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QUALITY PROPERTIES OF NON-FAT YOGHURT WITH ADDITION OF WHEY PROTEIN CONCENTRATE**

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**Original scientific paper

Abstract: The aim of the study was investigation of quality of fat-free, set-type yoghurt made at 5% (w/w) protein level with addition of skim milk powder (SMP) and whey protein concentrate (WPC) blends. The ratio SMP/WPC in used blends was: 1/0; 2/1; 1/2; 0/1. On 1st, 7th and 21st day of refrigerated storage yoghurt was analysed for sensory properties, titrable acidity, pH, free fatty acids (FFA), acetaldehyde, and diacethyl. Also enumeration of viable *L. delbrueckii ssp. bulgaricus* and *S. thermophilus* was carried out. During the whole storage period products with SMP/WPC ratio at 1/2 and 2/1 obtained the best sensory score. Addition of WPC to yoghurt significantly decreased lactic acid concentration which positively influenced its stability during shelf life. It was shown that during storage acidity of yoghurt was growing up in concern of pH level. The acetaldehyde content tended to increase significantly in the yoghurt fortified with higher proportion of WPC opposite to diacethyl level which was the lowest in yoghurt with an SMP/WPC addition at 0/1. During storage concentration of both volatile compounds were the highest on 7th day and the lowest on 21st day. The maximum concentration of FFA was stated in products with SMP/WPC ratio 1/2. During the storage period FFA content significantly increased after 7th days and had no changed during next 14 days. The total number of yoghurt bacteria during the whole storage time was up to 10^{10} and did not vary depending on SMP/WPC ratio. Amount of *L. delbrueckii ssp. bulgaricus* was about one log cycle lower than *S. thermophilus* in all kinds of yoghurt.

Key words: quality, fat-free yoghurt, whey protein concentrate,

Introduction and literature review

Consumption of low- and non-fat dairy products has increased in recent

years because of potential health benefits and nutritional advantages (*Haque & Ji* 2003). Traditional yoghurt contains high amount of fat and protein. These compounds play an important role in formation of its sensory properties. During production of fat-free natural yoghurt the use of optimal method for concentration of total solids level is fundamental for quality of final products. Weak body, syneresis and poor taste and flavor impair acceptability of fat-free yoghurt (*Kahkonen & Tuorila* 1999). Traditionally, skim milk powder is used for milk fortification, however, availability and the low cost of whey protein concentrates make them attractive and they are now commonly used to replace skim milk powder in yoghurt formulation (*Sodini et al*. 2006). The use of whey protein concentrate as partial substitutes from skim milk powder in yoghurt manufacture has been investigated by a number of workers but a range of effects have been reported. Additionally previous studies mainly concerned only structure and visco-elastic properties of yoghurts (*Puvanenthrian et al. 2002*, Singh 1999, *Augustin et al 2003*).

The aim of the study was investigation of quality of fat-free, set-type yoghurt made at 5% (w/w) protein level with addition of skim milk powder (SMP) and whey protein concentrate (WPC) blends.

Material and methods

For the preparation of yoghurt the bulk milk obtained from local farm was used. The experiment was performed during pasture feeding of cows. The starter culture YC-180 contains *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* was supplied by Christian Chansen (Denmark). Skim milk powder (SMP) obtained from the local market and whey protein concentrate (WPC) A235 (Lacma, Poland) were used to prepare formulations. Both products contain 34g protein per 100g.

For the yoghurt production whole milk was heated to 45 °C and centrifuged. Next, skim milk was standardized to content of 5g per 100g of protein. The fortification process was carried out by adding blends of skim milk powder and whey protein concentrate. The ratio SMP/WPC in used blends was: 1/0; 2/1; 1/2; 0/1. Prepared yoghurt formulations were then heated to 65°C, twice homogenized at a pressure of 7 MPa. Afterwards the mixtures were pasteurised at 90°C for 10 minutes, cooled to incubation temperature and inoculated with starter culture in a quantity of 2g/100g.
After mixing of milk, the sterilized containers were filled and incubated at

42 °C until the mixtures gelled and pH reached 4.6. Then, prepared yoghurts were cooled to 5°C and stored at this temperature until analysed. On 1st, 7th and 21st day of refrigerated storage the following determinations were carried out: pH (CP-215, Electron pH meter), titrable acidity (*AOAC,* 1995), free fatty acids (extraction-titrable Dole method by *Deeth et al.,* 1976), diacethyl (spectrophotometric method by *Pien,* 1974) and acetaldehyde content (spectrofotometric method by *Lees & Jago,* 1965). The number of *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* was evaluated by plate count method (*IDF Standard,* 1988). The experiment was carried out in four independent replicates and obtained data was analysed statistically with the two-factor ANOVA and differences between means were assessed with the Duncan test.

Results and discussion

Obtained results are presented in table 1 and 2. The best sensory quality revealed yoghurt fortified with mixture SMP/WPC at ratio 2/1, it was similar to products with ratio 1/0 (control) and 1/2. The addition of mixture with ratio 0/1 significantly decreased sensory properties of yoghurt. During the storage period all kind of yoghurt decreased their sensory quality. Statistically significant differences were observed at 21st day of storage period. But during all storage time analysed yoghurt got high score over 4 points. The phenomenon of getting worse of sensory characteristics of yoghurts during storage is commonly known and confirmed by many authors (*Tamime & Robinson,* 1999). It's mainly due to acidity growth, decomposition and evaporation of aroma substances, releasing of some proteolysis and lipolysis products, which influence negatively the taste and aroma, and shrinking of curd with whey release and getting worse of consistency.

The pH value of all yoghurts was similar. It was due to the fermentation process was carried out until pH fixed at 4.6. The fermentation times of the yoghurts with grater proportions of WPC were reduced (data not shown). This result agrees with those obtained by *Antunes et al*. (2004) who stated that yoghurt standardised by WPC addition achieve final pH=4.6, 45 minute before those standardised by SMP. The concentration of lactic acid significantly decreased when SMP/WPC ratio was reduced. A similar trend was observed by *Amatayakul et al*. (2006). They suggest that milks with low casein to whey protein ratios have low buffering capacity. It was shown

that during storage acidity of yoghurts was growing up in concern of pH level and lactic acid concentration and reached the highest value at 21st day of storage. That phenomenon is commonly known and it is a result of acidifying microflora activity (*Tamime & Robinson*, 1999).

Table 1. Influence of storage time on quality properties of yoghurts with different ratio of SMP/WPC in standardization blends ($x \pm s_e$)

Parameter	Day of storage	Ratio SMP/WPC			
		1/0	2/1	1/2	0/1
Sensory evaluation [points]	1	4.63±0.04	4.68±0.05	4.60±0.05	4.32±0.02
	7	4.61±0.08	4.63±0.05	4.57±0.04	4.28±0.03
	21	4.42±0.08	4.51±0.04	4.40±0.03	4.08±0.03
pH	1	4.66±0.02	4.51±0.06	4.53±0.07	4.60±0.06
	7	4.58±0.05	4.60±0.09	4.55±0.11	4.60±0.09
	21	4.38±0.09	4.49±0.13	4.43±0.10	4.46±0.14
Lactic acid [%]	1	0.81±0.06	0.85±0.03	0.84±0.03	0.80±0.03
	7	0.98±0.04	0.87±0.03	0.81±0.02	0.72±0.01
	21	0.95±0.01	0.91±0.03	0.87±0.03	0.73±0.03
Acetaldehyde [mg/dm^3]	1	6.60±0.70	6.40±0.29	6.97±0.29	7.94±0.65
	7	6.63±0.65	7.10±0.49	8.04±0.57	8.71±0.54
	21	5.35±0.89	5.37±0.53	6.38±0.82	6.40±0.51
Diacetyl [mg/dm^3]	1	0.70±0.03	0.51±0.05	0.24±0.03	0.37±0.09
	7	0.78±0.07	0.67±0.06	0.54±0.08	0.47±0.07
	21	0.53±0.01	0.29±0.08	0.21±0.04	0.23±0.06
FFA [mEq/dm^3]	1	4.61±0.23	5.32±0.22	6.14±0.42	5.19±0.27
	7	7.66±0.95	7.35±0.35	7.21±0.46	6.57±0.27
	21	6.27±0.76	6.23±0.38	8.97±0.46	7.03±0.77
S. thermophilus log [c.f.u./g]	1	10.46±9.21	9.91±8.27	10.35±9.17	10.12±8.89
	7	9.86±8.30	10.18±8.93	9.90±8.41	10.24±8.94
	21	9.68±9.42	10.13±8.58	10.36±9.07	10.32±9.05
L. delbr. ssp. bulgaricus log [c.f.u./g]	1	8.57±7.37	8.44±7.22	8.74±6.77	9.29±8.18
	7	8.43±7.09	8.36±6.86	8.53±6.91	8.85±6.95
	21	8.35±6.95	8.59±7.07	8.96±7.71	8.84±7.62

The composition of milk blends significantly influenced acetaldehyde and diacethyl level – the main aroma and flavour compounds. The concentration of acetaldehyde increased when the SMP/WPC ratio was reduced, what positively influenced on aroma of these products. Opposite trend was observed for diacethyl level. The lowest content of that compound was stated in yoghurt with SMP/WPC ratio 0/1. Acetaldehyde production starts at pH=5.0 and gets equilibrium at pH=4.0 (*Ziajka*, 1997). The

acetaldehyde amount in yoghurts should be not less than 10 mg/dm^3 and not more than 50 mg/dm^3 (*Tamime & Robinson*, 1999). Yoghurts assessed in our experiment had low levels of acetaldehyde, what, with presence of diacethyl, formed the mild and nice taste and smell of those drinks. According to *Hugunin* (1999) higher acetaldehyde concentrations in yoghurt produced with whey proteins may result from the higher concentrations of non-protein nitrogenous compounds in whey.

Table 2. Mean of the smallest squares from variance analysis concerning effect of SMP/WPC ratio and storage time on quality properties of yoghurt

Parameter	Ratio SMP/WPC				Time of storage		
	1/0	2/1	1/2	0/1	1	7	21
Sensory evaluation [points]	4.55 A	4.61 B	4.52	4.23 AB	4.56 A	4.52 B	4.35 AB
pH	4.54	4.56	4.51	4.55	4.60 a	4.58 b	4.44 ab
Lactic acid [%]	0.93 A	0.89 B	0.84 A	0.75 AB	0.84	0.85	0.87
Acetaldehyde [mg/dm^3]	6.19 A	6.28 B	7.13	7.68 AB	6.97	7.62	5.88
Diacetyl [mg/dm^3]	0.67 AB	0.49 Aa	0.33 A	0.36 Ba	0.46 A	0.62 A	0.32 A
FFA [mEq/dm^3]	6.18 A	6.30 B	7.44 ABa	6.26 a	5.31 AB	7.20 A	7.13 B
S. thermophilus log [c.f.u./g]	10.00	10.07	10.20	10.23	10.21	10.04	10.12
L. delbr. ssp. bulgaricus log [c.f.u./g]	8.46	8.47	8.78	9.00	8.76	8.54	8.68

A,B – statistically highly significant differences between means ($p \leq 0{,}01$) marked by the same letter in the rows

a,b – statistically significant differences between means ($p \leq 0{,}05$) marked by the same letter in the rows

There was observed significant influence of composition of milk base and storage time on free fatty acids (FFA) content in yoghurt. The maximum concentration of FFA was stated in products with SMP/WPC ratio 1/2. During the storage period FFA content significantly increased after 7th days and had no changed during next 14 days. The level of FFA is a measure of

lipolytic changes in yoghurts. Because of the analysed yoghurt was produced from skimmed milk there was probably other than lipolysis source of origin of this compounds. According to *Tamime & Robinson* (1999) probably the most important precursors of FFA in fat-free yoghurts are amino acids. It means that growth of FFA level during storage period was related to proteolytic activity of starter bacteria.

It was proved that the form and kind of additive had not influenced the microbiological quality of yoghurts. Analysed products was characterised by high number of yoghurt typical microflora. The total number of yoghurt microflora during the whole storage time was up to 10^{10}. The number of *S. thermophilus* was about 2 log cycle higher than *L. delbrueckii ssp. bulgaricus*. Investigated products met demands of international standard (*FAO/WHO*, 1994; *FIL/IDF*, 1997), where the number of viable cells of typical microflora in yoghurts must be no lower than 10^7. *Bury et al*. (1998) proved that WPC can stimulate the growth of lactic acid bacteria in whey or UF whey permeate broths. They suggest that components responsible for the increase growth might be α-nucleotides, non-protein nitrogen or some specific peptides. *McComas & Gilliland* (2003) investigated the growth of yoghurt cultures in milk supplemented with whey protein hydrolysates and concluded that it had no effect on the growth of various types of *L. delbrueckii ssp. bulgaricus* and *S. thermophilus*, whereas the probiotic bacteria were grown in combination with different yoghurt cultures.

Conclusion

The best sensory quality had yoghurt fortified with mixture SMP/WPC at ratio 2/1, it was similar to products with ratio 1/0 (control) and 1/2. The addition of mixture with ratio 0/1 significantly decreased sensory properties of yoghurt. Addition of WPC to yoghurt significantly decreased lactic acid concentration which positively influenced its stability during shelf life. The concentration of acetaldehyde increased when the SMP/WPC ratio was reduced, what positively influenced on aroma of these products. There was observed significant influence of composition of milk base and storage time on free fatty acids (FFA) content in yoghurt. The increase of FFA during storage was probably caused by proteolytic activity of yoghurt bacteria. Analysed yoghurts was characterised by high number of yoghurt typical microflora, which was not influenced by type of milk base and storage time.

OSOBINE KVALITETA NEMASNOG JOGURTA UZ DODATAK KONCENTRATA PROTEINA SURUTKE

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Rezime

Tradicionalno, obrano mleko u prahu se koristi za učvršćivanje jogurta, međutim, dostupnost i niska cena koncentrata proteina surutke ih čine atraktivnim kao zamene za mleko u prahu u proizvodnji fermentisanih mlečnih proizvoda. Cilj istraživanja je bio ispitivanje kvaliteta nemasnog jogurta sa nivoom proteina od 5% (w/w) uz dodatak mešavine obranog mleka u prahu (SMP) i koncentrata proteina surutke (WPC). Odnos SMP/WPC u korišćenim mešavinama je bio: 1/0; 2/1; 1/2; 0/1. Za proizvodnju jogurta mleko je centrifugirano, standardizovan protein, homogenizovano pod pritiskom od 7 MPa, pasterizovano na 90°C u trajanju od 10 minuta, ohlađeno do temperature inkubiranja i inokulisano starter kulturom YC-180 (DVS). Nakon mešanja mleka, sterilisani kontejneri su punjeni i inkubirani na 42 °C dok se smeše nisu želatinizirale i pH vrednost 4.6. Zatim, pripremljeni jogurti su ohlađeni na 5°C i čuvani na toj temperature. Prvog, sedmog i dvadesetprvog dana čuvanja u frižideru analizirane su senzorne osobine jogurta, kiselost, pH, slobodne masne kiseline (FFA), acetaldehid i diacetil. Takođe, urađena je enumeracija živih *L. delbrueckii ssp. bulgaricus* i *S. thermophilus*. Tokom čitavog perioda skladištenja, proizvodi sa odnosom SMP/WPC 1/2 i 2/1 su dobili najbolje senzorne ocene. Dodavanje WPC u jogurt je signifikantno smanjilo koncentrciju mlečne kiseline što je imalo pozitivan uticaj na stabilnost proizvoda tokom skladištenja. Tokom perioda skladištenja, kiselost jogurta se povećavala vezano za pH nivo. Sadržaj acetaldehida je pokazivao tendenciju signifikantnog povećanja u jogurtu u čijoj preradi je korišćen najveći udeo WPC, suprotno nivou diacetila gde je najniža vrednost utvrđena u jogurtu dobijenom korišćenjem SMP/WPC u odnosu 0/1. Tokom perioda skladištenja, koncentracija oba isparljiva jedinjenja je bila najviša 7.dana i najniža 21. dana. Maksimalna koncentracija FFA je zabeležena u proizvodima sa SMP/WPC u odnosu 1/2. Tokom perioda skladištenja sadržaj FFa je povećan signifikantno nakon 7. dana i više se nije menjao

tokom narednih 14 dana. Povećanje FFA tokom perioda skladištenja neamsnih proizvoda je verovatno izazvano proteolitičkom aktivnošću bakterija jogurta. Ukupan broj bakterija u jogurtu tokom čitavog perioda skaldištenja je iznosio do 10^{10} i nije varirao zavisno od odnosa SMP/WPC. Količina *L. delbrueckii ssp. bulgaricus* je bila za jedan ciklus niža od *S. thermophilus* u svim vrstama jogurta.

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