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CHARACTERISTICS OF PROBIOTIC YOGHURTS SUPPLEMENTED WITH PU-ERH TEA INFUSION

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ABSTRACT

Background. Recently, interest in the development of functional foods enriched with bioactive components has increased. Dairy products supplemented with tea extracts known for their health-promoting properties are good examples of such products. However, most of the scientific studies and applications focus on green tea. The present study was established to estimate the effect of Pu-erh tea supplementation on the viability of starter microorganisms and selected physico-chemical and sensory properties of probiotic ABT-yoghurt.
Material and methods. ABT-yoghurts (*Lactobacillus acidophilus* La-5, *Bifidobacterium animalis* ssp. *lactis* BB-12, *Streptococcus thermophilus*) were produced from cow's milk with 0%, 5%, 10% or 15% (v/v) of Pu-erh tea infusion added before the fermentation stage. The products obtained were subjected to the following analyses one day after production (colour profile) and after 7, 14 and 21 days of cold storage: ferric reducing antioxidant power (FRAP) and anti-radical power (ARP) measured against DPPH radical, titratable acidity, pH, texture parameters (back extrusion test), viability of starter cultures and sensory quality (hedonic scale experiment).
Results. Pu-erh tea supplementation significantly enhanced the antioxidant potential of probiotic yoghurts as a 3–6.5-fold increase in FRAP and a 10–24-fold increase in ARP values were observed in comparison to plain ABT-yoghurt. Pu-erh tea slightly enhanced the viability of *L. acidophilus* and reduced the pH of probiotic yoghurts. Higher concentrations of Pu-erh tea caused decreased firmness and consistency while cohesiveness and index of viscosity remained unaffected upon supplementation. The addition of Pu-erh tea infusion modified the colour and sensory properties of the probiotic yoghurts but the sensory quality of the tea yoghurts was rated lower when compared to the plain one. Among all tea yoghurts, the one with 15% Pu-erh tea additive received the highest scores in sensory assessment.
Conclusion. Pu-erh tea may be successfully applied as a functional additive to probiotic yoghurts, significantly enhancing the antioxidant properties of fermented milk and ensuring a high rate of starter bacteria viability during storage. However, the level of fortification must be carefully chosen as some doses negatively influence texture parameters and sensory quality.

Keywords: probiotic yoghurt, Pu-erh tea, texture, antioxidant properties, sensory quality

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INTRODUCTION

Nowadays, due to the increased demand for food with functional and health-promoting properties, plant extracts rich in polyphenolic compounds have been extensively studied as functional additives in food systems (Cutrim and Cortez, 2018). Dairy products enriched with single phenolics or plant extracts are good examples of such products. Among different natural, bioactive additives, tea extracts or single phenolic compounds derived from tea have great potential as supplements in milk matrices. Generally, previously conducted studies have confirmed that such enrichment results in increased antioxidant properties of fermented milks (Amirdivani and Baba, 2015; Jeong et al., 2018; Liu, 2018; Muniandy et al., 2016; Najgebauer-Lejko et al., 2011; 2014). This is due to the fact that tea brings a high content of phenolic compounds to the milk systems, which are known for their excellent antioxidant and free radical-scavenging, as well as antimutagenic and antimicrobial abilities, and are connected with a reduced risk of cardiovascular disease, coronary heart disease and atherosclerosis (Cutrim and Cortez, 2018; Lee and Foo, 2013). However, while developing novel functional foodstuffs enriched with bioactive additives (e.g. polyphenols), not only should the health benefits be taken into account, but also their appearance, texture and sensory properties, as these attributes have a great influence on consumer acceptance (Day et al., 2009). Therefore, many studies have been conducted in order to estimate the effect of tea addition on the sensory properties, acidity, textural and rheological parameters, and starter culture viability in fermented dairy products. However, these studies are usually limited to green tea (Amirdivani and Baba, 2015; Dönmez et al., 2017; Han et al., 2011; Najgebauer-Lejko, 2014; Najgebauer-Lejko et al., 2011; 2014), occasionally black tea (Jaziri et al., 2009; Muniandy et al., 2016; 2017) and dark, post-fermented tea (Liu, 2018; Najgebauer-Lejko et al., 2011; 2014).

Different types of tea are produced worldwide. The major types comprise non-fermented green tea, partially fermented oolong tea, black tea fully-fermented by oxidizing enzymes, and dark tea post-fermented using microbes. These types differ in their production procedure, composition and properties. Pu-erh tea is a post-fermented dark tea produced in the Yunnan Province of China by natural long-term storage (aging) or by ripening under conditions of high temperature and humidity of the raw tea leaves of *Camellia sinensis* var. *assamica* (Zhang et al., 2011; 2013). The microorganisms involved in the post-fermentation processes, which significantly change chemical composition and play a key role in the formation of the specific sensory characteristics in Pu-erh tea, are mainly fungi from the genera *Aspergillus* and *Penicillium* (Zhang et al., 2013). Recently, this type of tea has gained much attention due to its unique taste and aroma, as well as its health-promoting properties. Among multiple health benefits usually connected with tea consumption, the anti-obesity, anti-hyperglycaemic and hypolipidaemic effects of Pu-erh tea have been scientifically confirmed and are reported to be much higher than in other teas (Lin et al., 2018; Lv et al., 2015).

In light of this, the present study was established to estimate the effects of Pu-erh tea addition in different concentrations on the selected physico-chemical properties (acidity, texture and colour parameters), antioxidant properties and microbiological and sensory qualities of probiotic ABT-yoghurt.

MATERIAL AND METHODS

Material

Raw cow's milk (Holstein-Friesian breed) was obtained from a local milk farm (Dziekanowice, Poland). Pu-erh tea (individually packed tea blocks, 3.0 ±0.2 g; Yunnan Haichao Group, Haichao Tea Blocks Co., Ltd., China) and skim milk powder (Dairy Company in Gostyn, Poland) were purchased from the local super-market. ABT-1 starter culture (DVS), consisting of *Lactobacillus acidophilus* (La-5), *Bifidobacterium animalis* ssp. *lactis* (BB-12) and *Streptococcus thermophilus*, was obtained from Chr. Hansen (Hoersholm, Denmark).

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Fluka (Copenhagen, Denmark), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-tris(2-pyridyl)-*s*-triazine (TPTZ) from Sigma-Aldrich (Steinheim, Germany and Buchs, Switzerland). MRS agar and M17 agar for enumeration of bacteria were supplied by Biocorp (Warszawa, Poland). All other chemicals used were of analytical-reagent grade.

Preparation of tea infusion and ABT-yoghurts

Pu-erh tea water extract was prepared by infusion of 40 g tea leaves in 0.8 dm^3 of freshly boiled water in a covered glass beaker. After 15 min, the tea leaves were removed using a sieve and the fresh infusion, after cooling to an ambient temperature, was added to milk in the amount of 5%, 10% or 15% (v/v). Freshly boiled and cooled water was applied in the proper amount to 0%, 5% and 10% treatments to ensure the same milk dry matter content in all ABT-yoghurts.

The preparation of milk for probiotic yoghurts comprised the following stages: centrifugation (3500 × g, 45°C; LWG24E milk separator, Spomasz, Gniezno, Poland) to reach a 2% fat level, standardization with NMP (non-fat milk powder) to achieve 15% dm content in the final products (~63 $g \cdot dm^{-3}$), homogenization (60°C, 6 MPa; FT9 homogeniser Armfield Ltd., Ringwood, England), batch pasteurization (85°C, 15 min; Kochstar automat 2500, Merten & Storck, Drensteinfurt, Germany) and cooling to 38°C. Subsequently, the bulk milk was inoculated with the ABT-1 starter (0.08 g per 1 dm^3 of milk), then it was divided into four equal portions, mixed with proper amounts of Pu-erh tea infusion (5, 10, 15% v/v) or/and water and poured into 0.2 dm^3 sterile glass jars. The incubation proceeded at 37°C for 10–12 h until visible symptoms of coagulation occurred (4.6–4.8 pH). After that, the ABT-yoghurts were cooled and stored in a refrigerator at 4°C. Analyses were performed on the 1^{st}, 7^{th}, 14^{th} and 21^{st} days after production.

Analyses

Titratable acidity was determined according to the Soxhlet-Henkel method (Polish Standard, PN-A--86061:2002) and expressed as % of lactic acid. The Elmetron (Zabrze, Poland) CP-411 pH-meter was used for measuring the pH of the ABT-yoghurts.

Determinations of ferric reducing antioxidant power (FRAP) and the scavenging rate of 2,2-diphenyl--1-picrylhydrazyl (DPPH) radical were conducted by the procedures described by Najgebauer-Lejko et al. (2011). The FRAP value was expressed as mmol Fe^{2+} E (equivalents)$\cdot dm^{-3}$ and the DPPH antiradical activity as ARP (anti-radical power) calculated relative to the ARP of Trolox in μmol of Trolox equivalents (TE)$\cdot g^{-1}$ sample.

The level of *S. thermophilus* in ABT-yoghurts was assessed using M17 agar under aerobic conditions. Enumeration of *L. acidophilus* was performed under aerobic conditions using MRS-maltose (pH 6.4). The count of bifidobacteria was evaluated using NNLP--MRS agar (NNLP: nalidixic acid, neomycin sulfate, lithium chloride, and paromomycin sulfate, 5%) in anaerobic incubators filled with CO_2. All cultures were incubated at 37°C for 48 h (*S. thermophilus*) or 72 h (*L. acidophilus*, *B. lactis*).

Texture analysis of the ABT-yoghurts was carried out on undisturbed samples in glass jars (0.2 dm^3, 60 mm sample height; 55 mm internal diameter) by means of the back extrusion test (BET) using a TA.XTPlus texture analyser (Stable Micro Systems; Haslemere, Surrey, England). The ABT-yoghurts were studied immediately after removal from cold storage (4°C). BET was performed by one-cycle compression of a plastic disc of 50 mm diameter (P/50 cylinder probe), which was thrust through the sample to a depth of 30 mm at a speed of 1 $mm \cdot s^{-1}$. The following parameters were calculated from the obtained force *vs* time curves using Texture Exponent 32 software: firmness, N; consistency, N·s; cohesiveness, |N| – the absolute value of N, and index of viscosity, |N·s| – the absolute value of N·s. Firmness and cohesiveness are defined as a maximum and minimum force (respectively) obtained during extrusion of the yoghurt sample through the annular gap between the probe and jar wall; consistency and index of viscosity are related to the respectively positive and negative areas of the force *vs* time curve (Sánchez and Pérez, 2012).

Colour parameters were measured in a reflectance mode using a Minolta CM-3500d Spectrophotometer (Konica Minolta Sensing Inc., Japan). The following parameters in CIE $L^*a^*b^*$ space were monitored: L^* – lightness (luminance) on a scale from 0 (black) to 100 (white), a^* – negative and positive values are are indicative respectively for a green and red colour; b^* – values represent colours from blue (negative values) to yellow (positive values). Colour differences (ΔE) were also calculated between the tea ABT-yoghurts and the plain control yoghurt using the following equation:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Colour analysis was performed once on the first day after ABT-yoghurt production.

The sensory evaluation was performed according to the PN-ISO 6658 standard (1998) using a 5-point scale (from 1 – very bad to 5 – excellent) by a trained panel of 6 assessors (four women and two men between 24–45 years old). The ABT-yoghurt samples were presented to panellists in identical plastic cups labelled with random numbers and in a random order. The following properties were assessed: colour, taste, odour, consistency and general appearance, and the overall preference was calculated taking into account the following indexes of importance: 0.10, 0.35, 0.15, 0.25, 0.15 (respectively).

ABT-yoghurts were produced in three independent series, analyses were conducted in duplicate (except sensory analysis) and results were expressed as means ±SD. The effect of tea addition (colour analysis) or tea addition and storage time was estimated using ANOVA and, where a significant effect was found ($P \leq 0.05$), the significance of differences between the means was estimated using the Duncan test at a significance level of $P \leq 0.05$. Statistical analysis was performed using Statistica 12.0 software (StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Antioxidant properties

The antioxidant activity of the ABT-yoghurts measured by both methods, i.e. FRAP (ferric reducing antioxidant power) and DPPH radical scavenging activity (ARP), significantly increased upon supplementation with Pu-erh tea extracts (Fig. 1). The positive effect of Pu-erh tea was strongly concentration-dependent. Non-supplemented ABT-yoghurts were characterized by 0.80–1.13 mmol Fe^{2+} E·dm^{-3} FRAP and 0.05–0.16 μmol TE·g^{-1} ARP values. The average FRAP values were respectively 2.8, 4.8 and 6.5 times higher for ABT-yoghurts enriched with increasing amounts of Pu-erh tea additive. The scavenging activity against DPPH radical in yoghurts with 5, 10 and 15% Pu-erh tea infusion was on average enhanced 10.3, 18.2 and 24.1 times when compared to the plain ABT-yoghurt. Both studied antioxidant parameters were not affected by storage time. Generally, the same results were stated for ABT-yoghurts enriched with green tea extracts (Najgebauer-Lejko, 2014) but,

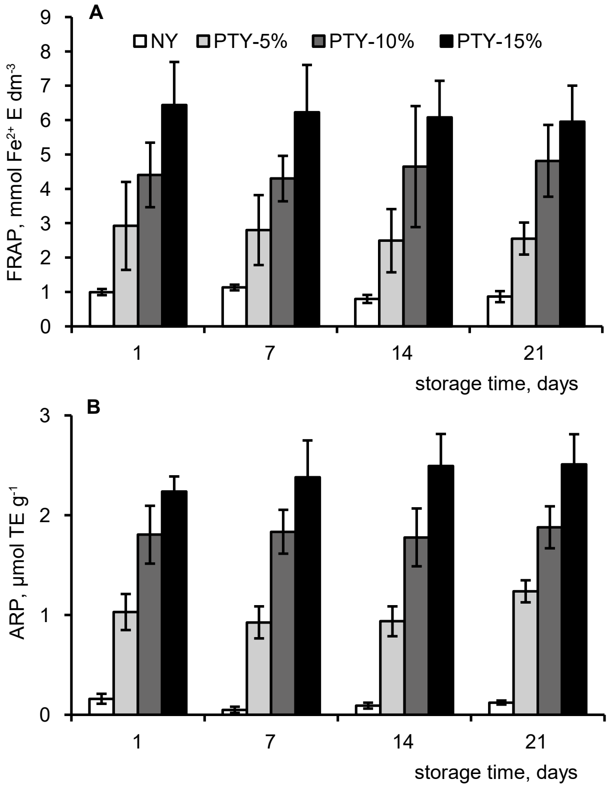

Fig. 1. Ferric reducing antioxidant power (FRAP) and anti-radical power (ARP) of ABT-yoghurts as affected by Pu-erh tea concentration and storage time: NY – natural ABT-yoghurt, PTY-5% – ABT-yoghurt with 5% (v/v) of Pu-erh tea infusion, PTY-10% – ABT-yoghurt with 10% (v/v) of Pu-erh tea infusion, PTY-15% – ABT-yoghurt with 15% (v/v) of Pu-erh tea infusion; means ±SD, $n = 6$

in this case, ARP and FRAP values were two times or more higher than in the Pu-erh tea yoghurts. The difference between the antioxidant power of green and Pu-erh tea is probably due to the differences in their chemical composition. Comparison of both tea types revealed that Pu-erh tea is not as good a source of catechins and total phenolic content as green tea (Najgebauer-Lejko et al., 2011; 2014). Zhang et al. (2011) and Lv et al. (2013) reported that, after fermentation by microorganisms, the level of catechins in Pu-erh tea dramatically decreased, whereas the post-fermentation process increased the concentration of gallic acid and caffeine, as well as highly polymerized flavan-3-ols derivatives such as theabrownin. Catechins, especially epigallocatechin gallate (EGCG) are generally recognized as potent antioxidant compounds (Zhang et al., 2011). Thus, lowering their concentration may result in decreased antioxidant properties. On the other hand, the antioxidant potential of Pu-erh tea may be related to the presence of active constituents like gallic acid and theabrownin (Lee and Foo, 2013).

Colour parameters

Calculated for tea ABT-yoghurts in reference to the natural yoghurt ΔE values, i.e. 7.02 (PT-5%), 10.30 (PT-10%), 13.30 (PT-15%) revealed that the addition of Pu-erh tea significantly changed the colour of yoghurts, and the differences between products with various levels of supplementation were also considerable. Plain ABT-yoghurt was characterized by a high average lightness (L^*) value, and a yellowish (b^*+) and slightly greenish (a^*–) colour (Fig. 2). Increasing amounts of added tea infusions caused a lower L^* as well as higher a^* and b^* chromatic components. These results were to be expected as Pu-erh tea infusion is described as reddish to brownish red (Gramza-Michałowska et al., 2017; Lee and Foo, 2013) due to the high content of polymerized pigments specific to this type of tea, namely theabrownin (brown), and thearubigin and theaflavin (red) (Najgebauer-Lejko et al., 2014). The results are also consistent with the previously reported colour profiles for yoghurts with Pu-erh tea (Najgebauer-Lejko et al., 2014).

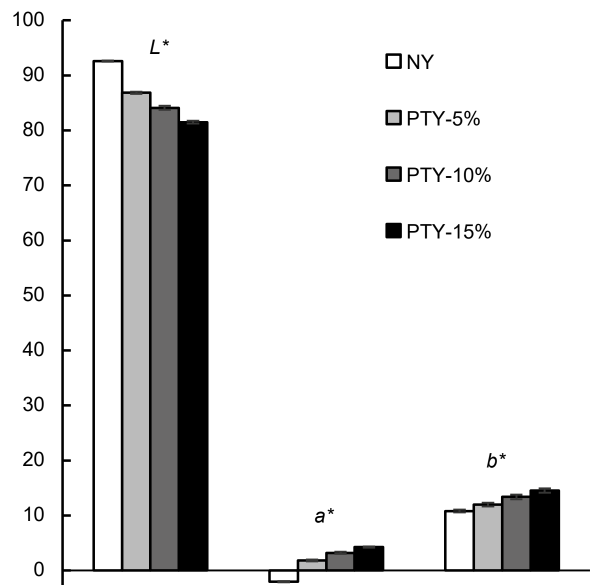

Fig. 2. Colour profile parameters: L^* – lightness, a^* – green-red colour, b^* – blue-yellow colour of ABT-yoghurts; NY – natural ABT-yoghurt, PTY-5% – ABT-yoghurt with 5% (v/v) of Pu-erh tea infusion, PTY-10% – ABT-yoghurt with 10% (v/v) of Pu-erh tea infusion, PTY-15% – ABT-yoghurt with 15% (v/v) of Pu-erh tea infusion; means ±SD, $n = 6$

Textural properties

The texture parameters of ABT-yoghurts determined by means of a back extrusion test (BET) are presented in Table 1. The firmness of the analysed ABT-yoghurts was not affected by the Pu-erh tea enrichment until the 14^{th} day of storage. After three weeks, a significant increase in the firmness value was observed in probiotic yoghurt with 5% Pu-erh tea which was much higher than the firmness of the other treatments. Generally, when mean values calculated for the whole storage period are taken into consideration, 15% Pu-erh tea addition produced yoghurt gels with a lower firmness than the 5% treatment, but both yoghurt types differed significantly from the plain one. Higher doses of Pu-erh tea infusion also influenced a loss in ABT-yoghurt consistency but only after 14 and 21 days of storage. After two weeks, all tea treatments were characterized by a lower consistency than the natural one, whereas at the end of the experiment the consistency of the 5% treatment was similar to that stated for natural yoghurt but much higher than those measured for 10% and 15% Pu-erh tea treatments. The cohesiveness and index of viscosity of ABT-yoghurts were not affected by the Pu-erh tea addition and remained unchanged throughout the whole storage period. The concentration-dependent effect of tea addition on the yoghurt gel mechanical properties was also observed by Dönmez et al. (2017), who noticed modified consistency and syneresis rate in yoghurts supplemented with green tea powder and green coffee powder in comparison to the control product. The effect was dependent on the type of polyphenolic substances and their concentration. The authors concluded that the addition of tea may enhance the strength of the yoghurt gel and stabilize the structure of the protein network but only when added in certain amounts.

Sensory properties

The unique aroma of tea is composed of a complicated mixture of more than 630 aroma compounds identified so far (Chaturvedula and Prakash, 2011). Lee and Foo (2013) described Pu-erh tea as having a refreshing taste and attractive aroma, reddish to brownish-red colour, and as full-bodied and notably fragrant. The specific aroma of this type of tea is formed by volatile compounds, such as methoxy-phenolic compounds, alcohols and hydrocarbons, which contribute to the stale, woody, fruity and floral notes of Pu-erh tea's flavour. According to Zhang et al. (2011), during fermentation, Pu-erh tea develops a "wonderfully complex, silky smooth and mellow" taste. However, tea consumption is also associated with astringency and bitterness related with its considerable content of polyphenolic compounds, caffeine and some amino acids (Chaturvedula and Prakash, 2011). The results of the sensory assessment presented in Table 1 revealed that Pu-erh tea yoghurts, especially those with 5–10% of tea incorporation, gained lower notes than probiotic yoghurt without tea. Interestingly, among tea yoghurts, a 15% treatment was more appreciated by the panellists (notes above 4.0 in a 5-point scale). Moreover, an increased sensory quality was observed with storage time. It is postulated that perception of the bitterness and astringency together with Pu-erh tea's specific, less acceptable, fungal and earthy flavour (Gramza-Michałowska et al., 2017) contributed to the lower ratings received by the Pu-erh tea probiotic yoghurts. In the study by Liu (2018), dark Fuzhuan brick tea addition did not influence the overall sensory notes of the probiotic yoghurts and the received scores were sufficient to consider supplemented yoghurts acceptable. However, yoghurts with higher doses of Fuzhuan tea gained lower scores for appearance and higher ones for aroma than the control.

Acidity

Generally, the titratable acidity of ABT-yoghurt was not affected by Pu-erh tea addition, but tea yoghurts were characterized by a slightly more visible post-acidification effect as significant increment of acidity was noticed at the 21st day of storage, which was not observed for the plain fermented milk (Table 1). On the other hand, the pH of ABT-yoghurts decreased upon supplementation with Pu-erh tea. However, the only significant difference occurred between all tea treatments and the natural one after 7 days of cold storage. The significant drop in pH value during the storage period was observed in all treatments, however, Pu-erh tea caused higher rate of pH reduction in the first studied time interval in contrast to the plain yoghurt. The observed effect may result from the increased activity of lactobacilli, whose viability was slightly stimulated by the Pu-erh tea extracts, as revealed in the microbiological assessment. These findings are consistent with previously published studies for conventional yoghurt, which revealed that Pu-erh tea supplementation influenced a lower pH (Najgebauer-Lejko et al., 2011). Muniandy et al. (2017) also observed a reduction of pH and increment of lactic acid production in yoghurt with green, black and white tea (in order: green tea > black tea > white tea). In contrast, the addition of 1–3% of Fuzhuan brick dark tea had no significant effect on the pH or titratable acidity of probiotic yoghurt during fermentation and refrigerated storage (Liu, 2018). The same was stated for ABT-yoghurt enriched with 5–10% of green tea infusion (Najgebauer-Lejko, 2014).

Viability of starter bacteria

Statistical analysis (ANOVA) revealed that Pu-erh tea fortification in doses of 5% and 15% positively affected the level of *L. acidophilus* La-5 in ABT-yoghurt ($P \leq 0.05$) when the mean values for the whole storage period (the least square means) were calculated (7.93 log cfu·g^{-1} for plain yoghurt vs. 8.20 log cfu·g^{-1} and 8.21 log cfu·g^{-1} for yoghurt with 5% and 15% of Pu-erh tea). However, the differences between *L. acidophilus* counts for the specific time intervals were not statistically significant (Fig. 3A). The viability of bifidobacteria and streptococci was not affected by Pu-erh tea supplementation (Fig. 3B and 3C). Generally, all starter bacteria counts were stable during the whole three weeks of storage and the number of probiotic bacteria remained at a therapeutic level i.e. above 10^6 cfu·g^{-1} to the end of the experiment (Shori, 2016). These findings are in concordance with previously reported results for green tea bio-yoghurts, which did not differ significantly from the plain control in the number of bifidobacteria and streptococci (Najgebauer-Lejko, 2014). However, in the mentioned study, higher doses of green tea extracts (10% and 15%)

Table 1. Changes in pH, titratable acidity, sensory notes and texture (back extrusion) parameters of ABT-yoghurts during cold storage (means ±SD, *n* = 6)

Parameter	Treatment	Day of storage				Mean for the whole storage period*
		1	7	14	21	
pH	NY	4.70 ±0.09^{Ab}	4.64 ±0.04^{Bb}	4.54 ±0.04^{Aa}	4.50 ±0.01^{Aa}	4.60 ±0.09^{B}
	PTY-5%	4.68 ±0.11^{Ab}	4.51 ±0.08^{Aa}	4.47 ±0.06^{Aa}	4.46 ±0.05^{Aa}	4.53 ±0.12^{A}
	PTY-10%	4.63 ±0.12^{Ab}	4.52 ±0.05^{Aa}	4.48 ±0.06^{Aa}	4.48 ±0.04^{Aa}	4.53 ±0.09^{A}
	PTY-15%	4.61 ±0.14^{Ab}	4.53 ±0.04^{Aab}	4.48 ±0.06^{Aa}	4.49 ±0.02^{Aa}	4.53 ±0.09^{A}
Titratable acidity % lactic acid	NY	0.85 ±0.04^{Aa}	0.83 ±0.07^{Aa}	0.83 ±0.06^{Aa}	0.91 ±0.07^{Aa}	0.85 ±0.07^{A}
	PTY-5%	0.84 ±0.09^{Aab}	0.80 ±0.03^{Aa}	0.85 ±0.09^{Aab}	0.91 ±0.04^{Ab}	0.85 ±0.08^{A}
	PTY-10%	0.81 ±0.04^{Aa}	0.84 ±0.07^{Aab}	0.85 ±0.07^{Aab}	0.91 ±0.02^{Ab}	0.85 ±0.06^{A}
	PTY-15%	0.87 ±0.07^{Aab}	0.84 ±0.05^{Aa}	0.89 ±0.09^{Aab}	0.93 ±0.05^{Ab}	0.88 ±0.07^{A}
Sensory notes	NY	4.67 ±0.13^{Cab}	4.59 ±0.30^{Ca}	4.79 ±0.06^{Cb}	4.71 ±0.18^{Cab}	4.69 ±0.19^{C}
	PTY-5%	3.78 ±0.1^{Aa}	3.90 ±0.13^{Aa}	4.10 ±0.11^{Ab}	4.23 ±0.07^{Ab}	4.00 ±0.21^{A}
	PTY-10%	3.71 ±0.11^{Aa}	3.82 ±0.08^{Aa}	4.03 ±0.09^{Ab}	4.43 ±0.05^{Bc}	4.00 ±0.29^{A}
	PTY-15%	4.20 ±0.18^{Ba}	4.36 ±0.09^{Bab}	4.40 ±0.20^{Bb}	4.69 ±0.02^{Cc}	4.41 ±0.22^{B}
Firmness, N	NY	7.47 ±0.88^{Aa}	7.38 ±0.10^{Aa}	8.04 ±0.14^{Aa}	8.12 ±0.32^{Aa}	7.75 ±0.53^{AB}
	PTY-5%	7.50 ±0.59^{Aa}	8.01 ±0.40^{Aa}	7.63 ±0.41^{Aa}	9.13 ±0.48^{Bb}	8.07 ±0.78^{B}
	PTY-10%	7.52 ±0.38^{Aa}	7.58 ±0.35^{Aa}	7.70 ±0.33^{Aa}	7.62 ±1.15^{Aa}	7.61 ±0.56^{AB}
	PTY-15%	6.92 ±0.30^{Aa}	7.38 ±0.56^{Aa}	7.45 ±0.49^{Aa}	7.30 ±1.26^{Aa}	7.26 ±0.67^{A}
Consistency, N·s	NY	167.03 ±15.82^{Aa}	158.20 ±10.28^{Aa}	176.93 ±4.48^{Ba}	172.13 ±3.54^{ABa}	168.57 ±11.10^{C}
	PTY-5%	156.83 ±13.19^{Aa}	165.39 ±2.83^{Aa}	155.10 ±8.29^{Aa}	178.20 ±19.83^{Ba}	163.88 ±14.43^{BC}
	PTY-10%	156.96 ±5.35^{Aa}	160.62 ±4.19^{Aa}	159.07 ±0.96^{Aa}	158.69 ±10.30^{Aa}	158.84 ±5.45^{AB}
	PTY-15%	150.86 ±1.86^{Aa}	152.98 ±9.17^{Aa}	155.88 ±4.14^{Aa}	155.76 ±6.97^{Aa}	153.87 ±5.69^{A}
Cohesiveness, |N|	NY	4.58 ±0.30^{Aa}	4.16 ±0.19^{Aa}	4.07 ±0.51^{Aa}	4.16 ±0.30^{Aa}	4.24 ±0.36^{A}
	PTY-5%	4.86 ±0.13^{Aa}	4.32 ±1.10^{Aa}	4.15 ±0.35^{Aa}	4.73 ±0.43^{Aa}	4.52 ±0.61^{A}
	PTY-10%	4.69 ±0.12^{Aa}	4.61 ±0.40^{Aa}	4.40 ±0.43^{Aa}	4.21 ±0.21^{Aa}	4.48 ±0.33^{A}
	PTY-15%	4.82 ±0.38^{Aa}	4.51 ±0.30^{Aa}	4.16 ±0.35^{Aa}	4.16 ±0.40^{Aa}	4.41 ±0.42^{A}
Index of viscosity, |N·s|	NY	9.12 ±0.78^{Aa}	9.22 ±0.59^{Aa}	8.14 ±0.69^{Aa}	8.53 ±0.59^{Aa}	8.73 ±0.69^{A}
	PTY-5%	10.00 ±0.20^{Aa}	8.92 ±0.88^{Aa}	8.83 ±0.78^{Aa}	8.92 ±0.29^{Aa}	9.12 ±0.69^{A}
	PTY-10%	9.51 ±0.69^{Aa}	9.81 ±1.27^{Aa}	9.51 ±0.59^{Aa}	9.32 ±1.27^{Aa}	9.51 ±0.88^{A}
	PTY-15%	9.71 ±0.49^{Aa}	9.51 ±0.29^{Aa}	8.53 ±0.78^{Aa}	9.32 ±0.69^{Aa}	9.32 ±0.68^{A}

NY – natural (plain) ABT-yoghurt; PTY-5%, PTY-10%, PTY-15% – ABT-yoghurts with addition of 5%, 10% or 15% (v/v) of Pu--erh tea water extract.

*The least square mean value.

Means for a given parameter within each column/row followed by different capital/lower-case letters (respectively) are statistically different at $P \leq 0.05$.

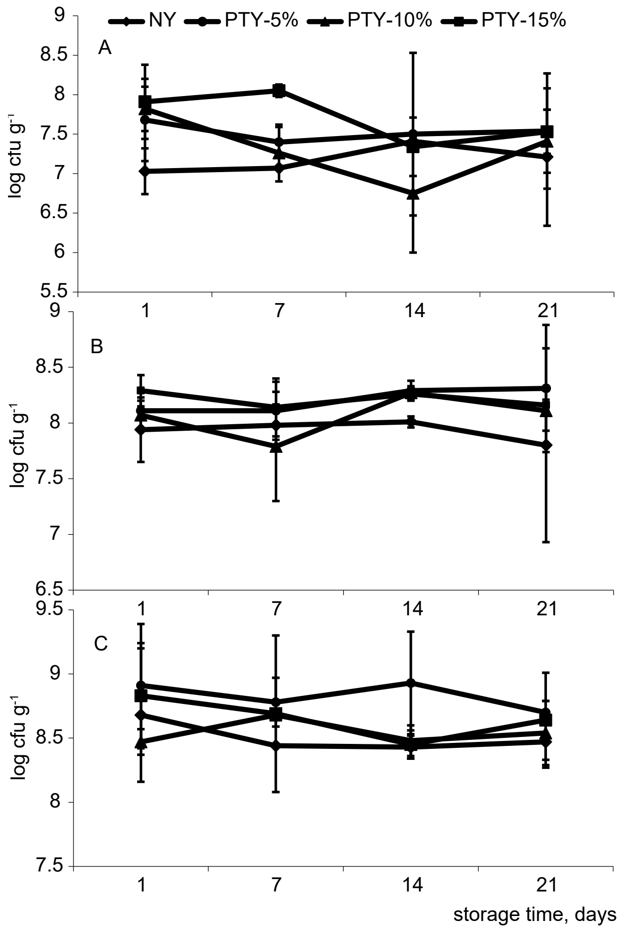

Fig. 3. The number of starter bacteria in ABT-yoghurts during cold storage: *B. lactis* BB-12 (A), *L. acidophilus* La-5 (B), *S. thermophilus* (C); NY – natural ABT-yoghurt, PTY-5% – ABT-yoghurt with 5% (v/v) of Pu-erh tea infusion, PTY-10% – ABT-yoghurt with 10% (v/v) of Pu-erh tea infusion, PTY-15% – ABT-yoghurt with 15% (v/v) of Pu-erh tea infusion; means ±SD

resulted in higher numbers of *L. acidophilus* than in the lowest level (5%) of supplementation. No impact of green or black tea on the yoghurt bacteria was stated by Jaziri et al. (2009). Neither were significant differences between yoghurt bacteria numbers observed by Muniandy et al. (2017) upon supplementation of green, black or white tea. On the contrary, Najgebauer-Lejko et al. (2011) reported the positive influence of green and Pu-erh tea additives on the viability of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* in conventional yoghurt. Amirdivani and Baba (2015) also observed the positive effects of green tea on the growth of *Lactobacillus* ssp. and *S. thermophilus* in probiotic yoghurt inoculated with *L. acidophilus* La-5, *B. lactis* BB-12, *L. casei* LC-01, *S. thermophilus* and *L. bulgaricus*. Increased levels of *S. thermophilus* and *L. acidophilus* in probiotic yoghurt with added extracts of dark Fuzhuan brick-tea accompanied with slightly improved β-galactosidase and proteolytic activities was demonstrated by Liu (2018). These discrepancies between the results obtained by different authors may have arisen from different types, varieties and origins of the tea, the tea infusions or dry extracts applied, different conditions of brewing (proportions of tea leaves to water, water quality, temperature, time of brewing, brewing in water or directly in milk), different modes of addition (before or after heat treatment of milk), differences in the raw materials and preparation of fermented milk (milk base, heat treatment etc.), and different starter cultures used for milk inoculation (species, strains of bacteria).

CONCLUSION

Pu-erh tea can be successfully applied as a functional additive to probiotic yoghurt as it significantly increases the antioxidant properties of the final product. This supplementation also ensures a high stability of starter cultures during cold storage. Moreover, Pu-erh tea may be considered as an additive with the potential to enhance the viability of probiotic strains. On the other hand, the concentration of Pu-erh tea infusion must be carefully chosen as in some doses it negatively influences the textural parameters and sensory acceptance of probiotic yoghurts.

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