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CHANGES IN ISOFLAVONES CONCENTRATIONS IN CHEESE DURING PROCESSING AND RIPENING

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Abstract

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The aim of the study was to determine possible changes in isoflavones concentration in cheese made from either control or isoflavone-enriched milk during manufacturing and ripening. The experiment was carried out on four high-yielding lactating Holstein cows that were divided into two groups with similar mean milk yield. The control group of cows was fed a diet based on extruded rapeseed cake (C) while the experimental group of animals was fed a diet based on extruded full-fat soya (S). The experiment was carried out in the form of a cross-over design and was divided into 2 periods of 14 days (a 10-d preliminary period and a 4-d experimental period). Cows were fed individually twice daily *ad libitum* the diet based on maize silage, lucerne hay and supplemental mixture. In each period 20 kg of morning milk was collected from each group for cheese processing. After pasteurisation (65 °C, 30 min.) a total of 5 kg of milk from each sample in each period was weighed out to make cheese with a low-heated curd. Cheeses were salted in 20% solution of NaCl for 3.5 h and allowed to ripen for 90 days at 15 °C. During technological processing samples were taken to determine isoflavones content. Data concerning the nutrients intake, milk yield and concentration of isoflavones were analysed by means of multifactor analysis of variance using the GLM procedure of the Statgraphics 7.0 package. Average daily isoflavones intake in S (1284.7 mg/d) was higher than in C (2.9 mg/d, $P < 0.001$). Milk yield expressed in 4% FCM did not differ significantly between groups ($P > 0.05$). Concentration of daidzein, genistein and glycitein in pasteurised full fat milk was similar in both groups. Milk from S group had higher concentration of equol (26.7 µg/L) in comparison to C group (4.0 µg/L). After processing cheese in C contained 32.1 µg/kg daidzein and 5.6 µg/kg of equol while cheese in S contained 17.5 µg/kg of daidzein and 24.3 µg/kg of equol. During a 90-day ripening percentage decrease in isoflavones concentration was lower in S than in C. Concentration of daidzein was reduced by 47% in C and 37% in S. Concentration of genistein decreased by 51% in C and 31% in S, concentration of glycitein by 46% in C and 29% in S and concentration of equol by 50% in C and 38% in S.

soybean, equol, milk, dairy products

Soybean-derived isoflavones daidzein and genistein have attracted a lot of attention due to their diverse pharmacological and antioxidant properties including anticarcinogenic (e.g. Messina and Barnes, 1991; Hirano *et al.*, 1994), antimutagenic (Hartman and Shankel, 1990), and antioxidant (Jha *et al.*, 1985) activities, as well as antiproliferative effects against tumor cells (Hirano *et al.*, 1989), reducing risk of heart disease (Wong *et al.*, 1998) and menopausal symptoms (Chiechi, 1999) and improving bone health (Anderson and Garner, 1997). However, it has been proposed that clinical effectiveness of soy protein in cardiovascular, bone, and menopausal health is influenced by the ability of human to biotransform daidzein to equol (Setchell *et al.*, 2002). Recent studies found that equol is in vitro more bio-active

than its precursor daidzein, it has a higher oestrogenicity (e. g. Muthyala *et al.,* 2004; Kostelac *et al.,* 2003; Setchell *et al.,* 2002; Morito *et al.,* 2001), is a more potent antioxidant (e. g. Arora *et al.,* 1998; Rimbach *et al.,* 2003; Turner *et al.,* 2004) and possesses antiandrogenic properties (Lund *et al.,* 2004). Furthermore, equol has a higher effective free fraction circulating in human serum (Nagel *et al.,* 1999) and a slower plasma clearance (Setchell *et al.,* 2002) compared to daidzein.

Equol is exclusively formed during digestion by the intestinal microbiota (e. g. Atkinson *et al.,* 2004; Bowey *et al.,* 2003). Nevertheless studies (e.g. Lampe *et al.,* 1998; Rowland *et al.,* 2000) have shown that there are substantial inter-individual variations in the bacterial metabolism of isoflavones in the gut resulting in a low proportion of adult population (30–50%), so called equol producers, that is able to convert daidzein into equol (Atkinson *et al.,* 2005). However, oral administration of equol seems to be an alternative strategy for obtaining the health-promoting benefits of this substance in non-equol producers as documented by Setchell *et al.* (2002) who has reported that an oral dose of 25 mg of equol was rapidly absorbed with maximum plasma concentration observed after 4–6 h. Also Walsh *et al.* (2003) and Walsh and Faila (2009) demonstrated stability of equol during simulated gastric and small intestinal digestion and its rapid bioaccessibility. Recent studies suggest (Mustonen *et al.,* 2009; Steinshamn *et al.,* 2008; Kuhnle *et al.,* 2008) that bovine milk and some dairy products can be considered as a potential dietary source of equol for non-equol producing human. Although concentrations of isoflavones in bovine milk and dairy products have been reported e.g. in latter mentioned studies, little is known about changes in isoflavones content during technological processing of milk and dairy products. Only effect of heat treatment of milk on isoflavones concentration has been reported previously (King *et al.,* 1998; Uzzan and Labuza, 2004).

The aim of the study was to determine possible changes in isoflavones concentration in cheese during manufacturing and ripening.

MATERIAL AND METHODS

Animals and diets

The experiment was carried out on four high-yielding lactating Holstein cows (lactation 2, 22–26. week of lactation) with similar milk production (18.0 ± 1.1 kg/d) that were divided into two groups with similar mean milk yield. The control group of animals was fed a diet based on extruded rapeseed cake (C) while the experimental group of animals was fed a diet based on extruded full-fat soya (S). The experiment was carried out in the form of a cross-over design and was divided into 2 periods of 14 days. Each period consisted of a 10-d preliminary period and a 4-d experimental period. Cows were fed individually twice daily (6.30 and 16.30 h) *ad libitum* the diet based on maize silage, lucerne hay and supplemental mixture (Table I). Prior the experiment there was a 1-week period to adaptation to the type of diet.

I: *Composition of diets (g/kg, dry matter basis)*

Components		C[1]	S[1]
Maize silage	g/kg	508	508
Lucerne hay	g/kg	92	92
Supplemental mixture C	g/kg	400	200
Supplemental mixture S	g/kg		200
Composition of supplemental mixtures			
Barley	g/kg	266.0	266.0
Oat	g/kg	266.0	266.0
Sugarbeet chippings	g/kg	150.0	96.0
Extruded full-fat soya	g/kg		336.0
Extruded rapeseed meal	g/kg	282.0	
Rapeseed oil	g/kg	10.5	
Sodium chloride (NaCl)	g/kg	5.5	4.0
Dicalciumphosphate (DCP)	g/kg	7.5	14.0
Limestone ($CaCO_3$)	g/kg	10.5	11.6
Sodium bicarbonate ($NaHCO_3$)	g/kg	1.0	4.5
Magnesiumphosphate (MgP)	g/kg		0.9
Blend-s minerals	g/kg	0.5	0.5
Blend-s vitamins	g/kg	0.5	0.5
Total	g/kg	1000.0	1000.0

[1] treatments were as follows: C – control group fed extruded rapeseed cake, S – experimental group fed extruded full-fat soya

Cheese manufacturing and ripening

Cows were milked twice a day (7.00 and 17.00 h). Milk yield was recorded at each milking. In each period 20 kg of morning milk was collected from each group for technological processing. Immediately after the collection, milk was cooled to 6 °C, transported to experimental pilot plant and stored overnight at 6–8 °C prior processing. Milk was centrifuged on EleCrem 1 (Elecrem, France) to remove solid impurities and to separate cream from skim milk. After centrifugation skim milk and cream was recombined to obtain again full-fat milk that was subsequently pasteurised at 65 °C for 30 min. A total of 5 kg of pasteurised milk from each group in each period was weighed out to make cheese with a low-heated curd. To curdle the milk, 1.5 ml of saturated solution of calcium chloride and 1% of cream culture FD was added and milk was then curdled at 32–33 °C with liquid rennet (1:15000, MILCOM a.s, Czech Republic). After 80–90 min, 1/3 of the whey was drained and the remaining material was supplied with 1/3 of washing water. The curdling milk was then reheated and drained at 37–38 °C for 40–50 min. After the draining, the curds were poured into the moulds and the cheeses were pressed for 1h under increasing pressure into its final shape.

Cheeses were salted for 3.5 h (20% solution of NaCl, pH 5.25) and then they were allowed to ripen for 90 days at 15 °C.

Samples of cheese after manufacturing and ripening were taken to determine possible isoflavones losses.

Analytical procedures

Dry matter of feeding components was determined by drying at 55 °C for 24 h, followed by milling through a 1 mm screen and drying for another 4h at 103 °C. Dry matter content of milk and dairy products was determined according to Czech National Standards (ČSN 570104-3, 1998, ČSN 570107, 1987, ČSN 570107-3, 1987) by drying sample with laboratory silica sand at 102 °C until constant weight.

Determination of isoflavones in feed and milk has been described previously (Třináctý *et al.,* 2009). Briefly, levels of targeted compounds were determined after their releasing from bonded forms. High purity standards of daidzein (≥ 98%), glycitein (≥ 97%) and genistein (≥ 95%) were purchased from Sigma-Aldrich (Germany), equol (≥ 99%) and internal standard 4-hydroxybenzofenon (4-HBPE) (≥ 99%) were purchased from Fluka (Germany).

Feed samples: Homogenised samples were hydrolysed with 6 mol/l hydrochloric acid and ethanol under the reverse condenser at the boiling point of ethanol. After hydrolysis the extract was cleaned up by SPE procedure on Oasis HLB, Waters (UK) cartridges. The analytical column used for experiments was LichroCART LiChrospher 100 RP8 (250 × 4 mm, 5 µm) with analytical precolumn LichroCART LiChrospher 100 RP8 (4 × 4 mm, 5 µm) (Merck, Germany). Mobile phase methanol and 0.1% acetic acid water solution (v/v) with gradient elution at a flow-rate 0.7 ml/min was used. The absorption maxima using for detection of total daidzein, glycitein and genistein was 260 nm. The HPLC analysis was carried out on an HP 1200 liquid chromatograph coupled with a diode array detector (DAD) (Hewlett Packard, USA). The limit of detection (LOD) for total isoflavones obtained under the described method was 0.5 mg/kg for daidzein, 0.5 mg/kg for glycitein, and 0.4 mg/kg for genistein. The repeatability expressed as a relative standard deviation (RSD%, n = 6) was 6%, 3% and 3%, respectively.

Milk and milk products samples: Target analytes were hydrolysed from possible conjugates by enzymatic hydrolysis with Helix pomatia enzyme β-glucuronidase/sulfatase in sodium acetate buffer (pH 5) at 37 °C. After hydrolysis the analytes were extracted by ethylacetate. The analytical column used for experiments was Discovery C18, (150 × 3 mm, 5 µm) with analytical precolumns Discovery C18 Guard column (20 × 4 mm, 5 µm) (Supelco, Germany). Mobile phase methanol and 0.1% acetic acid water solution (v/v) with gradient elution at a flow-rate 0.7 ml/min was used. For MS/MS detection APCI at positive ionization mode was used with monitoring of transitions (m/z) 255.3 → 199.3 for daidzein, 285.3 → 270.2 for glycitein, 271.4 → 215.3 for genistein, 243.1 → 123.1 for equol, and 199.2 → 121.2 for 4-HBPE. Analytes were quantified by the method of internal standard. Liquid chromatograph HP 1100, (Hewlett Packard, USA) coupled with mass spectrometry detector - ion trap, Finnigan LCQ Deca, (Finnigan, USA) operated in selected reaction monitoring (SRM) mode was used for analysis. The limit of detection (LOD) obtained under the described method was 2 ng/ml for daidzein and glycitein, 5 ng/ml for genistein, and 0.7 ng/ml for equol for both milk and milk products samples. The repeatability expressed as relative standard deviation (RSD%, n = 6) was 5% for daidzein, 7% for genistein and equol, and 4% for glycitein in milk and milk products samples.

Statistical analysis

Mean daily intake of isoflavones was calculated from the analytically determined isoflavones concentrations of individual dietary components (silage, hay, supplemental mixture) and their respective intakes. When the concentration of isoflavones was so low that it could not be detected, the concentration was estimated to be half the detection limit before statistical analysis.

Data concerning the nutrients intake, milk yield and concentration of isoflavones obtained in the experiment were analysed using the GLM procedure of the Statgraphics 7.0 package (Manugistics Inc. and Statistical Graphics Corporation, Rockville, Maryland, USA) according to the following model:

$$Y_{ijkl} = \mu + T_i + C_j + P_k + D_l + \varepsilon_{ijkl},$$

where μ = general mean, T_i = treatment effect (i = 2), C_j = cow effect (j = 4), P_k = period effect (k = 2), D_l = day of sampling effect (l = 4) and ε_{ijkl} = error term.

RESULTS AND DISCUSSION

Nutrient intake, milk yield and concentration of isoflavones in milk

The average daily intake of dry matter and isoflavones is presented in Table II. Dry matter intake in S was higher than in C ($P < 0.05$). Extruded full-fat soya used in the present experiment contained 377.9 mg/kg of daidzein, 558.2 mg/kg of genistein and 129.6 mg/kg of glycitein. These values are considerably higher than reported for the same feedstuff in Třináctý *et al.* (2009). However, concentration of total isoflavones in soybeans can vary from 1.2 up to 4.2 mg/kg (Kurzer and Xu, 1997; Nakamura *et al.*, 2000) in dependence on the environmental factors, growth, harvesting and processing (Wang and Murphy, 1994). Average daily isoflavones intake in S was 1284.7 mg/d while calculated average daily intake of isoflavones in C was considerably lower, being 2.9 mg/d ($P < 0.001$). Compared to our previous study (Třináctý *et al.*, 2009) average daily isoflavones intake in S was lower although concentration of individual isoflavones was higher. Nevertheless, proportion of extruded full-fat soya in S in the present experiment was lower than in above mentioned study.

Milk yield and isoflavones concentration in milk is given in Table III. Although milk yield in S was higher than in C ($P < 0.05$), milk yield expressed in 4% FCM did not differ significantly between groups ($P > 0.05$). Similarly, in the study of Komprda *et al.* (2000) or Kudrna and Marounek (2006) no difference in milk yield between cows receiving rapeseed cake and extruded soybean meal or extruded soybeans, respectively, was determined.

Daidzein, genistein and glycitein were detected in milk of both groups, C and S. While concentrations of daidzein and genistein were not influenced by the treatment ($P > 0.05$), concentrations of equol and glycitein were higher in S than in C ($P < 0.001$). Findings concerning the differences in milk concentrations of genistein and equol between experimental groups are in accordance with Třináctý *et al.* (2009). In comparison to latter study, the concentration of equol in milk in S was considerably lower. This discrepancy was probably caused by a lower rumen degradability of extruded full-fat soya currently used in the experiment in comparison with other extruded soybean-derived feeding components (data not shown). Although the daily isoflavones intake in C was very low (2.9 mg/d) relatively high concentration of isoflavones was detected in milk of control animals (C). Similar findings were also reported by Třináctý *et al.* (2009), Mustonen *et al.* (2009), Andersen *et al.* (2009) or Steinshamn *et al.* (2008) who suggested that the transfer rate of phytoestrogens from feed to milk is rate-limiting, diminishing the amount of phytoestrogens transferred to the milk with increasing content of phytoestrogens in the feed.

Isoflavones concentration in milk and cheese during processing

Isoflavones content in pasteurised full fat milk prior cheese manufacturing is given in Table IV. Concentration of daidzein, genistein and glycitein was similar in both groups. Milk from S group had

II: *Average daily intake of dry matter and isoflavones*

Intake of	Units	C[1]	S[1]	SEM	P
Dry matter	kg/d	16.8	17.8	0.28	0.01
Daidzein	mg/d	1.1	438.7	7.62	< 0.001
Genistein	mg/d	0.8	681.8	11.44	< 0.001
Glycitein	mg/d	1.1	164.2	5.18	< 0.001
Total isoflavones	mg/d	2.9	1284.7	24.24	< 0.001

[1] treatments were as follows: C – control group fed extruded rapeseed cake, S – experimental group fed extruded full-fat soya

III: *Milk yield and concentration of isoflavones*

Item	Units	C[1]	S[1]	SEM	P
Milk yield	kg/d	17.6	19.5	0.50	0.01
4% FCM yield	kg/d	19.0	20.9	0.68	0.06
Daidzein	μg/L	36.5	40.3	1.88	0.17
Genistein	μg/L	170.6	175.8	8.36	0.67
Glycitein	μg/L	23.4	27.9	0.77	< 0.001
Equol	μg/L	3.6	15.6	1.08	< 0.001
Total	μg/L	234.1	259.6	9.88	0.08

[1] treatments were as follows: C – control group fed extruded rapeseed cake, S – experimental group fed extruded full-fat soya

IV: *Concentration of isoflavones in control (C) and isoflavone-enriched (S) pasteurised milk prior cheese processing (μg/L, wet weight basis)*

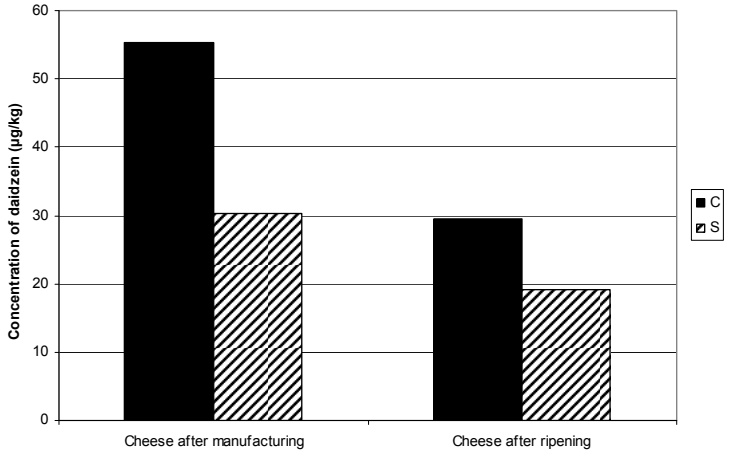
Isoflavones	Units	C[1]	S[1]
Daidzein	μg/L	50.8	47.3
Genistein	μg/L	169.4	156.1
Glycitein	μg/L	15.4	16.4
Equol	μg/L	4.0	26.7
Total	μg/L	239.6	246.5

[1] treatments were as follows: C – control group fed extruded rapeseed cake, S – experimental group fed extruded full-fat soya

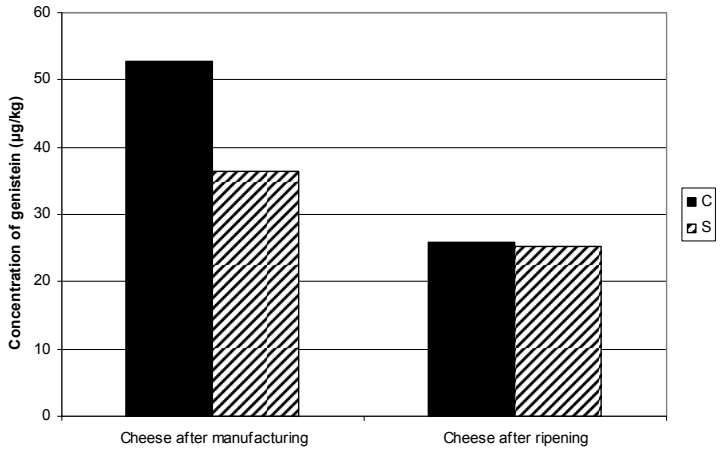
higher concentration of equol (26.7 μg/L) in comparison to C group (4.0 μg/L). Resulting concentration of total isoflavones was 239.6 μg/L in C and 246.5 μg/L in S. Due to the fact that the concentration of isoflavones in milk varies in dependence on a variety of factors, such as the composition and intake of diet and the season, the concentration of equol originating from clover-based diets can range from 14 to 643 μg/L (King *et al.,* 1998; Antignac *et al.,* 2004; Purup *et al.,* 2005; Hoikkala *et al.,* 2007; Steinshamn *et al.,* 2008; Mustonen *et al.,* 2009) while concentration of equol originated from dietary soybean was 55 μg/L (Třináctý *et al.,* 2009). Furthermore, it has been documented that isoflavones are not destroyed by heat treatment but rather are subject to intra-conversions between the different forms (e. g. Grun *et al.,* 2001; Jackson *et al.,* 2002). And no effect of pasteurisation or thermal treatment up to 140 °C for 20 sec has been observed (King *et al.,* 1998; Uzzan and Labuza, 2004 or Uzzan *et al.,* 2007). Thus, concentration of isoflavones in pasteurised milk found in our study was within the range of values reported in the literature and no effect of pasteurisation on isoflavones content was observed (Křížová *et al.,* unpublished data).

Concentration of isoflavones in cheese during manufacturing and ripening is presented in Table V. Milk used for cheese manufacturing contained 131.8 and 130.6 g/kg of dry matter, 45.0 and 44.7 g/kg of fat and 35.6 and 33.3 g/kg of protein in C and S group, respectively. From a total of 5 kg of pasteurised milk used for cheese processing it was produced 0.68 and 0.67 kg of cheese in C and S group, respectively. Total amount of whey drained during curdling was 5.58 and 5.62 kg in C and S, respectively. Cheeses after processing contained 576.0 and 581.9 g/kg of dry matter and 14.4 and 14.3 g/kg of salt in C and S group, respectively. Concentration of individual isoflavones in whey as given in Table V is relatively high confirming the findings reported in literature that isoflavones are during processing of soybeans at least partially transferred to whey or molasses that can be remarkably rich in isoflavones (Jackson *et al.,* 2002; Waggle and Bryan, 2003).

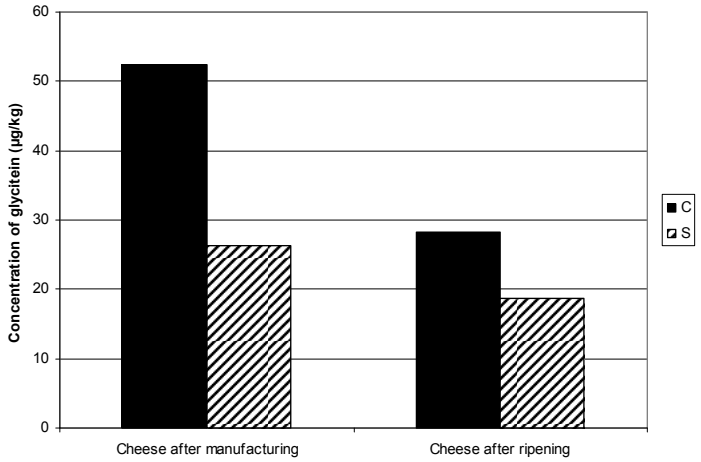
There was a decline in pH during curdling from initial 6.44 and 6.42 determined in milk prior renneting to 6.08 and 6.10 in cheese after pressing in moulds in C and S, respectively. Cheese pH determined at the beginning (d 1) and at the end of ripening (d 90) were 5.17 and 5.40 in C and 5.2 and 5.35 in S, respectively. The changes in pH were consistent in both groups. To our knowledge, no effect of cheese processing and ripening on changes in isoflavones content has been described previously and there are no comparable data to isoflavones content in immature cheese immediately after processing. Also data concerning the content of isoflavones in dairy products are scarce. However, Kuhnle *et al.* (2008) anal-



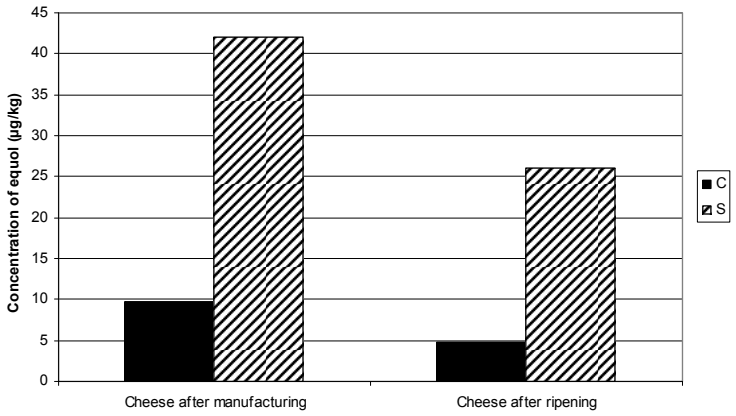
1: *Changes in daidzein concentration (μg/kg, dry weight basis) in cheese made from control (C) or isoflavone-enriched milk (S) during ripening*



2: *Changes in genistein concentration (µg/kg, dry weight basis) in cheese made from control (C) or isoflavone-enriched milk (S) during ripening*



3: *Changes in glycitein concentration (µg/kg, dry weight basis) in cheese made from control (C) or isoflavone-enriched milk (S) during ripening*



4: *Changes in equol concentration (µg/kg, dry weight basis) in cheese made from control (C) or isoflavone-enriched milk (S) during ripening*

V: *Concentration of isoflavones (on wet weight basis) in milk and cheese during technological processing*

		C[1]				S[1]			
		Dein[2]	**Gein[2]**	**Glein[2]**	**Equol**	**Dein[2]**	**Gein[2]**	**Glein[2]**	**Equol**
Pasteurised milk	µg/L	50.8	169.4	15.4	4.0	47.3	156.1	16.4	26.7
Whey	µg/L	43.3	156.4	12.1	4.3	42.3	151.2	13.2	11.3
Cheese									
- after processing	µg/kg	32.1	30.5	30.5	5.6	17.5	21.1	15.1	24.3
- after ripening	µg/kg	18.0	15.8	17.3	3.0	11.7	15.5	11.4	16.0

[1] treatments were as follows: C – control group fed extruded rapeseed cake, S – experimental group fed extruded full-fat soya
[2] Dein = daidzein, Gein = genistein, Glein = glycitein

ysed total of 115 samples of food of animal origin and their corresponding vegetarian substitutes for phytoestrogens content including total isoflavones and equol. They reported low content of isoflavones and equol in all samples of various commercially available milk and dairy products except butter where equol was not detected. The levels of total isoflavones determined in their study in 27 various types of cheese purchased from different food outlets, i. e. mature cheese, ranged from 1 to 17 µg/100g of wet weight and concentration of equol varied from 1 to 14 µg/100g of wet weight. These concentrations are similar to those found in our study.

Ripening involves three primary biochemical events: glycolysis of residual lactose and its constituent monosaccharides (glucose and galactose), lipolysis, and proteolysis (Hayaloglu *et al.,* 2002). The changes in individual isoflavones (on a dry weight basis) are shown in Figures 1–4. During a 90-day ripening percentage decrease in isoflavones concentration was lower in group S than in group C. Concentration of daidzein was reduced by 47% in C and 37% in S. Concentration of genistein decreased by 51% in C and 31% in S, concentration of glycitein by 46% in C and 29% in S and concentration of equol by 50% in C and 38% in S. Although losses of isoflavones during ripening were high, concentration of individual isoflavones namely equol (16 µg/kg) in cheese produced form isoflavone-enriched milk are still high enough to participate on oral administration of equol to human.

CONCLUSION

Bovine milk can be considered as a potential source of equol in human nutrition (Mustonen *et al.,* 2009). Results of the present study show that besides milk, cheese can be also included among possible sources of equol in human nutrition although losses of isoflavones during ripening are high. Relatively high concentrations of isoflavones were also detected in whey. Further study is needed to determine kinetics of isoflavone degradation during cheese processing and ripening.

SUMMARY

The aim of the study was to determine possible changes in isoflavones concentration in cheese made from either control or isoflavone-enriched milk during manufacturing and ripening. The experiment was carried out on four high-yielding lactating Holstein cows with average milk production of 18.0 ± 1.1 kg/d that were divided into two groups with similar mean milk yield. The control group of animals was fed a diet based on extruded rapeseed cake (C) while the experimental group of animals was fed a diet based on extruded full-fat soya (S). The experiment was carried out in the form of a cross-over design and was divided into 2 periods of 14 days (a 10-d preliminary period and a 4-d experimental period). Cows were fed individually twice daily (6.30 and 16.30 h) *ad libitum* the diet based on maize silage, lucerne hay and supplemental mixture.

Cows were milked twice a day (7.00 and 17.00 h). Milk yield was recorded at each milking. In each period 20 kg of morning milk was collected from each group for technological processing. Immediately after the collection, milk was cooled to 6 °C, transported to experimental pilot plant and stored overnight at 6–8°C prior processing. Milk was centrifuged to remove solid impurities and to separate cream from skim milk and then recombined to obtain again full-fat milk that was subsequently pasteurised at 65 °C for 30 min. A total of 5 kg of pasteurised milk from each group in each period was weighed out to make cheese with a low-heated curd. To curdle the milk, 1.5 ml of saturated solution of calcium chloride and 1% of cream culture FD was added and milk was then curdled at 32–33 °C with liquid rennet (1:15000, MILCOM a.s, Czech Republic). After the draining, the curds were poured into the moulds and the cheeses were pressed for 1h under increasing pressure into its final shape and subsequently salted for 3.5 h in 20% solution of NaCl and then they were allowed to ripen for 90 days at 15 °C. Samples of cheese after manufacturing and ripening were taken to determine possible isoflavones losses.

Dry matter of feeding components was determined by drying at 55 °C for 24 h, followed by milling through a 1 mm screen and drying for another 4h at 103 °C. Dry matter content of milk and dairy products was determined according to Czech National Standards (ČSN 570104-3, 1998, ČSN 570107, 1987, ČSN 570107-3, 1987) by drying sample with laboratory silica sand at 102 °C until constant weight. Concentration of isoflavones in feed and milk were determined using HPLC. Data concerning the nutrients intake, milk yield and concentration of isoflavones obtained in the experiment were analysed using the GLM procedure of the Statgraphics 7.0 package according to the following model: $Y_{ijkl} = \mu + T_i + C_j + P_k + D_l + \varepsilon_{ijkl,}$ where μ = general mean, T_i = treatment effect (i = 2), C_j = cow effect (j = 4), P_k = period effect (k = 2), D_l = day of sampling effect (l = 4) and ε_{ijkl} = error term.

Dry matter intake in S (17.8 kg/d) was higher than in C (16.8 kg/d, $P < 0.05$). Extruded full-fat soya used in the present experiment contained 377.9 mg/kg of daidzein, 558.2 mg/kg of genistein and 129.6 mg/kg of glycitein resulting in average daily isoflavones intake of 1284.7 mg/d in S and 2.9 mg/d in C ($P < 0.001$). Milk yield in S (19.5 kg/d) was higher than in C (17.6 kg/d, $P < 0.05$), milk yield expressed in 4% FCM did not differ significantly between groups ($P > 0.05$). Isoflavones were detected in milk of both groups, C and S. While concentrations of daidzein and genistein were not influenced by the treatment ($P > 0.05$), concentrations of equol and glycitein were higher in S than in C ($P < 0.001$).

Concentration of daidzein, genistein and glycitein in pasteurised full fat milk prior cheese manufacturing was similar in both groups. Milk from S group had higher concentration of equol (26.7 µg/L) in comparison to C group (4.0 µg/L). From a total of 5 kg of pasteurised milk used for cheese processing it was produced 0.68 and 0.67 kg of cheese in C and S group, respectively. There was a decline in pH during curdling from initial 6.44 and 6.42 determined in milk prior renneting to 6.08 and 6.10 in cheese after pressing in moulds in C and S, respectively. Cheese pH determined at the beginning (d 1) and at the end of ripening (d 90) were 5.17 and 5.40 in C and 5.2 and 5.35 in S, respectively. During a 90-day ripening percentage decrease in isoflavones concentration was lower in group S than in group C. Concentration of daidzein was reduced by 47% in C and 37% in S. Concentration of genistein decreased by 51% in C and 31% in S, concentration of glycitein by 46% in C and 29% in S and concentration of equol by 50% in C and 38% in S.

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