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INFLUENCE OF INULIN AND POTENTIALLY PROBIOTIC *LACTOBACILLUS PLANTARUM* STRAIN ON MICROBIOLOGICAL QUALITY AND SENSORY PROPERTIES OF SOFT CHEESE

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The influence of inulin HPX and potentially probiotic *Lactobacillus plantarum* 14 strain on microbial quality and organoleptic properties of soft cheese were studied. Also the effect of inulin on probiotic concentration was examined during 45 days of storage at 6°C. Four versions of soft cheese were produced: (1) control without synbiotic, (2) with *L. plantarum* 14, (3) with inulin HPX 2.5 g/100 g of cheese, (4) with inulin HPX 2.5 g/100 g of cheese and *L. plantarum* 14. The number of potentially probiotic bacteria was affected by the addition of inulin HPX ($p<0.05$). In all probiotic cheeses the concentration of potentially probiotic strain was at a recommended level of 10^6–10^7 cfu/g. Also sensory quality was positively affected by the presence of inulin in products. After production and 45 days of storage the most desirable properties possessed cheese produced with the addition of inulin HPX, followed by control cheese. Microbial quality of all cheeses was satisfactory.

INTRODUCTION

Probiotics are live microbial food supplements which benefit the health of consumers by maintaining or improving their intestinal microbial balance [Mattila-Sandholm *et al*., 2002]. A probiotic strain must be able to survive during passage through the upper gastrointestinal tract, colonize the intestine, and exert antimicrobial activity against pathogens. It has to be stable and maintain viability in food product and have a beneficial influence on sensory properties of the product [Saarela *et al*., 2000]. Probiotics have been used in numerous food products like yoghurts, kefir, ice cream, frozen fermented dairy products, Cheddar cheese, fruit and berry juices [Tharmaraj *et al*., 2004]. Cheese as a vehicle for live probiotic bacteria may be more effective than yoghurt-type products because of the higher pH, higher fat content and more solid consistency of cheese which offer protection to the probiotics in the gastrointestinal tract [Gardiner *et al*., 1998; Kasimoğlu *et al*., 2004].

Few papers report incorporation and survival of *Lactobacillus acidophilus*, *L. sake* and *Bifidobacterium* sp. in cheese [Kasimoğlu *et al*., 2004] but there are few scientific reports about lactobacilli of human origin as probiotic cheese additives [Gomez *et al*., 1996; Songisepp *et al*., 2004].

The recent trend in food technology is to combine probiotic with prebiotics. Products containing these both components are generically termed "synbiotics" [Holzapfel & Schillinger, 2002]. Prebiotics (inulin, lactulose, oligosaccharides *etc*.) are not digested by human enzymes and reach colon intake where they stimulate the growth and/or activity of one or a limited number of bacteria, thus improving the host's gut health [Losada & Olleros, 2002]. In the food technology carbohydrate prebiotics, like inulin, are used to improve body and mouthful, as stabilizers, fat replacers, and flavour enhacers [Voragen, 1998]. There are some reports concerning employing inulin in probiotic yoghurt [Crittenden *et al*., 2001; Lourens-Hattingh & Viljoen, 2001; Staffolo *et al*., 2004] but not many refer to synbiotic white cheese. Therefore the aim of the study was to obtain soft white synbiotic cheese containing *L. plantarum* 14 and heat-stable inulin HPX and examine the influence of pre- and probiotics on the microbial quality and sensory properties of cheese. The effect of inulin on the population of potentially probiotic strain was determined as well.

MATERIALS AND METHODS

Cheese production. Cheese was produced in a dairy plant located in central Poland according to manufacture instructions with commercial mesophilic lactic culture and rennet. After coagulation, curd was cut and cooled to 8–10°C to avoid over acidification and overcome thermization. Hot cheese was centrifuged and moisture content was established at a required level of 63–65%. After cooling, inulin and probiotic cultures were added. After stirring, the cheese was manually packed in 150-g containers and kept for 24 h at a room temperature (approx. 20°C) to enable probiotics proliferation and then cooled to 6–8°C and stored for 45 days.

Four versions of cheese were prepared: (1) control without synbiotic, (2) with *L. plantarum* 14, (3) with inulin HPX (Orafti) 2.5 g/100 g of cheese, and (4) with inulin HPX (Orafti) 2.5 g/100 g of cheese and *L. plantarum* 14. *Lactobacillus plantarum* 14 was previously isolated from the gastrointestinal tract of a healthy infant. The strain turned out to be acid and

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bile resistant and showed antimicrobial activity against Gram--positive and Gram-negative bacteria in agar well-diffusion method (data not shown). For cheese making the culture was prepared on reconstituted skimmed milk and incubated for 24 h at 37°C. To cheeses 2 and 4, a culture containing about 10^8 cfu/mL was added in the amount of 20 mL/1 kg of cheese. The same amount of skimmed milk was added to cheeses 1 and 3.

Physicochemical analysis of cheese. After production, cheese samples were determined for moisture and fat, protein and ash contents using standard methods. pH values of cheese were determined on duplicate samples after production, 14, 21, 28, 35 and 45 days of storage.

Microbial analysis. Viability of *Lactobacillus plantarum* was monitored after 2, 20 and 45 days of storage. To this end, 10-g portions of duplicate cheese samples were blended with 90 mL of peptone water in a laboratory blender (Seward Stomacher 400) and submitted to serial dilutions with the same diluent. The number of potentially probiotic microorganisms was enumerated using MRS with maltose and Bromocresol Purple after 48 h of anaerobic incubation (Anaerocult C, Merck) at 37°C. Counts of moulds and yeasts were enumerated on Petrifilm PYF (Noack) after 5 days of incubation at 25°C, coli rods on Petrifilm PCC (Noack) after 24 h at 30°C, *Staphylococcus aureus* on Petrifilm Staph Express (Noack) after 24 h at 37°C. The presence of *Salmonella* sp. in 25 g of cheese was examined using Tecra Unique Salmonella (Noack) after pre-enrichment in Buffered Peptone Water for 24 h at 37°C.

Sensory analysis. A comparison between cheese samples was conducted according to Polish Standard PN ISO 4121 [1998] after production and at the end of the storage time. Flavour, color and texture were evaluated by a panel of 5 trained persons using 6-point scale on the basis of a descriptor table for each attribute (6-very good, 1-very bad). Factors of significance for colour, smell, texture and taste were 0.15, 0.20, 0.30 and 0.35, respectively.

TABLE 1. Average composition of cheeses after production.

Cheeses	Moisture (%)	Proteins (%)	Fat (%)	Ash (%)
1	64.12	5.80	25.0	0.650
2	64.82	5.79	24.5	0.737
3	65.30	4.90	23.75	0.546
4	64.65	4.36	25.0	0.517

1 – control, 2 – with *L. plantarum* 14, 3 – with inulin HPX, 4 – with inulin HPX and *L. plantarum* 14

Statistical analysis. Statistical analysis was performed using Statistica 6.0 (StatSoft) Duncan's test at $p<0.05$.

RESULTS AND DISCUSSION

The average chemical composition of all cheeses is presented in Table 1. pH values of all cheeses did not change significantly ($p<0.05$) over the storage time (Table 2). These results were similar to findings of Staffolo *et al*. [2004] who studied changes in pH values of yoghurts produced with the addition of inulin and suggested that inulin addition to fermented dairy products did not influence its acidity.

Microbial quality of all cheeses was satisfactory. Pathogenic bacteria: *Salmonella* sp. and coagulase-positive *Staphylococcus aureus* were not present in any of the experimental cheeses. Numbers of coli forms, yeasts and moulds were <10 cfu/g over the entire storage time.

The number of potentially probiotic bacteria was affected by the addition of inulin HPX. In cheese samples containing inulin, the mean number of *L. plantarum* 14 was higher than in cheese samples without prebiotic over the entire period of storage (Table 3). After production, the number of potentially probiotic bacteria in cheese without inulin supplementation was at the level of 2.0×10^6 cfu/g. It increased after 20 days of storage to 2.9×10^7 cfu/g and remained at a statistically equal ($p<0.05$) level of 2.7×10^7 cfu/g till the end of shelf life. The initial concentration of *L. plantarum* 14 in cheese with inulin HPX was 1.9×10^7cfu/g. After 20 and 45 days of storage the number increased, reaching 4.6×10^7 cfu/g, and after 45 days slightly decreased (not significantly $p<0.05$) to 4.1×10^7 cfu/g. In order to exert the beneficial effects of probiotic foods, a minimum probiotic therapeutic daily dose of 10^8–10^9 cfu has been proposed, which corresponds to the daily intake of 100 g of a food product containing 10^6–10^7 cfu/g [Lee & Salminen, 1995]. In the present study, the counts of *Lactobacillus plantarum* in cheeses with and without inulin addition

TABLE 3. Effect of milk supplementation with inulin HPX on the concentration of probiotic *Lactobacillus plantarum* 14 strain in soft cheese during storage (the numbers of bacteria expressed as mean value of cfu/g were calculated from two duplicates).

Cheese with the addition of	Storage period (days)		
	2	20	45
L. plantarum 14	$^{a}2.0\times10^{6\,A}$	$^{a}2.9\times10^{7\,B}$	$^{a}2.6\times10^{7\,B}$
L. plantarum 14 and inulin HPX	$^{b}1.9\times10^{7\,A}$	$^{a}4.6\times10^{7\,B}$	$^{b}4.1\times10^{7\,AB}$

$^{a,\,b}$ column means with no common superscripts differ ($p<0.05$); $^{A,\,B}$ row means with no common superscripts differ ($p<0.05$)

TABLE 2. Changes of mean pH values of cheeses during storage at 6°C.

Cheeses	Storage time (days)					
	0	14	21	28	35	45
1	$^{a}4.58^{A}$	$^{a}4.60^{A}$	$^{a}4.61^{A}$	$^{a}4.61^{A}$	$^{a}4.62^{A}$	$^{a}4.60^{A}$
2	$^{a}4.59^{A}$	$^{a}4.60^{A}$	$^{a}4.59^{A}$	$^{a}4.58^{A}$	$^{a}4.58^{A}$	$^{a}4.58^{A}$
3	$^{a}4.59^{A}$	$^{a}4.59^{A}$	$^{a}4.58^{A}$	$^{a}4.57^{A}$	$^{a}4.56^{A}$	$^{a}4.60^{A}$
4	$^{a}4.59^{A}$	$^{a}4.62^{A}$	$^{a}4.62^{A}$	$^{a}4.61^{A}$	$^{a}4.60^{A}$	$^{a}4.61^{A}$

1 – control, 2 – with *L. plantarum* 14, 3 – with inulin HPX, 4 – with inulin HPX and *L. plantarum* 14; ^{a} column means with common superscripts do not differ ($p<0.05$); ^{A} row means with common superscripts do not differ ($p<0.05$)

were at the recommended level of 10^6–10^7 cfu/g, thus satisfying the criteria established for a probiotic food. An increase was observed in *L. plantarum* concentration up to 20 day of storage in cheeses with and without inulin. Similar findings concerning probiotic bacteria concentration were described by Buriti *et al*. [2005], who examined survival of *Lactobacillus paracasei* strain in Minas fresh cheese. Initial counts of probiotic microorganisms in cheeses were above the level of 10^6-10^7 cfu/g and increased by 2 log cycles during 21 days of storage.

All cheeses obtained high scores in the sensory analysis (Figures 1, 2). After production and 45 days of storage, the most desirable properties possessed cheese produced with the addition of inulin HPX, followed by control cheese. Cheeses with *Lactobacillus plantarum* 14 strain gained slightly lower notes. However, they were also accepted by panelists and were rated relatively high scores. Buriti *et al*. [2005] reported no significant differences in the sensory quality between Minas fresh cheeses manufactured with probiotic *Lactobacillus paracasei* and control ones without probiotic supplementation. Similar findings were reported by Gardiner *et al*. [1998] and Stanton *et al*. [1998], who studied the influence of *Lactobacillus* adjuncts on sensory properties of Cheddar cheese, indicating that lactobacilli had no adverse effect on the sensory quality of products. On the other hand, Menéndez *et al*. [2000] observed differences between Arzúa-Ulloa

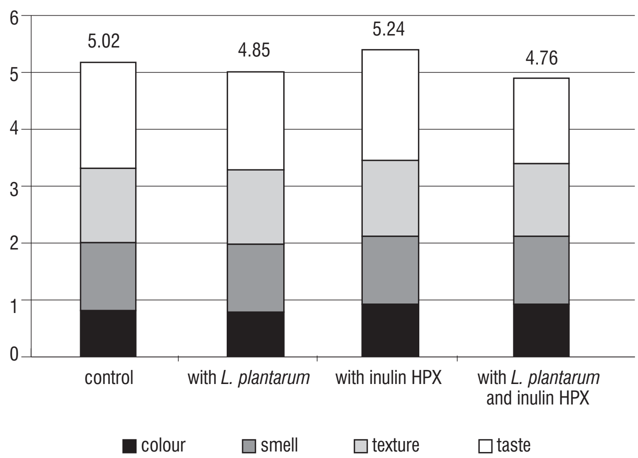

FIGURE 1. Total mean values of the sensory analysis of cheeses after production evaluated using a 6-point scale (6 – good, 1 – bad).

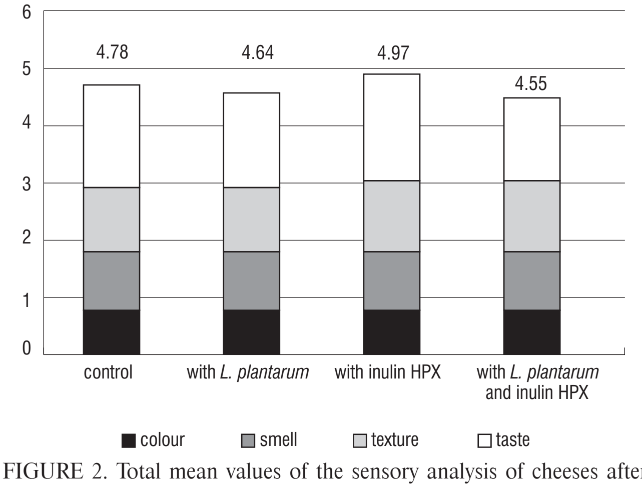

FIGURE 2. Total mean values of the sensory analysis of cheeses after 45 days of storage evaluated using a 6-point scale (6 – good, 1 – bad).

cheeses manufactured with the addition of *Lactobacillus casei* and *Lactobacillus plantarum* strains and control ones which presented less acid flavour and greater firmness.

CONCLUSIONS

The results obtained suggest that inulin addition to soft cheese advantageously affects the sensory profile of products and promotes growth of probiotic bacteria. Thus application of inulin HPX in soft cheese production will result in obtaining dairy products with new functional properties.

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WPŁYW INULINY I POTENCJALNIE PROBIOTYCZNEGO SZCZEPU *LACTOBACILLUS PLANTARUM* NA JAKOŚĆ MIKROBIOLOGICZNĄ I CECHY ORGANOLEPTYCZNE SERKÓW TWAROGOWYCH

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W pracy zbadano wpływ ciepłoopornej inuliny HPX (Orafti) i jelitowego szczepu *Lactobacillus plantarum* o właściwościach probiotycznych na cechy organoleptyczne i jakość mikrobiologiczną serków twarogowych. Badano także wpływ inuliny na populację bakterii *L. plantarum* podczas chłodniczego przechowywania wyrobów przez 45 dni. Przygotowano 4 wersje wyrobów: (1) bez inuliny i szczepu probiotycznego, (2) ze szczepem *L. plantarum*, (3) z inuliną HPX, (4) z inuliną HPX i szczepem *L. plantarum*. Wykazano, że liczba *L. plantarum* 14 w serkach z dodatkiem prebiotyku była wyższa niż w wersji kontrolnej ($p<0,05$), przy czym we wszystkich wersjach serków odpowiadała zalecanej dla wyrobów probiotycznych (poziom 10^6–10^7 jtk/g) (tab. 3). Dodatek inuliny korzystnie wpłynął na cechy sensoryczne – po produkcji i 45 dniach przechowywania najwyższe noty uzyskał produkt z udziałem inuliny HPX (rys. 1, 2). Jakość mikrobiologiczna wszystkich serków odpowiadała wymaganiom Polskiej Normy.