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# The influence of extrusion on loss and racemization of amino acids

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**Summary.** The influence of the operation conditions (temperature and residence time) of a thermic treatment on the total amount (free and protein-bound) of amino acid enantiomers of dry fullfat soya was investigated. Total amino acid content was determined using conventional ion-exchange amino acid analysis of total hydrolysates and chiral amino acid analysis was performed by HPLC after precolumn derivatization with *o*-phthaldialdehyde and 1-thio-β-D-glucose tetraacetate. Contrary to corn that was investigated previously, notable racemization was detected even at lower temperatures. At 140 °C the ratio of the D-enantiomer was 0.87% for glutamic acid, 2.81% for serine, and 1.92% for phenylalanine; at 220 °C the ratios of the D-enantiomer of the above amino acids were 1.43, 4.61, and 4.68%, respectively. The concentration of several L-amino acids decreased. At 220 °C there was 10% less L-glutamic acid, 17% less L-serine, 5% less L-phenylalanine, 6.6% less L-aspartic, acid and 21% less L-lysine than in the control; their loss can be assigned to different degrees of L – D conversion. While nearly complete transformation of L-phenylalanine can be attributed to racemization, the main cause of the loss of L-lysine is not racemization. The treatments in the same order of magnitude resulted in the formation of more D-amino acids and greater extent of racemization of amino acids in fullfat soya than that of maize.

**Keywords:** Racemization – D-amino acid – Fullfat soybeans – Extrusion temperature – Residence time

## Introduction

Epimerization (partial racemization) of protein-bound and free amino acids may occur during the processing of food if the operation conditions involve application of heating and/or alkaline conditions (Friedman, 1991, 1999; Zagon et al., 1994). Heating in alkaline medium has been shown to yield significant amounts of D-amino acids through the mechanism of base-catalyzed racemization (Masters and Friedman, 1980; Friedman et al., 1981; Liardon and Ledermann, 1986; Liardon and Friedman, 1987). Albeit the medium is usually neutral or weakly acidic during food processing, reducing sugars can induce partial racemization of free L-amino acids in the course of the Maillard reaction (Erbe and Brückner, 2000; Brückner et al., 2001), and D-amino acids can also be formed via the mechanism of acid-catalyzed racemization (Frank et al., 1981; Liardon et al., 1991). Epimerization of L-aspartic acid can also occur through transpeptidation reactions via a succinimide intermediate (Geiger and Clarke, 1987).

The digestibility of the proteins decreases when significant ratios of the protein-bound amino acids occur in the D-configuration due to the stereospecificity of the proteinases and peptidases (Friedman, 1991, 1999; De Vrese et al., 2000). The rate of absorption can be discriminative to D-amino acids (Oxender, 1965; Schwass et al., 1983) and the bioavailability due to the restricted efficiency of D-amino acid oxidase system can be diminished (Man and Bada, 1987; Nagata et al., 1991). The activity of D-amino acid oxidases depends on several factors (species, age, organ, tissue, substrate), and there is a big variation in the efficiency of utilization of the D-amino acids among species (Friedman, 1999). In the case of mammals only small ratios of the D-amino acids were utilized following oral consumption, and the D-stereoisomers of the essential amino acids in some cases caused growth inhibition and were mainly excreted in the urine (Man and Bada, 1987). The value of relative oral bioavailability (RBV) for D-Met is only 30% for humans (Baker, 2006). From a nutritional standpoint, racemization could result in the loss of protein that is one of the most valuable components of the food.

On the other hand the oral consumption of D-serine, lysinoalanine (Kaltenbach et al., 1979; Carone et al., 1985; Imai et al., 1998) and D-proline (Kampel et al., 1990) have been claimed to induce histological changes in the

rat kidney while others found no sign of organic disorders in the case of D-proline and D-aspartic acid (Schieber et al., 1997). Recently it has been shown that D-amino acids that are present in the different organs and tissues of animals and humans have specific biological functions. In the central nervous system, D-serine and D-aspartate occur in considerable concentrations (Fuchs et al., 2005). D-serine is synthesized and metabolized endogenously by human serine racemase (De Miranda et al., 2000) and the same might account for D-aspartate. Some part of D-serine in brain can originate from exogenous sources through the blood-to-brain transfer (Bauer et al., 2005).

The food industry is nowadays aware of the potential risk of the treatment of proteinaceous food, and the aim is to define conditions when the aim of the treatment is completed without significant change in the structure of the biological valuable components.

During thermic processing the integrity of food components is disrupted due to the effect of heat and pressure and a spongoid structure is formed. Heat-sensitive antinutritive factors are totally or partially inactivated, and the number of microorganisms is also diminished. The task is to determine the conditions of good manufacturing practice in which the above-mentioned aims are accomplished without significant loss of amino acids. The decrease of the amino acid content in corn grain due to extrusion has been investigated, but in these studies the ratio of the enantiomers was not determined (Ormainé Cserhalmi et al., 1988; Ormainé Cserhalmi and Czukor, 1991). In our previous experiment the influence of extrusion conditions on the D-amino acid content of corn was investigated (Vargáné et al., 2004). In the present work the thermic treatment of an important vegetal protein source is investigated and the results are compared.

## Materials and methods

*Extrusion*

The raw material of the extrusion was fullfat soya (Glycine max (L.) Merr., '*Borostyán*' variety). The basic chemical composition was as follows: the dry matter content was 98.1% and the ash content 4.5% (g/100 g sample). Fullfat soya consisted of 33.7% crude protein, 22.9% crude fat, 3.4% crude fiber, and 33.6% nitrogen-free extractable material. The starch content was 5.4% and the total sugar content 8.9%. The amino acid content of fullfat soya and the amino acid composition of its proteins are shown in Table 1.

**Table 1.** The amino acid content and the amino acid composition of untreated fullfat soybean grain

| Amino acid | Amino acid content (g amino acid/100 g sample) | Amino acid composition (g amino acid/100 g protein) |
|---|---|---|
| Asp | 3.83 | 11.6 |
| Thr | 1.25 | 3.8 |
| Ser | 1.77 | 5.3 |
| Glu | 6.39 | 19.3 |
| Pro | 1.73 | 5.2 |
| Gly | 1.57 | 4.7 |
| Ala | 1.51 | 4.6 |
| Cys | 0.38 | 1.1 |
| Val | 1.43 | 4.3 |
| Met | 0.50 | 1.5 |
| Ile | 0.96 | 2.9 |
| Leu | 2.57 | 7.8 |
| Tyr | 1.36 | 4.1 |
| Phe | 1.64 | 4.9 |
| Lys | 2.26 | 6.8 |
| His | 1.10 | 3.3 |
| Arg | 2.47 | 7.5 |
| $NH_3$ | 0.42 | 1.3 |

The raw material was ground with a hammer grinder and the particle size distribution was determined. Due to the high oil content conditioning was not necessary prior to extrusion. Ten kilogram of material was used for each trial. Extrusion was carried out using a Do-Corder DC 2001 type Brabender machine equipped with a 19 mm i.d. barrel (21:1 length to diameter ratio); a screw with the length of 400 mm with increasing screw diameter from 12 to 17 mm, and a cylindrical die which consists of two parts: a 55 mm long by 8 mm i.d. following a 22 mm long by 5 mm. The barrel and the die were heated by electrically controlled split ring resistance heaters, and the screw speed was also kept under control. The barrel and the die temperatures were monitored by thermocouples mounted in shallow wells. Extrusion trials with the full cross-classification of the applied nominal temperature and screw speed levels (Table 2) were repeated three times on three different days. From the two reported zone temperatures ($T_1$, $T_2$), one value was calculated (T) to characterize the effect of temperature. Minimum residence time was determined by introducing a small amount of dye into the feeding port and measuring the time required for the first colored extrudate to exit the die. Prior to sampling, the machine was allowed to equilibrate to the desired temperature, then approx. 200 g sample was collected and allowed to cool down before being homogenized, and sealed in polyethylene bags and stored at −20 °C.

**Table 2.** Nominal and measured properties of extrusion of fullfat soya

| Levels | Nominal temperature (°C) | Measured temperature (T) average ± s.d. (°C) (n = 12) | Levels | Screw speed ($s^{-1}$) | Residence time (s) average ± s.d. (n = 12) | Throughput (kg/h) average ± s.d. (n = 12) |
|---|---|---|---|---|---|---|
| 1 | 100 | 101 ± 4 | 1 | 50 | 29 ± 0.2 | 1.6 ± 0.4 |
| 2 | 140 | 140 ± 3 | 2 | 90 | 17 ± 0.2 | 2.8 ± 0.8 |
| 3 | 180 | 180 ± 3 | 3 | 130 | 12 ± 0.8 | 4.1 ± 1.1 |
| 4 | 220 | 220 ± 3 | 4 | 170 | 10 ± 1.4 | 4.8 ± 1.4 |

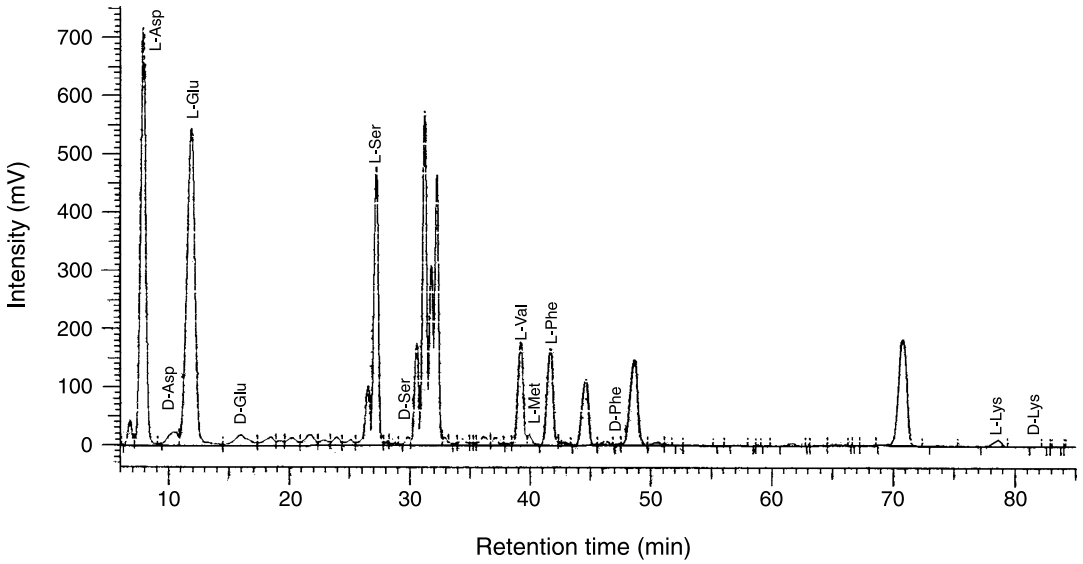


**Fig. 1.** The chromatogram of the OPA/TATG derivatives of the amino acid enantiomers obtained from hydrolyzed fullfat soya proteins. See conditions in the text

Control samples were taken from each batch and treated in the same way as extruded samples.

*Chemical analysis*

The moisture content was determined with the standard procedure of MSZ ISO 1442, the crude protein content measurement based on the basic method of Kjeldahl (MSZ EN ISO 5983-1:2005). Crude fat (MSZ 6369-15:1982), total ash (MSZ ISO 749:1992), crude fiber (MSZ ISO 6865:2000), starch (MSZ 6830-18:1988) and total sugar content examinations (MSZ 6830-26:1987) were carried out with the use of standard procedures approved by the Hungarian Standards Institution.

Prior to amino acid analysis the samples were dissolved in hydrochloric acid (6 M; 5 $cm^3$) and proteins were hydrolyzed at $105 \pm 1\,^{\circ}C$ for 24 h. The amino acid content and composition was determined with an INGOS AAA 400 amino acid analyzer (INGOS, Praha, Czech Republic) equipped with a $35 \times 0.37$ cm column packed with OSTION Lg ANB.

The concentration of the amino acid enantiomers was also determined from the total hydrolysate of the samples. After cooling, the pH was adjusted to pH = 7 with sodium hydroxide solution, then diastereomers were produced with OPA (*o*-phthaldialdehyde) and TATG (1-thio-β-D-glucose tetraacetate) (Sigma, St. Louis, MO, USA) during precolumn derivatization and separated by HPLC with detection using a fluorescence detector as described elsewhere (Einarsson et al., 1987; Csapó et al., 1995; Vargáné et al., 2004). A typical chromatogram of the derivatives of the examined L- and D-amino acids of soybean can be seen in Fig. 1. Before the analysis of soybean samples standard solutions of D- and L-amino acids were derivatized and analyzed, and calibration curves were established and response factors were calculated for each analyzed component. The amount of the amino acid enantiomers of the samples was calculated based on these calibration curves and corrected for hydrolysis-induced racemization. Moreover, the concentration of the enantiomers was determined after the hydrolysis of the total amount of samples; thus, the sum of free and protein-bound amino acids was determined.

*Statistical analysis*

Data analysis was carried out with the use of SPSS for Windows 10.0 (1999) statistical program (Statistical Package for Social Sciences). There were four levels of temperature factor and four levels of screw speed factor. The number of replications was three; sampling was repeated on three different days with the full cross-classification of the applied levels of factors. The extent of racemization was defined as the percentage of the D-enantiomer of the total (D + L) amino acid content ($\frac{D}{D+L} \cdot 100$) (Friedman, 1999). In the above formula 'D' and 'L' means the concentration of the D- and L-enantiomer of the given amino acid calculated with the use of the relevant response factors. The influence of temperature and residence time on the D-amino acid content and the degree of racemization were evaluated with multiple analysis of variance. The equation of the used linear model was the following:

$$Y_{ijk} = \mu + T_i + F_j + TF_{ij} + e_{ijk}$$

with

$Y_{ijk}$ = the $k^{th}$ observation in the $ij^{th}$ treatment combination,
$\mu$ = the least squares mean,
$T_i$ = the effect of the $i^{th}$ class of factor T (temperature) expressed as a deviation from $\mu$,
$F_j$ = the effect of the $j^{th}$ class of factor F (screw speed) expressed as a deviation from $\mu$,
$TF_{ij}$ = the interaction effect of the $i^{th}$ class of factor T and the $j^{th}$ class of factor F expressed as a deviation from $\mu + T_i + F_j$ and
$e_{ijk}$ = the random error associated with the $k^{th}$ observation in the $ij^{th}$ treatment combination.

If the treatment means differed significantly ($P < 0.05$), the comparison of that was accomplished with the Student-Newman-Keuls test.

## Results and discussion

### *The influence of extrusion on the D-amino acid content of fullfat soya*

Similarly to extrusion of corn, residence time did not change when the same screw speed was used at different temperatures (Vargáné et al., 2004). Thus residence time and screw speed could be regarded as traits substituting each other

without confounding with temperature when their influence on the D-amino acid formation was analyzed (Table 2).

In order to determine the D-amino acid producing capacity of extrusion, the measured D-amino acid values of treated samples have to be corrected for the D-amino acid content formed during acidic hydrolysis of control samples (De Vrese et al., 2000; Masters and Friedman, 1980; Csapó et al., 1997). There were significant differences in the D-glutamic acid, D-serine and D-phenylalanine content of soybean treated at different temperatures ($P < 0.05$). The amount of these amino acids showed a notable increase when the extrusion temperature was increased from 101 to 140 °C (Table 3), and their concentration was higher in the products extruded at 140 °C than in control samples without extrusion. The variance of D-aspartic acid content was higher than that of the other amino acids, and thus significant differences cannot be detected. In the case of corn samples the extrusion on the same instrument below 144 °C of 28–72 s did not induce significant ($P < 0.05$) racemization (Vargáné et al., 2004).

**Table 3.** Influence of the extrusion temperature on the total (free and protein-bound) D-amino acid content of fullfat soya (mg/100 g dry matter)

| Average ± s.d.[1,2] (n = 12) | Temperature (T) | | | |
|---|---|---|---|---|
| | 101 °C | 140 °C | 180 °C | 220 °C |
| D-Glu | $40^{a} \pm 9$ | $57^{b} \pm 13$ | $65^{b} \pm 24$ | $89^{c} \pm 20$ |
| D-Ser | $29^{a} \pm 27$ | $49^{b} \pm 20$ | $67^{c} \pm 12$ | $74^{c} \pm 12$ |
| D-Phe | $5^{a} \pm 23$ | $27^{b} \pm 16$ | $42^{b} \pm 18$ | $66^{c} \pm 10$ |
| D-Asp | $29^{a} \pm 17$ | $20^{a} \pm 56$ | $47^{a} \pm 92$ | $83^{a} \pm 106$ |

[a,b,c] Averages in one row with common superscript do not differ ($P \geq 0.05$).
The total amount of the amino acid enantiomers was determined after the hydrolysis of the extruded samples
[1] Corrected with control values obtained from untreated fullfat soya
[2] Averages and standard deviations of samples extruded at the same temperature with different residence times

**Table 4.** Influence of the extrusion temperature on the degree of partial racemization of the examined amino acids in corn ($\frac{D}{D+L} \cdot 100$)

| Average ± s.d.[1,2] (n = 12) | Temperature (T) | | | |
|---|---|---|---|---|
| | 101 °C | 140 °C | 180 °C | 220 °C |
| Glu | $0.57^{a} \pm 0.13$ | $0.87^{b} \pm 0.18$ | $0.99^{b} \pm 0.38$ | $1.43^{c} \pm 0.36$ |
| Ser | $1.51^{a} \pm 1.41$ | $2.81^{b} \pm 1.04$ | $3.90^{c} \pm 0.70$ | $4.61^{c} \pm 0.69$ |
| Phe | $0.23^{a} \pm 1.56$ | $1.92^{b} \pm 1.18$ | $2.88^{b} \pm 1.17$ | $4.68^{c} \pm 0.72$ |
| Asp | $0.69^{a} \pm 0.40$ | $0.44^{a} \pm 1.35$ | $1.06^{a} \pm 2.15$ | $1.93^{a} \pm 2.46$ |

[a,b,c] Averages in one row with common superscript do not differ ($P \geq 0.05$)
In the above formula 'D' and 'L' means the concentration of the D- and L-enantiomer of the given amino acid calculated with the use of the relevant response factors
The total (free and protein-bound) amount of the amino acid enantiomers was determined after the hydrolysis of the extruded samples
[1] Corrected with control values obtained from untreated fullfat soya
[2] Averages and standard deviations of samples extruded at the same temperature with different residence times

The influence of the screw speed (residence time) on the D-amino acid content was not significant at the investigated ranges.

High-temperature treatments relative to the low-temperature treatments resulted in more significant increase of the extent of racemization ($\frac{D}{D+L} \cdot 100$, Table 4) than that of the amount of D-amino acids (Table 3) because the L-amino acid concentration decrease (Table 5) exceeded the D-amino acid concentration increase. Namely, besides isomerization, the intensity of other processes that alter the structure of the amino acids was also significant. In the case of corn the loss of aspartic acid and lysine was reported during high temperature extrusion (Ormainé et al., 1988; Ormainé and Czukor, 1991; Vargáné et al., 2004).

The concentration of most of the L-amino acids under the scope of the study decreased when the temperature of the heat treatment increased (Table 5). The sample ex-

**Table 5.** The total (free and protein-bound) L-amino acid content of soybean treated at different temperatures (g/100 g dry matter)

| Average ± s.d.[1] (n = 12) | Control | Temperature (T) | | | |
|---|---|---|---|---|---|
| | | 101 °C | 140 °C | 180 °C | 220 °C |
| L-Asp | $3.70^{b} \pm 0.17$ | $3.77^{b} \pm 0.39$ | $3.59^{ab} \pm 0.21$ | $3.56^{ab} \pm 0.15$ | $3.38^{a} \pm 0.28$ |
| L-Glu | $6.74^{b} \pm 0.26$ | $6.73^{b} \pm 0.52$ | $6.41^{ab} \pm 0.42$ | $6.43^{ab} \pm 0.35$ | $6.05^{a} \pm 0.53$ |
| L-Ser | $1.83^{c} \pm 0.06$ | $1.82^{c} \pm 0.15$ | $1.69^{b} \pm 0.10$ | $1.66^{b} \pm 0.06$ | $1.52^{a} \pm 0.10$ |
| L-Val | $1.53^{bc} \pm 0.06$ | $1.57^{c} \pm 0.08$ | $1.50^{bc} \pm 0.09$ | $1.45^{ab} \pm 0.07$ | $1.38^{a} \pm 0.13$ |
| L-Met | $0.54^{a} \pm 0.01$ | $0.55^{a} \pm 0.07$ | $0.55^{a} \pm 0.05$ | $0.56^{a} \pm 0.03$ | $0.55^{a} \pm 0.04$ |
| L-Phe | $1.40^{bc} \pm 0.04$ | $1.46^{c} \pm 0.10$ | $1.37^{bc} \pm 0.16$ | $1.41^{bc} \pm 0.06$ | $1.34^{ab} \pm 0.08$ |
| L-Lys | $2.39^{b} \pm 0.39$ | $2.10^{b} \pm 0.30$ | $1.96^{a} \pm 0.19$ | $1.99^{a} \pm 0.22$ | $1.87^{a} \pm 0.23$ |

[a,b,c] Averages in one row with common superscript do not differ ($P \geq 0.05$)
[1] Averages and standard deviations of samples extruded at the same temperature with different residence times

truded at the highest temperature contained 6.6% less L-aspartic acid than the control. The amount of the D-enantiomer formed (0.08 g/100 g) accounted for 25% of the difference (0.32 g/100 g). In the case of L-glutamic acid and L-serine there was a concentration decrease of 10–17%, respectively, and 24% of the L-serine loss can be attributed to formation of the D-enantiomer. The degree of the decomposition of L-phenylalanine (5%) was almost the same as the extent of the formation of the D-enantiomer. The highest concentration decrease was detected for L-lysine (21%). Since the degree of racemization of lysine was less than 2%, racemization can account for not more than 8% of the concentration decrease of L-lysine (0.52 mg/100 g). Similarly, as in the case of corn, the main cause of the loss of L-lysine is not racemization.

*Comparison of the effect of extrusion on soya and corn with respect to the formation of D-amino acids*

Thermal treatment of fullfat soya resulted in higher amounts of D-amino acids relative to dry matter than in the case of corn. This can be attributed to the fact that the protein content of fullfat soya is about four-fold higher than that of corn. In contrast to D-amino acid content, the $\frac{D}{D+L} \cdot 100$ ratio does not depend on the absolute amount of protein. Since the ratios of the amino acids that are most susceptible to racemization (that is serine, glutamic and aspartic acid) are similar in soya and in corn proteins, their common transformation can be investigated. The degree of partial racemization of these amino acids in soya extruded at 180 °C was slightly higher than that of corn at 200 °C. Thus, similar heat treatment seems to cause a higher extent of L–D amino acid conversion in the soya proteins than in corn proteins.

The influence of the screw speed (residence time) on the D-amino acid content and racemization was not significant in the case of either of the raw materials. This can be attributed to the fact that in the function of screw speed there was only a three-fold change of the residence time. Ten degrees temperature increase resulted in 2.2–5.5-fold increase in the first order reaction rate constant (k) of amino acid racemization (Friedman, 1999). Due to the relationship of reaction time (t) and ''k'' in the first order reaction kinetic equitation of racemization, a three-fold residence time increase exerted about the same effect on the D-amino acid content as 10 degrees temperature increase. Therefore, within the examined temperature and time intervals, the change in the treatment temperature has only significant effect on the racemization of the proteins and the D-amino acid content of products.

*Comparison of the effect of extrusion on soya and corn with respect to the loss of L-amino acids*

The rate of contribution of racemization and the other processes to the loss of L-amino acids seems to depend both on the sort of the amino acid and the type of the protein source. In the case of soya the ''non-racemization loss'' of L-aspartic acid, L-glutamic acid, L-serine, and L-lysine related to the whole concentration decrease was 75, 87, 76 and 92%, respectively. In the case of corn 22% of the loss of L-Asp and 98% of the concentration decrease of L-Lys was not related to racemization. Heat treatment can also alter side chains of the amino acids and cause crosslink formation, e.g., serine (after β-elimination as dehydroalanine) and lysine can form lysinoalanine, the side chain of asparagine and glutamine can form an imide-type crosslink with lysine, the carboxyl group of acidic amino acids can esterify the hydroxyl group of serine. Furthermore, the loss of lysine can also be attributed to the reaction of the $\varepsilon$-amino group with reducing sugars in the Maillard reaction. In contrast to the above four amino acids, the degree of the concentration decrease of L-phenylalanine was practically the same as the amount of D-enantiomer formed, namely there was no significant concentration decrease due to other processes than racemization.

Both in soya and in corn the decomposition of L-lysine was the highest among amino acids. The loss of L-amino acids was more significant in case of soya than that of corn. High temperature (200 °C) extrusion of corn reduced the amount of L-lysine and L-aspartic acid, while in soya significant decreases of the following other amino acids were also detected such as L-serine, L-glutamic acid and L-phenylalanine. The ratio of lysine within the soya protein (6.8%) was almost three-fold higher than that of corn protein (2.5%), and the main cause of the loss of L-lysine was not the racemization but other processes. Supposedly the greater amount of lysine in the soya protein can form more crosslinks with serine and the acidic amino acids than in corn protein, and thus the L-amino acid loss could be higher in the case of soya. This hypothesis is supported by the fact that contrary to corn in which racemization is the main cause of the loss of L-aspartic acid (78%), the concentration decrease detected in soya can be attributed to a lesser extent to racemization (25%) than other reactions (75%).

In summary, dry extrusion of fullfat soybeans can result in significant loss of the amino acids. Within the decomposition the ratio of racemization and that of the other processes was evaluated. Among essential amino acids the concentration decrease of lysine was the most significant (21%). From a nutritional point of view one can avoid

drawing considerable consequences because analytical results only give gross values and utilization of amino acids depends on several factors. This study pointed out the need of conducting biological tests in order to estimate the possible loss of the bioavailability of amino acids of fullfat soybean due to dry extrusion at different species.

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