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The Effect of Technological Processing on the Content of Isoflavones in Bovine Milk and Dairy Products

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1. Introduction

Isoflavones that belong to a class of phytoestrogens have a relatively limited distribution in nature and from the aspects of human nutrition they are found in physiologically relevant amounts only in soybeans and soybean-derived foods (Franke et al., 1998). Isoflavones are phytoalexins that are formed by the host plant in response to physiological or biological stimuli and possess properties (i.e. antifungal, antimicrobial, and antioxidant) that enhance the survival of the soybean (Dakora & Phillips, 1996). For this reason, soybean isoflavone concentrations increase greatly in times of stress (e.g. limited moisture) and are influenced by the environmental conditions under which the soybean is grown (Eldridge & Kwolek, 1983, Wang & Murphy, 1994).

The major dietary phytoestrogens present in soya (Glycine max (L.) Merr.) are daidzein, genistein and glycitein. After ingestion these substances are subjected to biotransformation by gut microbiota to diverse metabolites that can be detected in human urine (Joannou et al., 1995, Lampe et al., 1998, Coldham et al., 1999, Rowland et al., 1999, Hur et al., 2000, Heinonen et al., 2003, Zheng et al., 2003, Simons et al., 2005). While glycitein has been found to be metabolically stable (Setchell et al., 2002), genistein is converted to 6'-hydroxy-O-desmethylangolensin, 2,4,6-trihydroxybenzoic acid and p-ethyl phenol (Heinonen et al., 1999, Steer et al., 2003, Wähälä et al., 1998), daidzein is metabolised by intestinal microflora to equol and O-desmethylangolensin (Heinonen et al., 1999, Adlercreutz et al., 1986). Especially equol has gained a lot of attention since Setchell et al. (2002) proposed a hypothesis that the ability to biotransform daidzein to equol may be the key factor to clinical effectiveness of soy protein in cardiovascular, bone, and menopausal health in so-called equol producers. Indeed, recent studies found that equol is in vitro more bio-active than its precursor daidzein: it has a higher oestrogenicity (Kostelac et al., 2003, Setchell et al., 2002, Morito et al., 2001, Muthyala et al., 2004, Sathyamoorthy & Wang, 1997, Schmitt et al., 2001), is a more potent anti-oxidant (Arora et al., 1998, Mitchell et al., 1998, Rimbach et al., 2003, Turner et al., 2004) and possesses anti-androgenic 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.

As mentioned above, equol is not of plant origin and is exclusively formed by the intestinal microbiota (Atkinson et al., 2004, Blair et al., 2003, Bowey et al., 2003, Hoey et al., 2004). 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 %) that is able to convert daidzein into equol (Atkinson et al., 2005). However, an alternative strategy for obtaining the health-promoting benefits of equol is oral administration. Setchell et al. (2002) has reported that an oral dose of 25 mg of equol was rapidly absorbed with maximum plasma concentration observed after 4 - 6 h. Walsh et al. (2003) and Walsh & Faila (2009) have found that equol is stable during simulated gastric and small intestinal digestion and is readily bioaccessible. This further supports the beneficial potential of orally administered equol to individuals classified as equol "non-producers". From the range of foods commonly consumed by humans, cow's milk is presumably the only nutritive that can contain appreciable amounts of equol itself (Mustonen et al., 2009, Steinshamn et al., 2008) thus bovine milk can be considered as a potential source of equol for non-equol producers. Furthermore, in a recent study, Kuhnle et al. (2008) reported low content of equol in various commercially available dairy products except butter. Thus, not only milk but also dairy products could be a source of equol in a human diet.

Although changes in isoflavones content during technological processing of soybean based products are extensively studied (e.g Prabhakaran & Perera, 2006, Uzzan & Labuza, 2004, Jackson et al., 2002), studies focused on changes in isoflavones, especially equol, during technological processing of bovine milk are scarce. To our knowledge, only effect of heat treatment on milk isoflavones has been reported previously. King et al. (1998) found no effect of pasteurization on concentration of equol and genistein in milk. Similarly, Uzzan & Labuza (2004) determined no effect of heat treatment at 72, 121, 140 and 140 °C for 120, 24, 2 and 20 sec, respectively on content of daidzein, genistein and glycitein in an isoflavone-enriched cow milk beverage.

The aim of the study was to determine possible changes in isoflavones content in milk and dairy products during technological processing.

2. Material and methods

2.1 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 $\pm$ 1.1 kg/d) that were divided into 2 groups with similar 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 1). Prior the experiment there was at least a 1-week period to adaptation to the type of diet.

Cows were milked twice a day (7.00 and 17.00 h). Milk yield was recorded at each milking. During the experimental period, samples of milk were taken at each milking. Samples for determination of basic constituents were conserved by 2-bromo-2-nitropropane-1.3-diol (Bronopol; D&F Control Systems, Inc. USA), cooled to the 6 °C and analysed by infrared analyser (Bentley Instruments 2000, Bentley Instruments Inc., USA). Milk samples for determination of isoflavones concentration were kept frozen at -20 °C.

Components		**C**	**S**
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 cake	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

Table 1. Composition of diet (g/kg, dry matter basis)

In each period a 20 kg of morning milk was collected from each group for technological 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. Full-fat milk was pasteurised at 65 °C for 30 min and used for manufacturing of plain yoghurt without any other ingredients. Pasteurised milk was warmed up to temperature of 37 °C, inoculated with a 1% of yoghurt cultures KAN IV (*Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus*, MILCOM a.s., Czech Republic) and packed into sterile bottles (180 ml volume) with twist-off lid and maintained in the thermoregulator at 37 °C for 16 – 18 h until coagulation. Then the coagulated products were cooled and stored in the thermoregulator at 6.5 °C for 1 month. During the above described technological processing samples were taken to determine isoflavones content.

2.2 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 4 h at 103 °C.

Dry matter content of milk and dairy products was determined according to czech national standards 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.

2.3 Calculations

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.

Apparent recovery of phytoestrogens from feed to milk was calculated according to the following formulas (based on Steinshamn et al., 2008):

Recovery of daidzein [μg/mg] = (sum of daidzein and equol secreted in milk)/sum of daidzein intake

Recovery of genistein [μg/mg] = sum of genistein secreted in milk/sum of genistein intake

Recovery of glycitein [μg/mg] = sum of glycitein secreted in milk/sum of glycitein intake

2.4 Statistical analysis

Data concerning the nutrients intake, milk yield, concentration, output and recovery 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.

3. Results and discussion

3.1 Nutrient intake, milk yield, concentration, output and recovery of isoflavones in milk

The average daily intake of dry matter and isoflavones is presented in Table 2.

Intake of	Units	**C**	**S**	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
Isoflavones total	mg/d	**2.9**	**1284.7**	24.24	< 0.001

Table 2. Average daily intake of dry matter and isoflavones

Various soybean products are commonly used as a dietary component of diets for high yielding dairy cows as an excelent source of high-quality protein and energy (Chouinard et al. 1997), however soybeans are also the richest source of isoflavones (Hollman, 2001) containing up to 1.2 – 4.2 mg/g dry weight of isoflavones (Kurzer & Xu, 1997). Intake of dry matter in S was higher than in C ($P<0.05$). The concentration of isoflavones in extruded rapeseed cake and individual dietary components was under the sensitivity level of used analytical method (see Material and methods), however very low intake of isoflavones was calculated. Mean concentrations of isoflavones in extruded full-fat soya used in the present experiment were as follows: daidzein 377.9 mg/kg, genistein 558.2 mg/kg and glycitein 129.6 mg/kg, resulting in average total isoflavones intake of 1285 mg/d in S. Although concentration of individual isoflavones was considerably higher than that used in our previous study (Třináctý et al., 2009), average daily isoflavones intake in S was lower than in above mentioned work. This discrepancy can be explained by lower proportion of extruded full-fat soya in experimental diet.

Milk yield and isoflavones concentration, output and apparent recovery in milk is given in Table 3. Although milk yield in S was higher than in C ($P<0.05$), milk yield expressed in 4% FCM (fat corrected milk) did not differ significantly between groups ($P>0.05$). This is in accordance with e. g. Komprda et al. (2000) or Kudrna & Marounek (2006) who did not find a difference in milk yield between cows receiving rapeseed cake and extruded soybean meal or extruded soybeans, respectively. All studied isoflavones were detected in milk of both groups, C and S. While concentrations of daidzein and genistein were similar in both groups and were not affected by the treatment ($P>0.05$), concentrations of equol and glycitein were higher ($P<0.001$) in S than in C, resulting in higher daily output of daidzein, glycitein, equol and total isoflavones in S compared to C ($P<0.01$). Findings concerning the differences in milk concentrations of genistein and equol between experimental groups are in accordance with Třináctý et al. (2009). However, based on the latter study, the concentration of equol in

milk in S was considerably lower than expected. A probable explanation to this discrepancy could be 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). Similarly to our previous study (Třináctý et al., 2009), isoflavones were detected in milk of control animals (C) although the daily isoflavones intake in this group was very low (3 mg/d). Thus, apparent recovery from feed to milk of daidzein was 2.5 μg/mg, of genistein was 5.0 μg/mg and of glycitein was 3.9 μg/mg in group S while apparent recoveries of individual isoflavones in C were enormously high. Similar findings were also reported by e. g. Mustonen et al. (2009), Andersen et al. (2009) or Steinshamn et al. (2008) who studied the recovery of red clover-derived phytoestrogens suggesting that the transfer rate of isoflavonoids from feed to milk is higher at low intake than at higher intake.

Item	Units	C	S	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
Output					
Daidzein	μg/d	**643.9**	**776.9**	33.72	0.01
Genistein	μg/d	**3008.6**	**3396.2**	144.37	0.07
Glycitein	μg/d	**417.1**	**543.2**	19.58	< 0.001
Equol	μg/d	**65.2**	**305.5**	21.92	< 0.001
Total	μg/d	**4134.9**	**5021.8**	173.41	0.001
Recovery of isoflavones					
Recovery of daidzein	μg/mg	**670.7**	**2.5**	26.06	< 0.001
Recovery of genistein	μg/mg	**3568.5**	**5.0**	160.79	< 0.001
Recovery of glycitein	μg/mg	**393.5**	**3.9**	13.00	< 0.001

Table 3. Milk yield, concentration and output of isoflavones, recovery of isoflavones

3.2 Concentration of isoflavones in bovine milk and dairy products

Although the concentration of isoflavones in many vegetal species and foodstuffs of plant origin are extensively documented (e. g. Umphress et al., 2005, Nurmi et al., 2002, Liggins et al., 2000 a, b), there are only a few studies focused on the transfer of isoflavones from feed to bovine milk. The isoflavones content in milk varies depending on a variety of factors, such as the composition of the diet and the season. The concentration of equol in milk of cows fed red clover based diets can range from 14 to 643 μg/L in dependence on isoflavones intake (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).

Data concerning the content of isoflavones in dairy products are scarce. However, Kuhnle et al. (2008) analysed 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 whole and skimmed milk, cream and plain yoghurt were considerably lower than that found in our study in C, but the content of equol in mentined products was higher than in C but lower than in S.
For technological processing samples of morning milk from each group in each period were taken, immediatelly after the collection, the milk was cooled to 6 °C, transported to experimental pilot plant, stored overnight at 6 – 8 °C and then processed. Isoflavones content in raw milk prior technological processing is given in Table 4.

Isoflavones	Units	C	S
Daidzein	μg/L	47.6	45.4
Genistein	μg/L	143.8	147.1
Glycitein	μg/L	13.2	16.1
Equol	μg/L	4.1	25.4
Total	μg/L	208.7	234.0

Table 4. Isoflavones content in raw milk prior technological processing (μg/L of wet weight)

Concentration of daidzein, genistein and glycitein was similar in both groups. Milk from S group had higher concentration of equol (25.4 μg/L) in comparison to C group (4.1 μg/L). Resulting concentration of total isoflavones was 208.7 μg/L in C and 234.0 μg/L in S.

3.3 Effect of technological processing

The effect of skimming on the concentration of isoflavones is given in Table 5.

		C		S	
		Skim milk	Cream	Skim milk	Cream
Concentration in wet weight					
Daidzein	μg/L	43.5	49.4	50.2	47.7
Genistein	μg/L	157.4	156.5	148.1	150.1
Glycitein	μg/L	14.1	16.9	15.4	22.8
Equol	μg/L	4.0	3.3	27.4	18.1
Total	μg/L	219.0	226.2	241.0	238.6
Concentration in dry weight					
Daidzein	μg/L	444.0	109.0	525.0	117.0
Genistein	μg/L	1605.1	345.3	1551.2	368.7
Glycitein	μg/L	144.0	37.1	161.2	56.5
Equol	μg/L	40.7	7.4	286.2	44.4
Total	μg/L	2233.9	498.7	2523.6	586.6

Table 5. Effect of skimming on concentration of isoflavones

In general, it is accepted 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, Uzzan et al., 2007). Losses of isoflavones determined during cooking were usually assumed to be a result of leaching into the discarded cooking water (Setchell, 1998, Frank et al., 1999, Grun et al., 2001, Hendrich & Murphy 2001, Jackson et al., 2002).
In our study pasteurisation at 65 °C for 30 min had no effect on concentrations of individual isoflavones neither in the C nor in S group (Table 6).

		C		S	
		Prior pasteurisation	After pasteurisation	Prior pasteurisation	After pasteurisation
Concentration in wet weight					
Daidzein	µg/L	52.9	50.8	48.2	47.3
Genistein	µg/L	170.4	169.4	154.9	156.1
Glycitein	µg/L	15.7	15.4	18.4	16.4
Equol	µg/L	4.4	4.0	27.9	26.7
Total	µg/L	243.4	239.6	249.3	246.5
Concentration in dry weight					
Daidzein	µg/L	369.0	385.2	366.0	362.2
Genistein	µg/L	1275.9	1285.3	1176.0	1194.7
Glycitein	µg/L	117.3	117.0	139.4	125.7
Equol	µg/L	32.7	30.3	211.8	204.7
Total	µg/L	1821.9	1817.9	1893.1	1887.3

Table 6. Effect of pasteurisation

Similar findings were reported by King et al. (1998) for equol and genistein in milk from red-clover based pasture although they did not report details about temperature and time. Also Uzzan & Labuza (2004) and Uzzan et al. (2007) determined no effect of thermal treatment at 72, 121, 140 and 140 °C for 120, 24, 2 and 20 sec, respectively on content of daidzein, genistein and glycitein in an isoflavone-enriched cow milk beverage.

The concentrations of isoflavones during the yoghurt manufacturing and storage are given in Table 7. There was a decline in pH during fermentation from initial 6.55 and 6.53 to 4.18 and 4.20 in C and S, respectively. The decrease in pH was consistent in both groups. To our knowledge, there is no study focused on the changes in isoflavone profile during fermentation and storage of isoflavone-enriched dairy products. However, the effect of fermentation of soybean products with various strain of bacteria and the effect of subsequent storage of fermented products has been studied in several recent studies (e. g. Tsangalis et al., 2002, Uzzan et al., 2007, Chen et al., 2010) mainly with a view to β-glucosidase activity of used bacterial strains and with a view to conversion of isoflavone glucosides to aglycones that are absorbed by humans faster and in greater amounts than the isoflavone glucoside (Izumi et al., 2000).

In the present study, concentration of total isoflavones in plain yoghurt after fermentation at 37 °C for 16 – 18 h was slightly decreased in C from 239.6 to 239.2 µg/L, while the total isoflavone concentration in S was reduced from 246.5 to 237.2 µg/L. Our findings are in agreement with e. g. Chen et al. (2010), Tsangalis et al. (2002) or King & Bignell (2000) who suggested that losses in total isoflavone concentration were caused by hydrolytic cleavage of the glucose moiety from the glucosides, which contributes to the mass of isoflavones when found as glucoside forms. Similarly, Tsangalis et al. (2002) reported that significant losses in total isoflavone concentration during fermentation of soymilks only occurred, when there were significant decreases in the concentration of isoflavone glucosides caused by enzymic hydrolysis.

During the fermentation (37 °C, 16 - 18 h), concentration of equol changed from 4.0 to 6.0 µg/L in C and from 26.7 to 26.8 µg/L in S. There are no comparable data to compare changes in equol concentration during fermentation in dairy products. However, recent findings suggest that equol can occur in fermented products as a result of fermentation by

		C	S
Concentration on a wet weight basis Daidzein			
Full-fat milk after pasteurisation	µg/L	50.8	47.3
Yoghurt after manufacturing	µg/L	50.8	49.5
Yoghurt after storage	µg/L	51.3	48.2
Genistein			
Full-fat milk after pasteurisation	µg/L	169.4	156.1
Yoghurt after manufacturing	µg/L	167.6	145.1
Yoghurt after storage	µg/L	165.8	147.6
Glycitein			
Full-fat milk after pasteurisation	µg/L	15.4	16.4
Yoghurt after manufacturing	µg/L	14.8	15.8
Yoghurt after storage	µg/L	13.6	14.8
Equol			
Full-fat milk after pasteurisation	µg/L	4.0	26.7
Yoghurt after manufacturing	µg/L	6.0	26.8
Yoghurt after storage	µg/L	5.7	20.5
Total isoflavones			
Full-fat milk after pasteurisation	µg/L	239.6	246.5
Yoghurt after manufacturing	µg/L	239.2	237.2
Yoghurt after storage	µg/L	236.3	231.1
Concentration on a dry weight basis Daidzein			
Full-fat milk after pasteurisation	µg/L	385.2	362.2
Yoghurt after manufacturing	µg/L	393.9	391.1
Yoghurt after storage	µg/L	399.2	381.8
Genistein			
Full-fat milk after pasteurisation	µg/L	1285.3	1194.7
Yoghurt after manufacturing	µg/L	1298.9	1145.8
Yoghurt after storage	µg/L	1290.4	1167.9
Glycitein			
Full-fat milk after pasteurisation	µg/L	117.0	125.7
Yoghurt after manufacturing	µg/L	114.9	124.7
Yoghurt after storage	µg/L	105.5	117.2
Equol			
Full-fat milk after pasteurisation	µg/L	30.3	204.7
Yoghurt after manufacturing	µg/L	46.4	211.2
Yoghurt after storage	µg/L	44.5	162.0
Total isoflavones			
Full-fat milk after pasteurisation	µg/L	1817.9	1887.3
Yoghurt after manufacturing	µg/L	1854.1	1872.8
Yoghurt after storage	µg/L	1839.6	1828.9

Table 7. Effect of yoghurt manufacturing and storage

certain bacterial strains. E.g. Tsangalis et al. (2002) found equol in soymilk fermented with *Bifidobacterium pseudolongum*, *Bifidobacterium longum*-a and *Bifidobacterium animalis*. In the present study, yoghurt cultures probably contributed to elevated levels of equol by transformation of daidzein to equol.

After a one-month storage concentration of total isoflavones in plain yoghurt declined in both groups. Similar results were reported by Otieno et al. (2006) for soymilk fermented by *Bifidobacterium animalis* and stored at 4°C for up to 8 weeks. Based on their findings, this decline is probably caused by the glucosides that were not stable during storage and incurred more losses in comparison to aglycone forms.

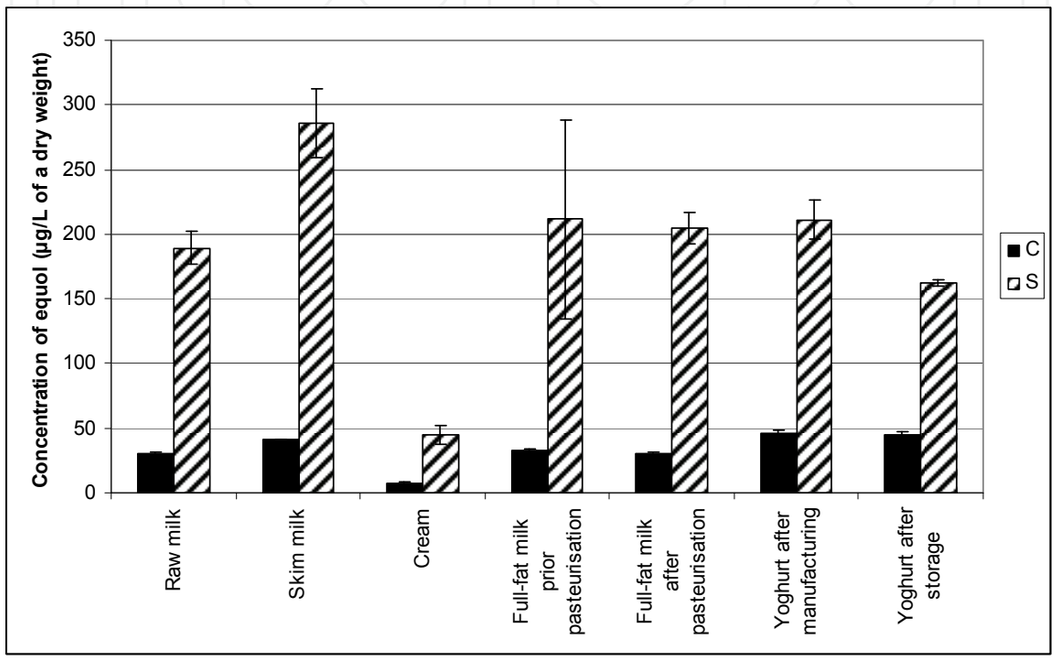


Fig. 1. Changes in equol content (µg/L of dry weight, mean ± standard deviation) during technological processing

After one-month storage the equol content decreased to 44.5 µg/L (dry weight) in C and to 162.0 µg/L (dry weight) in S. There is no comparable study focused on changes in equol concentration during storage of equol enriched products. However, Otieno et al. (2006) detected equol in trace amounts in soymilk fermented with *Bifidobacterium animalis* during storage and noted that equol was not detected until the third week of storage at -80 °C, fifth week at 4 °C, while it took only 2 weeks to be detected during storage at 24.8 °C and 37 °C.

Bovine milk can be considered as a potential source of equol in human nutrition (Mustonen et al., 2009). Figure 1 sumarises the changes in equol concentration expressed on a dry weight basis during various steps of technological processing determined in our study. As already mentioned, low concentrations of equol have been found in cream and relatively high concentrations in skimmed milk. From the previous discussion, it seems that the equol concentration is not altered by pasteurisation. Slight increase in equol concentration was noted during fermentation by yoghurt cultures consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus.* Losses during one-month storage of plain yoghurt reached for 23 % in S while in C were negligible.

4. Conclusion

It has been proved that bovine milk can be a potential source of equol for human especially for so called non-equol producers as an alternative strategy for obtaining the health-promoting benefits of equol. Besides red clover, soybean-derived feeding components can be also a potential source of isoflavones in bovine milk. Data suggest that the concentration of equol in milk can be manipulated by choosing an appropriate form of technologically processed soybeans. To our knowledge, this is the first study monitoring the changes in isoflavones concentrations in bovine milk during technological processing. Results of the present work show that studied dairy products, it is pasteurised milk, skim milk and yoghurt can be also included among possible sources of equol in human nutrition. Low concentrations of isoflavones (on a dry matter basis) were also detected in cream. After one-month storage, decrease in equol concentration was noted in isoflavones enriched yoghurt. Further study is needed to determine kinetics of isoflavone degradation during fermentation and storage of dairy products. With regards to differences in bioavailability of various forms of isoflavones (glucosides, aglycones, malonyl- and acetyl-forms) for human, further studies focused on possible intra-conversions between the different forms would be also useful.

5. Acknowledgements

This study was supported by the Ministry of Education, Youth and Sports, Czech Republic, projects No. 2B08073, MSM 2678846201, MSM 6046137305 and within specific university research MSMT No. 21/2010.

6. References

Adlercreutz, H.; Fotsis, T.; Bannwart, C.; Wähälä, K.; Makela, T.; Brunow, G. & Hase, T. (1986). Determination of urinary lignans and phytoestrogen metabolites, potential antiestrogens and anticarcinogens, in urine of women on various habitual diets. *Journal of Steroid Biochemistry and Molecular Biology,* 25, 791-797, ISSN: 0960-0760

Andersen, C.; Weisbjerg, M. R.; Hansen-Møller, J. & Sejrsen, K. (2009). Effect of forage on the content of phyto-oestrogens in bovine milk. *Animal,* 3, 617–622, ISSN: 1751-7311

Antignac, J. P.; Cariou, R.; Le Bizec, B. & Andre, F. (2004). New data regarding phytoestrogens content in bovine milk. *Food Chemistry,* 87, 275–281, ISSN: 0308-8146

Arora, A.; Nair, M. G. & Strasburg, G. M. (1998). Antioxidant activities of isoflavones and their biological metabolites in a liposomal system. *Archives of Biochemistry and Biophysics,* 356, 133-141, ISSN: 0003-9861

Atkinson, C.; Berman, S.; Humbert, O. & Lampe, J. W. (2004). In vitro incubation of human feces with daidzein and antibiotics suggests interindividual differences in the bacteria responsible for equol production. *Journal of Nutrition,* 134, 596-599, ISSN: 0022-3166

Atkinson, C.; Frankenfeld, C. L. & Lampe, J. W. (2005). Gut bacterial metabolism of the soy isoflavone daidzein: exploring the relevance to human health. *Experimental Biology and Medicine,* 230, 155-170, ISSN: 1535-3702

Blair, R. M.; Appt, S. E.; Franke, A. A. & Clarkson, T. B. (2003). Treatment with antibiotics reduces plasma equol concentration in Cynomolgus monkeys (Macaca fasicularis). *Journal of Nutrition,* 133, 2262-2267, ISSN: 0022-3166

Bowey, E.; Adlercreutz, H. & Rowland, I. R. (2003). Metabolism of isoflavones and lignans by the gut microflora: a study in germ-free and human flora associated rats. *Food and Chemical Toxicology,* 41, 631-636, ISSN: 0278-6915

Chen, T. R.; Su, R. Q. & Wei, Q. K. (2010). Hydrolysis of isoflavone phytoestrogens in soymilk fermented by *Lactobacillus* and *Bifidobacterium* cocultures. *Journal of Food Biochemistry,* 34, 1–12, ISSN: 0145-8884

Chouinard, P. Y.; Le´ Vesque, J.; Girard, V. & Brisson, G. J. (1997). Dietary soybeans extruded at different temperatures: milk composition and in situ fatty acid reactions. *Journal of Dairy Science,* 80, 2913–2924, ISSN: 0022-0302

Coldham, N. G.; Howells, L. C.; Santi, A.; Montesissa, C.; Langlais, C.; King, L. J.; MacPherson, D. D. & Sauer, M. J. (1999). Biotransformation of genistein in the rat: elucidation of metabolite structure by production mass fragmentology. *Journal of Steroid Biochemistry and Molecular Biology,* 70, 169–184, ISSN: 0960-0760

Dakora, F. D. & Phillips, D. A. (1996). Diverse functions of isoflavonoids in legumes transcend anti-microbial definitions of phytoalexins. *Physiological and Molecular Plant Pathology,* 49, 1–20, ISSN: 0885-5765

Eldridge, A. C. & Kwolek, W. F. (1983). Soybean isoflavones: effect of environment and variety on composition. *Journal of Agricultural and Food Chemistry,* 31, 394–396, ISSN: 0021-8561

Frank, A. A.; Hankin, J. H.; Yu, M. C.; Maskarinec, G.; Low, S. H. & Custer, L. J. (1999). Isoflavone level in soy foods consumed by multiethnic populations in Singapore and Hawaii. *Journal of Agricultural and Food Chemistry,* 47, 977–986, ISSN: 0021-8561

Franke, A. A.; Custer, L. J.; Wang, W. & Shi, C. Y. (1998). HPLC analysis of isoflavonoids and other phenolic agents from foods and from human fluids. *Proceedings of the Society for Experimental Biology and Medicine,* 217, 263–273, ISSN: 0037-9727

Grun, I. U.; Adhikari, K.; Li, C.; Li, Y.; Lin, B.; Zhang, J. & Fernando, L. N. (2001). Changes in the profile of genistein, daidzein, and their conjugates during thermal processing of tofu. *Journal of Agricultural and Food Chemistry,* 49, 2839–2843, ISSN: 0021-8561

Heinonen, S.; Wähälä, K. & Adlercreutz, H. (1999). Identification of isoflavone metabolites dihydrodaidzein, dihydrogenistein, 6 '-OH-O-dma, and cis-4-OH-equol in human urine by gas chromatography-mass spectroscopy using authentic reference compounds. *Analytical Biochemistry,* 274, 211-217, ISSN: 0003-2697

Heinonen, S.; Hoikkala, A.; Wähälä, K. & Adlercreutz, H. (2003). Metabolism of the soy isoflavones daidzein, genistein and glycitein in human subjects. Identification of new metabolites having an intact isoflavonoid skeleton. *Journal of Steroid Biochemistry and Molecular Biology,* 87, 285–299, ISSN: 0960-0760

Hendrich, S. & Murphy, P. A. (2001). Isoflavones: source and metabolism. In: *Handbook of nutraceuticals and functional foods.* Wildman R. E. C. (Ed.), 55–75, CRC Press, ISBN: 0849387345, Boca Raton, Fla

Hoey, L.; Rowland, I. R.; Lloyd, A. S.; Clarke, D. B. & Wiseman, H. (2004). Influence of soya-based infant formula consumption on isoflavone and gut microflora metabolite concentrations in urine and on faecal microflora composition and metabolic activity in infants and children. *British Journal of Nutrition,* 91, 607-616, ISSN: 0007-1145

Hoikkala, A.; Mustonen, E.; Saastmoinen, I.; Jokela, T.; Teponen, J.; Saloniemi, H. & Wähälä, K. (2007). High levels of equol in skimmed Finnish cow milk. *Molecular Nutrition & Food Research* 51, 782–786, ISSN: 1613-4125

Holman, P. (2001). Evidence for health benefits of plant phenols: local or systemic effects? *Journal of the Science of Food and Agriculture,* 81, 842–852, ISSN: 0022-5142

Hur, H. G.; Lay, J. O. J.; Beger, R. D.; Freeman, J. P. & Rafii, F. (2000). Isolation of human intestinal bacteria metabolizing the natural isoflavone glycosides daidzin and genistin. *Archives of Microbiology,* 174, 422–428, ISSN: 0302-8933

Izumi, T.; Piskula, M. K.; Osawa, S.; Obata, A.; Tobe, K.; Saito, M.; Kataoka, S.; Kubota, A. Y. & Kikuchi, M. (2000). Soy isoflavone aglycones are absorbed faster and in higher amounts than their glucosides in humans. *Journal of Nutrition, 130*, 1695–1699, ISSN: 0022-3166

Jackson, C. J. C.; Dini, J. P.; Lavandier, C.; Rupasinghe, H. P. V.; Faulkner, H.; Poysa, V.; Buzzell, D. & DeGrandis, S. (2002). Effects of processing on the content and composition of isoflavones during the manufacturing of soy beverage and tofu. *Process Biochemistry,* 37, 1117–1123, ISSN: 1359-5113

Joannou, G. E.; Kelly, G. E.; Reeder, A. Y.; Waring, M. & Nelson, C. (1995). A urinary profile study of dietary phytoestrogens: The identification and mode of metabolism of new isoflavonoids. *Journal of Steroid Biochemistry and Molecular Biology,* 54, 167–184, ISSN: 0960-0760

King, R. A.; Mano, M. M. & Head, R. J. (1998). Assessment of isoflavonoid concentrations in Australian bovine milk samples. *Journal of Dairy Research,* 65, 479–489, ISSN: 0022-0299

King, R. A. & Bignell, C. M. (2000). Concentrations of isoflavone phytoestrogens and their glucosides in Australian soya beans and soya foods. *Australian Journal of Nutrition and Dietetics, 57*, 70–78, ISSN: 1032-1322

Komprda, T.; Dvořák, R.; Suchý, P.; Fialová, M. & Šustová, K. (2000). Effect of heat treated rapeseed cakes in dairy cow diet on yield, composition and fatty acid pattern of milk. *Czech Journal of Animal Science,* 45, 325–332, ISSN: 1212-1819

Kostelac, D.; Rechkemmer, G. & Briviba, K. (2003). Phytoestrogens modulate binding response of estrogen receptors alpha and beta to the estrogen response element. *Journal of Agricultural and Food Chemistry,* 51, 7632-7635, ISSN: 0021-8561

Kudrna, V. & Marounek, M. (2006). Feeding oilseed products to dairy cows. *Journal of Animal and Feed Sciences,* 15, 36–69, ISSN: 1230-1388

Kuhnle, G. G. C.; Delcaqulla, C.; Aspinall, S. M.; Runswick, S. A.; Mulligan, A. A. & Bingham, S. A. (2008). Phytoestrogen content of foods of animal origin: dairy products, eggs, meat, fish, and seafood. *Journal of Agricultural and Food Chemistry,* 56, 21, 10099-10104, ISSN: 0021-8561

Kurzer, M. S. & Xu, X. (1997). Dietary phytoestrogens. *Annual Review of Nutrition,* 17, 353–381, ISSN: 0199-9885

Lampe, J. W.; Karr, S. C.; Hutchins, A. M. & Slavin, J. L. (1998). Urinary equol excretion with a soy challenge: influence of habitual diet. *Proceedings of the Society for Experimental Biology and Medicine,* 217, 335–339, ISSN: 0037-9727

Liggins, J.; Bluck, L. J. C.; Runswick, S.; Atkinson, C.; Coward, W. A. & Bingham, S. A. (2000a). Daidzein and genistein contents of vegetables. *British Journal of Nutrition,* 84, 717-725, ISSN: 0007-1145

Liggins, J.; Bluck, L. J. C.; Runswick, S.; Atkinson, C.; Coward, W. A. & Bingham, S. A. (2000b). Daidzein and genistein content of fruits and nuts. *Journal of Nutritional Biochemistry,* 11, 326-331, ISSN: 0955-2863

Lund, T. D.; Munson, D. J.; Haldy, M. E.; Setchell, K. D. R.; Lephart, E. D. & Handa, R. J. (2004). Equol is a novel anti-androgen that inhibits prostate growth and hormone feedback. *Biology of Reproduction,* 70, 1188-95, ISSN: 0006-3363

Mitchell, J. H.; Gardner, P. T.; McPhail, D. B.; Morrice, P. C.; Collins, A. R. & Duthie, G. G. (1998). Antioxidant efficacy of phytoestrogens in chemical and biological model systems. *Archives of Biochemistry and Biophysics,* 360, 142-148, ISSN: 0003-9861

Morito, K.; Hirose, T.; Kinjo, J.; Hirakawa, T.; Okawa, M.; Nohara, T.; Ogawa, S.; Inoue, S.; Muramatsu, M. & Masamune, Y. (2001). Interaction of phytoestrogens with estrogen receptors α and β. *Biological & Pharmaceutical Bulletin,* 24, 351-356, ISSN: 0918-6158

Mustonen, E. A.; Tuori, M.; Saastamoinen, I.; Taponen, J.; Wähälä, K.; Saloniemi, H. & Vanhatalo, A. (2009). Equol in milk of dairy cows is derived from forage legumes such as red clover. *British Journal of Nutrition,* 102, 1552–1556, ISSN: 0007-1145

Muthyala, R. S.; Ju, J.-H.; Sheng, S.; Williams, L. D.; Doerge, D. R.; Katzenellenbogen, B. S.; Helferich, W. G. & Katzenellenbogen, J. A. (2004). Equol, a natural estrogenic metabolite from soy isoflavones: convenient preparation and resolution of R- and S-equols and their differing binding and biological activity through estrogen receptors α and β. *Bioorganic & Medicinal Chemistry,* 12, 1559-1567, ISSN: 0968-0896

Nagel, S. C.; vom Saal, F. S. & Welshons, W. V. (1999). Developmental effects of estrogenic chemicals are predicted by an in vitro assay incorporating modification of cell uptake by serum. *Journal of Steroid Biochemistry and Molecular Biology,* 69, 343-357, ISSN: 0960-0760

Nurmi, T.; Mazur, W.; Heinonen, S.; Kokkonen, J. & Adlercreutz, H. (2002). Isoflavone content of the soy based supplements. *Journal of Pharmaceutical and Biomedical Analysis,* 28, 1-11, ISSN: 0731-7085

Otieno, D. O.; Ashton, J. F. & Shah, N. P. (2006). Stability of isoflavone phytoestrogens in fermented soymilk with *Bifidobacterium animalis* Bb12 during storage at different temperatures. *International Journal of Food Science and Technology,* 41, 1182–1191, ISSN: 0950-5423

Prabhakaran, M. P. & Perera, C. O. (2006). Effect of extraction methods and UHT treatment conditions on the level of isoflavones during soymilk manufacture. *Food Chemistry,* 99, 231–237, ISSN: 0308-8146

Purup, S.; Hansen-Møller, J.; Sejrsen, K.; Christensen, L. P.; Lykkesfeldt, A. E.; Leffers, H. & Skakkebæk, N. E. (2005). Increased phytoestrogen content in organic milk and the biological importance. Newsletter from Danish Research Centre for Organic Farming, no. 2. Danish Research Centre for Organic Farming, Tjele, Denmark.

Rimbach, G.; De Pascual-Teresa, S.; Ewins, B. A.; Matsugo, S.; Uchida, Y.; Minihane, A. M.; Turner, R.; VafeiAdou, K. & Weinberg, P. D. (2003). Antioxidant and free radical scavenging activity of isoflavone metabolites. *Xenobiotica,* 33, 913-925, ISSN: 0049-8254

Rowland, I.; Wiseman, H. & Sanders, T. (1999). Metabolism of oestrogens and phytoestrogens: role of the gut microflora. *Biochemical Society Transactions,* 27, 304–308, ISSN: 0300-5127

Rowland, I. R.; Wiseman, H.; Sanders, T. A.; Adlercreutz, H. & Bowey, E. A. (2000). Interindividual variation in metabolism of soy isoflavones and lignans: influence of

habitual diet on equol production by the gut microflora. *Nutrition and Cancer,* 36, 27–32, ISSN: 0163-5581

Sathyamoorthy, N. & Wang, T. T. Y. (1997). Differential effects of dietary phyto-oestrogens daidzein and equol on human breast cancer MCF-7 cells. *European Journal of Cancer,* 33, 2384–2389, ISSN: 0959-8049

Schmitt, E.; Dekant, W. & Stopper, H. (2001). Assaying the estrogenicity of phytoestrogens in cells of different estrogen sensitive tissues. *Toxicology In Vitro,* 15, 433-439, ISSN: 0887-2333

Setchell, K. D. R.; Brown, N. M. & Lydeking-Olsen, E. (2002). The clinical importance of the metabolite equol - a clue to the effectiveness of soy and its isoflavones. *Journal of Nutrition,* 132, 3577-3584, ISSN: 0022-3166

Setchell, K. D. R. (1998). Phytoestrogens: biochemistry, physiology, and implications for human health of soy isoflavones. *American Journal of Clinical Nutrition,* 68, 1333S–1346S, ISSN: 0002-9165

Simons, A. L.; Renouf, M.; Hendrich, S. & Murphy, P. A. (2005). Metabolism of glycitein (7,4'-dihydroxy-6-methoxy-isoflavone) by human gut microflora. *Journal of Agricultural and Food Chemistry,* 53, 8519–8525, ISSN: 0021-8561

Steer, T. E.; Johnson, I. T.; Gee, J. M. & Gibson, G. R. (2003). Metabolism of the soyabean isoflavone glycoside genistin in vitro by human gut bacteria and the effects of prebiotics. *British Journal of Nutrition,* 90, 635-642, ISSN: 0007-1145

Steinshamn, H.; Purup, S.; Thuen, E. & Hansen-Møller, J. (2008). Effects of clover-grass silages and concentrate supplementation on the content of phytoestrogens in dairy cow milk. *Journal of Dairy Science,* 91, 2715–2725, ISSN: 0022-0302

Třináctý, J.; Křížová, L.; Schulzová, V.; Hajšlová, J. & Hanuš, O. (2009). The effect of feeding soybean-derived phytoestrogens on their concentration in plasma and milk of lactating dairy cows. *Archives of Animal Nutrition,* 63, 1-11, ISSN: 1745-039X

Tsangalis, D.; Ashton, J. F.; McGill, A. E. J. & Shah, N. P. (2002). Enzymic transformation of isoflavone phytoestrogens in soymilk by β-glucosidase-producing *Bifidobacteria*. *Journal of Food Science,* 67, 3104–3113, ISSN: 0022-1147

Turner, R.; Baron, T.; Wolffram, S.; Minihane, A. M.; Cassidy, A.; Rimbach, G. & Weinberg, P. D. (2004). Effect of circulating forms of soy isoflavones on the oxidation of low density lipoprotein. *Free Radical Research,* 38, 209-216, ISSN: 1071-5762

Umphress, S. T.; Murphy, S. P.; Franke, A. A.; Custer, L. J. & Blitz, C. L. (2005). Isoflavone content of foods with soy additives *Journal of Food Composition and Analysis,* 18, 533-550, ISSN: 0889-1575

Uzzan, M. & Labuza, T. P. (2004). Critical issues in R&D of soy isoflavone–enriched foods and dietary supplements. *Journal of Food Science,* 69, R77–R86, ISSN: 0022-1147

Uzzan M.; Nechrebeki, J. & Labuza T. P. (2007). Thermal and storage stability of nutraceuticals in a milk beverage dietary supplement. *Journal of Food Science,* 72, E109-E114, ISSN: 0022-1147

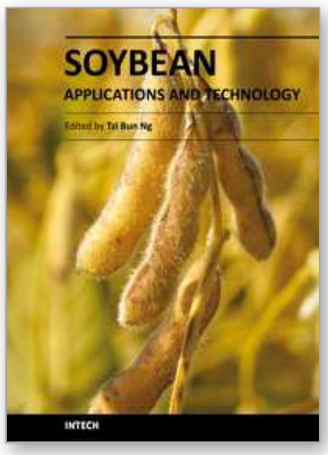
Wähälä, K.; Salakka, A. & Adlercreutz, H. (1998). Synthesis of novel mammalian metabolites of the isoflavonoid phytoestrogens daidzein and genistein. *Proceedings of the Society for Experimental Biology and Medicine,* 217, 293-299, ISSN: 0037-9727

Walsh, K. R. & Failla, M. L. (2009). Transport and metabolism of equol by Caco-2 human intestinal cells. *Journal of Agricultural and Food Chemistry,* 57, 8297–8302, ISSN: 0021-8561

Walsh, K. R.; Zhang, Y. C.; Vodovotz, Y.; Schwartz, S. J. & Failla, M. L. (2003). Stability and bioaccessibility of isoflavones from soy bread during in vitro digestion. *Journal of Agricultural and Food Chemistry,* 51, 4603–4609, ISSN: 0021-8561

Wang, H. J. & Murphy, P. A. (1994). Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year, and location. *Journal of Agricultural and Food Chemistry,* 42, 1674–1677, ISSN: 0021-8561

Zheng, Y.; Hu, J.; Murphy, P. A.; Alekel, D. L.; Franke, W. D. & Hendrich, S. (2003). Rapid gut transit time and slow fecal isoflavone disappearance phenotype are associated with greater genistein bioavailability in women. *Journal of Nutrition,* 133, 3110–3116, ISSN: 0022-3166



Soybean - Applications and Technology
Edited by Prof. Tzi-Bun Ng

ISBN 978-953-307-207-4
Hard cover, 402 pages
Publisher InTech
Published online 26, April, 2011
Published in print edition April, 2011

Soybean is an agricultural crop of tremendous economic importance. Soybean and food items derived from it form dietary components of numerous people, especially those living in the Orient. The health benefits of soybean have attracted the attention of nutritionists as well as common people.

How to reference
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Ludmila Křížová, Jiří Třináctý, Jana Hajšlová and Šárka Havlíková (2011). The Effect of Technological Processing on the Content of Isoflavones in Bovine Milk and Dairy Products, Soybean - Applications and Technology, Prof. Tzi-Bun Ng (Ed.), ISBN: 978-953-307-207-4, InTech, Available from: http://www.intechopen.com/books/soybean-applications-and-technology/the-effect-of-technological-processing-on-the-content-of-isoflavones-in-bovine-milk-and-dairy-produc

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