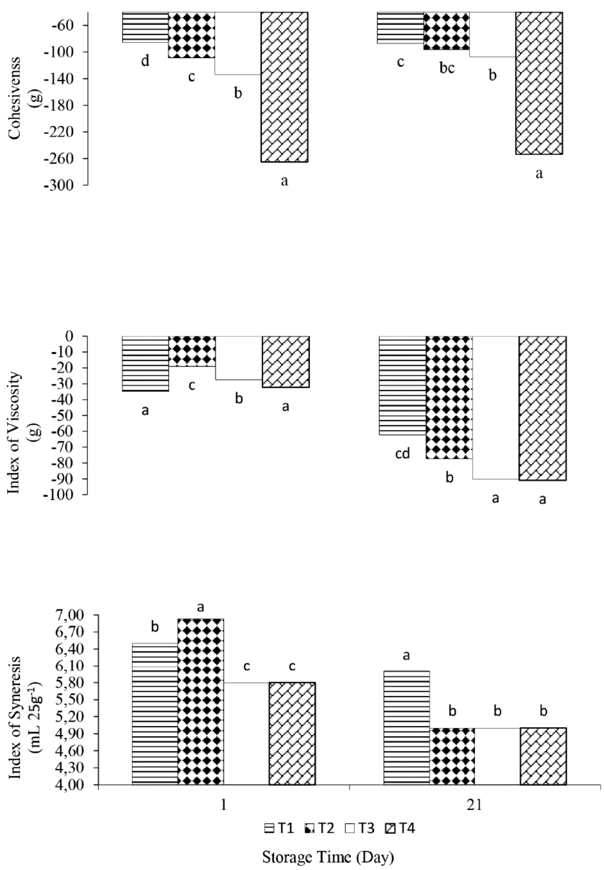

Figure 3. Textural properties of flavoured synbiotic yoghurt with acacia exudate gum

†Values are means of triplicates. Significance level: significant at P<0.01 (**) and P<0.05 (*), ns (non-significant) different superscript letters on the same column indicate significant differences.
BF, before fermentation; AF, after fermentation
Legend: T1: control; T2: synbiotic yoghurt with black carrot fibre; T3: synbiotic yoghurt with acacia exudate gum; and T4: synbiotic yoghurt with acacia exudate gum and black carrot fibre

Table 1. Growth proportion index (GPI) of lactic acid bacteria before (BF) and after (AF) fermentation (log10 cfu g-1)

		BF	AF	GPI^{BF-AF}
S. thermophilus	T1	9.20	9.48	1.03^{bc}
	T2	9.03	9.63	1.07^{a}
	T3	9.28	9.65	1.04^{b}
	T4	9.30	9.76	1.05^{b}
L. delbrueckii* subsp. *bulgaricus	T1	8.70	9.15	1.05^{b}
	T2	8.49	9.45	1.11^{a}
	T3	9.08	9.16	1.01^{c}
	T4	8.79	9.26	1.05^{b}
L. acidophilus	T1	9.03	9.17	1.02^{b}
	T2	8.74	9.20	1.05^{a}
	T3	9.06	9.32	1.03^{ab}
	T4	9.03	9.39	1.04^{a}
B. animalis subsp. lactis	T1	8.51	8.77	1.03^{a}
	T2	8.45	8.68	1.03^{a}
	T3	8.60	8.88	1.03^{a}
	T4	8.77	8.83	1.01^{b}

†Values are means of triplicates. Significance level: significant at P<0.01 (**) and P<0.05 (*), ns (non-significant) different superscript letters on the same column indicate significant differences.
‡GPI = Final cell population (log10 cfu /mL^{-1})/Initial cell population (log10 cfu g^{-1}).
Legend: *T1: control; T2: synbiotic yoghurt with black carrot fibre; T3: synbiotic yoghurt with acacia exudate gum; and T4: synbiotic yoghurt with acacia exudate gum and black carrot fibre*

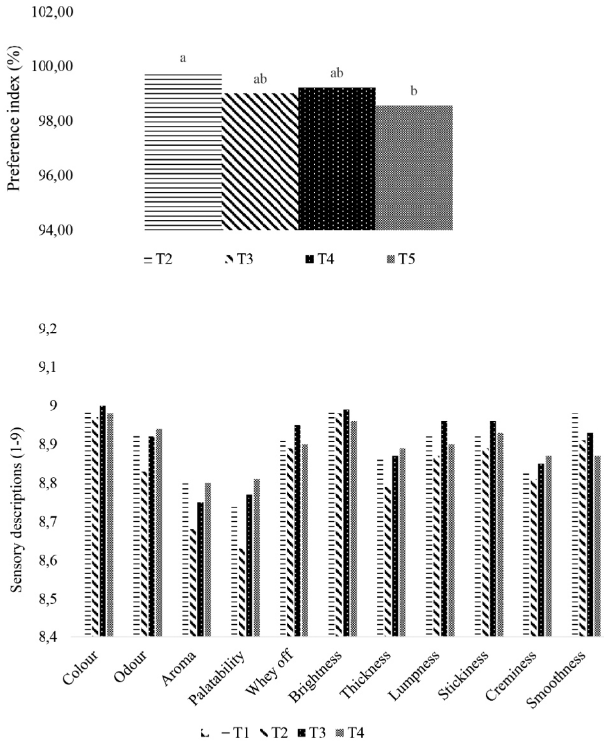

Figure 4. Sensory preference index and sensory descriptions of flavoured synbiotic yoghurt with acacia exudate gum

†Values are means of triplicates. Significance level: significant at P<0.01 (**) and P<0.05 (*), ns (non-significant) different superscript letters on the same column indicate significant differences.
BF, before fermentation; AF, after fermentation
Legend: *T1: control; T2: synbiotic yoghurt with black carrot fibre; T3: synbiotic yoghurt with acacia exudate gum; and T4: synbiotic yoghurt with acacia exudate gum and black carrot fibre*

It is known that the addition of fruits and vegetables to fermented milk gels decreases syneresis and increases the water-holding capacity because these ingredients increase the ratios of both dry matter, pectin and high dietary fibre (Yildiz and Ozcan, 2019; Mendes et al., 2019; Ozdemir and Ozcan, 2020). Yao et al. (2017) stated that while starter culture, incubation time and plant extracts were effective on viscosity, incubation kinetics were more effective on water holding capacity with the polysaccharides and casein interactions.

Sensory evaluation of fermented products has an important influence to define the product properties, which are prominent concerning the product acceptability for customer choice (Pereira et al., 2003; Chetachukwu et al., 2019). Especially *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* live in symbiosis and shape the specific sensory characteristics of fermented milk with the formation of these aromatic compounds and fermentation products. In this study, the effect of exudate acacia gum and black carrot pulp addition and storage time on the sensorial properties of synbiotic yoghurts was represented by the descriptive analysis for better conception in Figure 4 (p<0.01). The mean panel score obtained from the sensory analysis showed that the milk fermented with probiotic culture, prebiotic exudate acacia gum and black carrot pulp fibre were characterized by the highest score in all the parameters.

Although sensory preference index (PI) score of exudate acacia gum and black carrot pulp enriched yoghurt samples is lower than control sample. However, it seems that the addition of functional additives into yoghurt could improve the sensory characteristic of the products. HCA analysis clearly indicated a significant influence of sensory properties on sample groupings and relationships for their characteristics. The purpose of cluster analysis (data segmentation) is to measure the distance between each pair of objects in terms of the variables suggested in the study, and then to group objects which are close together (Li Vigni et al., 2013). Figure 5 shows the result of HCA of the data. According to the results obtained, acacia exudate

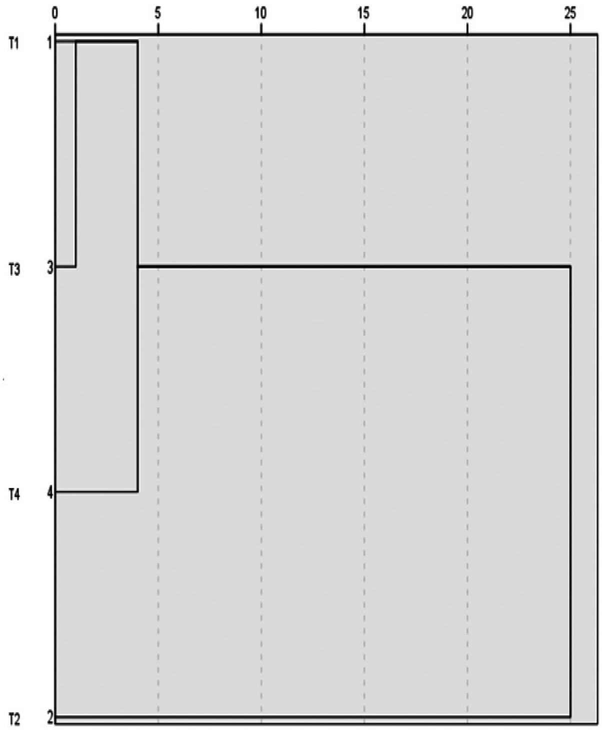

Figure 5. Hierarchical cluster analysis (HCA) of flavoured synbiotic yoghurt with acacia exudate gum

Legend: *T1: control; T2: synbiotic yoghurt with black carrot fiber; T3: synbiotic yoghurt with acacia exudate gum; and T4: synbiotic yoghurt with acacia exudate gum and black carrot fiber*

gum and the effect of black carrot and its natural flavour/colour components on bacterial fermentation changed the sensory HCA classification by affecting the textural and aromatic properties. As a result, T1 (control), T3 (synbiotic yoghurt with acacia exudate gum) and T4 (synbiotic yoghurt with acacia exudate gum and black carrot fibre) samples clustered together, while T2 samples containing black carrot fibre formed a separate cluster (T2) but correlated with other groups.

Conclusion

Consumption of functional food products containing probiotics and dietary fibre/prebiotics with micronutrient may prevent or decrease chronic disease. Moreover, it is necessary to deeply understand how exudate gums or mixing them with different gelation agents and probiotic bacteria, can change gel properties when added to foods. In addition to this, in some cases, the food matrix can positively affect the stability and bioavailability of the bioactive components and dairy foods should be fortified with *identified* bioactive compounds, selected from different specific starters and natural bio-preservatives and nutraceuticals.

Utjecaj dodatka gumastog sekreta bagrema na fermentaciju mlijeka u proizvodnji aromatiziranog sinbiotičkog jogurta obogaćenog dodatkom vlakana iz crne mrkve (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef)

Sažetak

Obogaćivanje jogurta i drugih mliječnih proizvoda dodatkom vlakana i probiotičkih bakterija poprima sve veći interes u proizvodnji funkcionalne hrane s dodanom vrijednosti. U ovom istraživanju mlijeko fermentirano uz dodatak gumastog sekreta bagrema i pulpe crne mrkve (*Daucus carota L. ssp. sativus var. atrorubens* Alef*)* imalo je puno kraće vrijeme fermentacije u usporedbi s drugim vrstama mlijeka fermentiranim sojevima *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus* i *Bifidobacterium animalis* subsp. *lactis*. Dodatak potencijalnih prebiotika povećao je kohezivnost i indeks viskoznosti te smanjio indeks sinereze u uzorcima sinbiotičkog jogurta. Ocjenjivanje senzorskih svojstava je pokazalo da dodatak funkcionalnih sastojaka poboljšava teksturu te su tijekom hladnog skladištenja tako obogaćeni proizvodi postizali veće ocjene.

Ključne riječi: jogurt; probiotik; prebiotik; gumasti sekret bagrema, crna mrkva, *Daucus carota* L. ssp. *sativus* var. *atrorubens* alef

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