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Review

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Factors Causing Compositional Changes in Soy Protein Hydrolysates and Effects on Cell Culture Functionality

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ABSTRACT: Soy protein hydrolysates significantly enhance cell growth and recombinant protein production in cell cultures. The extent of this enhancement in cell growth and IgG production is known to vary from batch to batch. This can be due to differences in the abundance of different classes of compounds (e.g., peptide content), the quality of these compounds (e.g., glycated peptides), or the presence of specific compounds (e.g., furosine). These quantitative and qualitative differences between batches of hydrolysates result from variation in the seed composition and seed/meal processing. Although a considerable amount of literature is available that describes these factors, this knowledge has not been combined in an overview yet. The aim of this review is to identify the most dominant factors that affect hydrolysate composition and functionality. Although there is a limited influence of variation in the seed composition, the overview shows that the qualitative changes in hydrolysate composition result in the formation of minor compounds (e.g., Maillard reaction products). In pure systems, these compounds have a profound effect on the cell culture functionality. This suggests that the presence of these compounds in soy protein hydrolysates may affect hydrolysate functionality as well. This influence on the functionality can be of direct or indirect nature. For instance, some minor compounds (e.g., Maillard reaction products) are cytotoxic, whereas other compounds (e.g., phytates) suppress protein hydrolysis during hydrolysate production, resulting in altered peptide composition, and, thus, affect the functionality.

KEYWORDS: *enzymatic hydrolysis, compositional changes, Maillard reaction, racemization, meal processing, soybeans*

■ INTRODUCTION

The performance of a mammalian cell culture system is primarily determined by the product yield (production of recombinant proteins, such as immunoglobulins (IgG) and interferon γ) and product quality (glycosylation of recombinant proteins).[1] In many cell culture studies, viable cell growth is used as a second parameter to describe the cell culture performance.[1,2] This is because the recombinant protein production can be affected by cell growth. In some studies, recombinant protein production has been reported to increase with an increase in cell growth,[3] whereas in other studies, recombinant protein production increased following a suppression in cell growth.[4]

To improve the performance of mammalian cell culture processes, fetal calf serum has been used as a supplement for several decades. This supplement provides growth factors, proteins, lipids, attachment factors, minerals, hormones, and several trace elements that are important for promoting cell growth and enhancing recombinant protein production.[1,5] However, the use of fetal calf serum has become restricted due to the risk of transmissible diseases such as bovine spongiform encephalopathy. As a result, substantial research has been performed to identify alternatives for fetal calf serum. Currently, two alternatives have been described in the literature: (1) using a chemically defined medium[6−9] or (2) supplementing the basal chemically defined medium with plant protein hydrolysates.[10−13] The latter approach has become a common practice in the biopharma industry. In Table 1, an overview of the influence of soy protein hydrolysate supplementation to chemically defined medium on cell growth and recombinant protein production is provided. In this overview, the cell growth and recombinant protein production for chemically defined medium is set to 100%. The cell growth and IgG production in hydrolysate-supplemented chemically defined medium ranged from 90 to 178% and from 95 to 300%, respectively, as compared to that in the chemically defined medium (100%) (Table 1).

Commercially, plant protein hydrolysates from several sources such as soy, cotton, rice, wheat gluten, pea, and rapeseed proteins are available for cell culture applications.[14−16] The cell growth of the Chinese hamster ovary (CHO, CHO DG44/*dhfr*−/−) cells cultivated in HyQ CDM4-CHO medium supplemented with 16 hydrolysates obtained from different sources varied from 106 to 144% relative to that in the chemically defined medium (100%).[2] Although the enhancement in cell growth in culture supplemented with soy protein hydrolysate was the highest, the reasons why soy protein hydrolysates performed better than other hydrolysates were not explained.

Several cell culture studies have been performed, in which various cell lines (e.g., CHO 320, ME 750, and WuT3)[11,12,17,18] and chemically defined media (e.g., RPMI 1640, 5:5:1 (v/v) of IMDM:Ham's F12:NCTC, and 2:1:1 (v/v) of DMEM:F12:RPMI)[7,9,10] supplemented with soy protein hydrolysates[10,12,13,17] produced using different processes have been tested. The comparison of results obtained in these studies is limited, because these chemically defined media and hydrolysates have different chemical compositions and different cell lines have diverse nutritional requirements.[1] This comparison is further complicated by the fact that although the chemically defined media to

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Table 1. Effect of Supplementation of Soy Protein Hydrolysates to Chemically Defined Media on the Cell Growth and Recombinant Protein Production

cell line	hydrolysate concn % (w/v)	cell growth[a]	recombinant protein[a,b]	specific production[c]	reference
CHO 320	0.2	150	272	1.81	3
ProCHO5	0.2	178	300	1.69	3
CHO 320	0.2	91	121	1.33	4
CHO 320	0.1	100	116	1.15	10
CHO 320	0.1	91	108	1.19	10
ME-750	0.2	107	112	1.05	12
CHO 320	0.2	98	135	1.38	17
WuT3	0.1−1.0	94−152	95−155	0.87−1.02	18
CHO	1.0	145	160	1.10	119, 120
CHO	1.0	145	135	0.93	121, 122
SP 2/0 hybridoma	0.1−0.5	90	126	1.40	15
SP 2/0 hybridoma	0.1−0.5	154	115	0.75	15
	chemically defined media	100	100	1.00	

[a]Cell growth and recombinant protein production are expressed as percent relative to chemically defined media. [b]Values shown for recombinant protein production are for interferon-γ, IgG, and unspecified recombinant proteins. [c]Specific production = (recombinant protein)/(cell growth).

which hydrolysates are supplemented are called "*chemically defined*", an exact definition, or composition, of such a medium is not reported in the scientific literature. This lack of comparability limits the understanding of the role of soy protein hydrolysates in enhancing cell culture functionality. Moreover, this results in wide variability in the functionality of soy protein hydrolysates in cell culture (Table 1). For several studies, the cell growth was low in hydrolysate-supplemented cultures as compared to the chemically defined media, but the recombinant protein production was always higher in the former than in the latter. The beneficial effects of soy protein hydrolysate are attributed to its complex composition, wherein it contains a large variety of different classes of compounds (e.g., peptides and carbohydrates). Typically, soy protein hydrolysates (60% peptides/amino acids and 20% carbohydrates)[17] are supplemented to a chemically defined medium at 0.1−1.0% (w/v). A typical chemically defined medium such as Iscove's modified Dulbecco's medium has amino acid and glucose contents of 1.2 and 4.5 g/L, respectively.[19] The hydrolysate supplementation to chemically defined medium significantly increases the protein content (50−500%) and carbohydrate content (4−44%) of the supplemented medium. As a result, these compounds considerably enhance the cell culture functionality of the chemically defined medium. First, these compounds provide carbon and nitrogen to cells resulting in enhanced cell growth and/or recombinant protein production;[16,20] Second, there might be certain specific (key) compounds in hydrolysates that specifically enhance cell growth, recombinant protein production, or both.[13,17] In previous work, phenyllactate, lactate, trigonelline, chiro-inositol, and X-190 (an unannotated peptide) were identified as key compounds in soy protein hydrolysates that correlated positively with CHO (CRL-11397) cell growth.[13] Lactate, ferulate, syringic acid, galactarate, adenine, and X-198 (an unannotated peptide) were shown to be the key compounds that correlated positively with IgG production.[13,21,22] In these studies, the variation in the concentration of the key compounds was linked to the batch-to-batch variability in the cell culture performance of soy protein hydrolysates. A comparison of supplementation of 30 batches of soy protein hydrolysate to chemically defined media to culture CHO (CRL-11397) cells showed that the cell growth and IgG production varied from 148 to 438% and from 117 to 283% relative to the chemically defined medium (100%), respectively.[13] For 29 batches of another soy protein hydrolysate, the recombinant protein production ranged from 52 to 164% of the average recombinant protein production of all hydrolysates.[23]

The extent of variability in the cell culture performance between different batches of a hydrolysate also depends on the cell lines used and the test concentration of hydrolysates. The IgG production in CHO cells ranged from 103 to 138% and that in avian cells ranged from 93 to 115% as measured for 16 batches of a soy protein hydrolysate.[24] When the same hydrolysates were supplemented at 0.5% (w/v) to CHO cells, the IgG production ranged from 103 to 138%, whereas it was 83−138% when hydrolysates were supplemented at 1.5% (w/v) concentration, respectively.[24]

In addition to cell line and hydrolysate concentration, batch-to-batch variation in the hydrolysate functionality is influenced by variability in the *gross composition* and *qualitative changes* that occur in the chemical composition of hydrolysates. The *gross composition* includes the total content and composition of each class of compound present in the hydrolysate, for instance, total peptide content and content of individual peptides. *Qualitative changes* refer to the chemical modification reactions (e.g., cross-linking and glycation of proteins) that occur in hydrolysates. These reactions result in the formation of minor compounds, such as furosine, lysinoalanine (LAL), peptide−phytate complexes, and peptide−polyphenol complexes. Conventionally, these compounds are not included in the gross compositional analysis of hydrolysates. Therefore, qualitative changes could result in large differences in the hydrolysate functionality, whereas the gross composition of different batches of hydrolysate stays identical. Both gross and qualitative compositional changes occur in soy protein hydrolysates due to variations in processing and raw material (seed and/or meal) composition. Although there is a lot of literature available on compositional (gross and qualitative) changes in soybean and its derived products, this knowledge, in relation to cell culture functionality, has not been combined in an overview yet.

In this review, we first discuss the effects of hydrolysate composition, both *gross* and *qualitative*, on cell growth and recombinant protein production in cell culture applications. Subsequent discussion focuses on how variations in the hydrolysate composition may be induced by variations in the raw material and processing.

■ CHEMICAL COMPOSITION OF SOY PROTEIN HYDROLYSATES

The major compounds present in soy protein hydrolysates are peptides, carbohydrates, and minerals. The typical gross composition of a commercial soy protein hydrolysate produced from defatted soybean meal is 60 ± 5% (w/w) peptides/amino acids, 20 ± 5% (w/w) carbohydrates, and 10% (w/w) minerals (variation in the mineral concentration was not specified).[17] Whereas the total carbohydrate and mineral contents of soy protein hydrolysate are known, the monosaccharide and mineral compositions are not yet reported. For 30 batches of another commercial soy protein hydrolysate, Proyield Soy SE50MAF-UF, produced from defatted soybean meal, the protein content varied in a narrow range of 56−58% (w/w).[13] This suggests that the batch-to-batch variability in the gross composition of a specific hydrolysate produced by a particular manufacturer is relatively small (<5%).

In addition to peptides, carbohydrates, and minerals, several compounds such as phytates (<0.05−0.2% in soy protein hydrolysates),[25] Maillard reaction products (absolute concentrations are not available) (MRP, e.g., pyrazines),[26] phenolic acids (227 mg/100 g protein in soy protein hydrolysates),[27] LAL (absolute concentrations are not available), and racemized amino acids/peptides (absolute concentrations are not available) are expected to be present in low concentrations. These minor compounds either are present in the raw material (soybean or meal) or are produced as a result of chemical modification reactions that occur during processing. Although some of these compounds (e.g., racemized peptides) are still determined as "proteins" in the total nitrogen analysis (e.g., Dumas method), their functionality is not the same as that of unmodified peptides. The concentrations of these minor compounds may be low, but they may have a large influence on the hydrolysate functionality.

■ EFFECT OF COMPOUNDS PRESENT IN SOY PROTEIN HYDROLYSATES ON CELL CULTURE FUNCTIONALITY

Typically, soy protein hydrolysates are supplemented at 0.1−1.0% (w/v) concentration to the chemically defined medium in cell culture assays (Table 1). The optimal hydrolysate concentration varies depending on the cell line and the experimental setup. The optimal hydrolysate concentrations are determined from dose (hydrolysate concentration)−response (cell growth/IgG production) curves. For CHO DG44/−*dhfr*− cells, the cell growth at 0.2, 0.4, 0.6, 0.8, and 1.2% (w/v) hydrolysate concentration in chemically defined medium was 131, 155, 152, 133, and 105%, respectively.[2] The recombinant protein production was not reported. As the peak cell growth is observed at 0.4% (w/v) hydrolysate concentration, this was considered as an optimum concentration. In WuT3 hybridoma cells, at 0.1, 0.2, 0.3, 0.5, and 1.0% (w/v) hydrolysate concentration, the cell growth was 134, 150, 151, 152, and 94%, respectively.[18] At 0.1, 0.2, 0.3, 0.5, and 1.0% (w/v) hydrolysate concentration, the recombinant protein production was 117, 133, 145, 155, and 95%, respectively.[18] Thus, 0.5% (w/v) was the optimum concentration when both cell growth and recombinant protein production were highest as compared to other hydrolysate concentrations. In addition to cell line and experimental setup, the optimal hydrolysate concentration depends on the chemical composition of hydrolysates. Surprisingly, the composition of hydrolysates has not been described in any of the cell culture studies that evaluated the effect of hydrolysate supplementation. Consequently, there is no knowledge of the effect of compositional differences between different batches of a hydrolysate on the functionality. This limits the understanding of the role of compounds present in hydrolysate on the cell culture functionality. Nevertheless, recently some systematic studies such as chemometrics in combination with LC-MS or NMR, RP-HPLC fractionation methods, and supplementation or removal of pure and specific compounds have been performed to start understanding the role of individual compounds on the functionality. Whereas the presence of peptides and carbohydrates contributes positively toward cell growth and IgG production, minor compounds such as racemized peptides, phytates, MRP, LAL, and polyphenols reduce this positive effect. These are discussed below.

Peptides. The role of peptides in cell culture is two-fold. First, the role of peptides is of nutritional character, where they act as a nitrogen source and support cell growth. Heidemann et al. demonstrated this nutritional effect by supplementing basal medium with 0.5% (w/v) wheat protein hydrolysate. The hydrolysate supplementation increased the cell growth and recombinant protein production by 105 and 114%, respectively, relative to the basal medium (100%).[16] These effects on functionality due to hydrolysate supplementation could be reproduced by supplementing the basal medium with twice the amount of glutamine and asparagine and 4 times the amount of serine present in the basal medium. The cell growth and recombinant protein production in the fortified medium were 114 and 130%, respectively, as compared to the nonfortified basal medium (100%).[16]

Second, certain peptides/peptide fractions exert a specific influence on cell growth and/or recombinant protein production. Franek et al. used liquid chromatography to fractionate soy protein hydrolysate. From these fractions, a specific peptide fraction (0.2% w/v) exhibited 141% cell growth and 213% IgG production as compared to the unfractionated hydrolysate (100%) in ME-750 hybridoma cells.[12] The other two fractions obtained exhibited lower cell growth (88 and 84%) and IgG production (78 and 102%) as compared to the unfractionated hydrolysate (100%).[12] In another study with synthetic peptides, supplementation at 0.2% (w/v) with Gly-Lys-Gly and Gly-His-Gly enhanced IgG production to 148 and 160%, whereas the cell growth was suppressed to 88 and 80% relative to the chemically defined medium (100%).[28] Conversely, supplementation at 0.2% (w/v) with Gly-Gly-Gly enhanced cell growth to 148% as compared to chemically defined medium (100%), whereas IgG production was unaffected.[28]

The specific effects are different from the nutritional effects because they cannot be reproduced by supplementing additional amino acids in the cell cultures.[29] In the literature, the specific effects of peptides are referred to as survival factors, anti-apoptotic factors, or growth factor-like activity. These specific effects can probably be explained by an energetically efficient peptide transport into the cell as compared to that of free amino acids supplied by chemically defined medium.[16,30] However, these mechanisms are not well understood.

The nutritional and specific effect of peptides depends on the molecular size. In general, hydrolysates with small peptides are functionally better than hydrolysates with large peptides. For example, extensively hydrolyzed (77% peptides <1 kDa; 1% peptides >10 kDa) and less extensively hydrolyzed (18% peptides <1 kDa; 26% peptides >10 kDa) rapeseed protein hydrolysates supplemented to chemically defined media showed cell growth of 133 and 85%, respectively.[30] Similar observations

were reported when rapeseed protein hydrolysates made with Esperase 7.5L, Neutrase 0.8L, and Orientase 90N were tested in cell culture. Esperase is a serine protease from *Bacillus lentus*, Neutrase is a neutral metallo-protaese from *Bacillus amyloliquefaciens*, and Orientase is a serine protease from *Bacillus subtilis*.[30] All of the enzymes had low specificities.[30] The activity of the enzymes was standardized using a hemoglobin standard, allowing comparison of different enzymes. The hydrolysates had similar molecular size distribution of peptides, but the cell growths observed were not similar. For example, the hydrolysate made with Esperase enhanced the cell growth 1.5 times higher than hydrolysates made with Neutrase.[30] This showed that hydrolysis with different enzymes resulted in the formation of different peptides which affected the functionality of hydrolysates.

Carbohydrates. Carbohydrates are a source of carbon that supports cell growth. Different carbohydrates differ in the rates at which they are consumed by cells and, hence, the maximum achievable cell growth differs.[31,32] Glucose, mannose, galactose, and fructose at 20 mM concentration were tested in a medium formulation used for CHO-TF-70R cells. Whereas the average cell concentration after 140 h of culturing in medium containing glucose or mannose was 1.2×10^6 cells/mL, it was 0.7×10^6 cells/mL in medium with galactose or fructose.[31] Furthermore, the consumption rate of glucose or mannose was much faster than that of galactose or fructose. After 150 h of culturing, 12.5 mM glucose/mannose and 2.5 mM galactose/fructose were consumed by CHO-TF-70R cells.[31] In a similar study, Barngrover et al. studied the influence of fructose and galactose concentration on cell growth of Vero African green monkey cells. After 6 days of culture, the cell growth was 27.5×10^5, 23.8×10^5, 11.3×10^5, and 16.3×10^5 cells/mL in 5, 10, 15, and 20 mM galactose in L-15 (Leibovitz) medium.[33] On the other hand, the cell growth was 23.8×10^5, 27.5×10^5, 23.8×10^5, and 16.8×10^5 cells/mL in 5, 10, 15, and 20 mM fructose concentration in L-15 (Leibovitz) medium.[33] Therefore, a strategic selection of carbohydrates and concentration in cell culture can be used to enhance the recombinant protein production.[34] Whereas the maximum cell growth and tissue plasminogen activator production in 20 mM glucose medium were 1.6×10^6 cells/mL and 3.9 μg/mL, respectively, they were 1.9×10^6 cells/mL and 4.5 μg/mL in 5 mM glucose and 20 mM galactose medium.[34]

Racemized Amino Acids/Peptides. The influence of racemized amino acids on cell growth has been investigated in few studies. In HeLa[35] and chick embryo cell culture[36] racemized D-amino acids did not inhibit or enhance cell growth. Similarly, Naylor et al. tested racemized amino acids in several mammalian cell lines (e.g., LM (TK^-), A9, HTC^+, 6TG-11, CHO, and B16) and reported that racemized amino acids did not affect cell growth.[37]

Chelating Compounds (LAL, Phytates, and MRP). In human and animal studies, LAL,[38,39] phytates,[40] and MRP[41,42] have been shown to chelate cations. Cations have an important role in mammalian cell culture; for example, magnesium controls the activities of many glycolytic enzymes in the Krebs cycle,[43] and calcium and zinc are essential for cell growth and differentiation, apoptosis, and recombinant protein production.[44,45] For instance, supplementation of phytic acid (0.27−2.7 mM) resulted in decreased BALB/c mouse 3T3 fibroblast cell growth (from 93 to 45%) as compared to the nonsupplemented control (100%).[46]

Maillard Reaction Products (MRP). The Maillard reaction results in the loss of essential amino acids (e.g., lysine), as the MRP cannot be metabolized in cell cultures. Moreover, Maillard reaction also results in the formation of new compounds, such as pyrazines and hydroxymethylfurfural, which affect cell cultures. Pyrazine and its derivatives at 1% (w/v) concentration induced genotoxicity in the range of 0.4−29% in CHO cells.[47] More than 1% (w/v) pyrazine concentration was toxic and completely inhibited cell growth. Another MRP, hydroxymethylfurfural, at 0.1% (w/v) concentration exhibited similar genotoxic and growth inhibition effects in chick-embryo fibroblasts.[48]

Peptide−Polyphenol Complexes. The influence of peptide−polyphenol complexes was investigated by supplementing growth medium with sunflower meal hydrolysates containing 1 and 6% (w/w) of polyphenols. Whereas the cell growth and recombinant protein production for the former hydrolysate were 9 g biomass/L and 180 units of streptokinase/mL^{-1} h^{-1}, respectively, they were only 2 g biomass/L and 40 units of streptokinase/mL^{-1} $hour^{-1}$ for the latter, respectively.[49] This clearly showed that high levels of polyphenol in hydrolysates inhibited cell growth and productivity. Although a large but similar extent of variability is reported for polyphenol content in sunflower (1.4−6.1%)[49−52] and soybean meal (0.02−9%),[53−55] the polyphenol compositions between the two meals differ greatly. Whereas the sunflower meal primarily contains chlorogenic acid as well as caffeic and quinic acids, soybean meals are rich in isoflavones such as daidzein, glycitein, and genistein. In chemically defined cell cultures, the influence of soy isoflavones on the cell culture functionality has also been investigated. Supplementation with genistein at >10 μM concentration (2.7 μg/mL) to the cell culture media resulted in a drastic reduction of CHO cell viability, and apoptosis and genotoxicity were induced.[56] In addition to cytotoxicity, genotoxic effects on cell cultures due to polyphenols are reported. A 40-fold increase in chlorogenic acid concentration from 0.01 to 0.4 mg/mL resulted in a 40-fold increase in genotoxicity in CHO cells.[57] These effects increased further in the presence of metal ions such as manganese and copper.[57]

In addition to a direct effect, the above-mentioned minor compounds influence the cell culture functionality by affecting hydrolysate composition. These compounds interfere with the production of peptides during enzymatic hydrolysis in hydrolysate production (Figure 1).

■ PRODUCTION OF SOY PROTEIN HYDROLYSATES

Soy protein hydrolysates for cell culture applications are produced by enzymatic hydrolysis of defatted soybean meal, soy protein concentrate, or protein isolate, followed by enzyme inactivation, coarse filtration, ultrafiltration, and spray-drying (Figure 2).[58] The industrial enzyme preparations that are used for hydrolysis may contain carbohydrase side activities. Such side activities in Neutrase (0.025% on protein basis) resulted in the release of 14% neutral sugars from the water-unextractable solids fraction obtained from toasted soybean meal.[59] The hydrolysis reaction is stopped by inactivating the enzyme using a heat treatment. After enzyme inactivation, the solution is coarse-filtered to remove large and insoluble impurities, mainly polysaccharides, nonhydrolyzed proteins, and aggregated peptides. Coarse filtration is usually performed using filter aids, which are available in a wide range of particle sizes (median particle size from 1.5 to 26 μm).[60] The coarse filtration can be substantially hindered by polysaccharides and protein−lysophospholipid complexes.[61] After coarse filtration, the hydrolysate solution is ultrafiltered, typically using a 10 kDa membrane, to remove large molecular weight compounds, such as partially hydrolyzed peptides and endotoxins.[58] Endotoxins are complex lipopolysaccharides, which

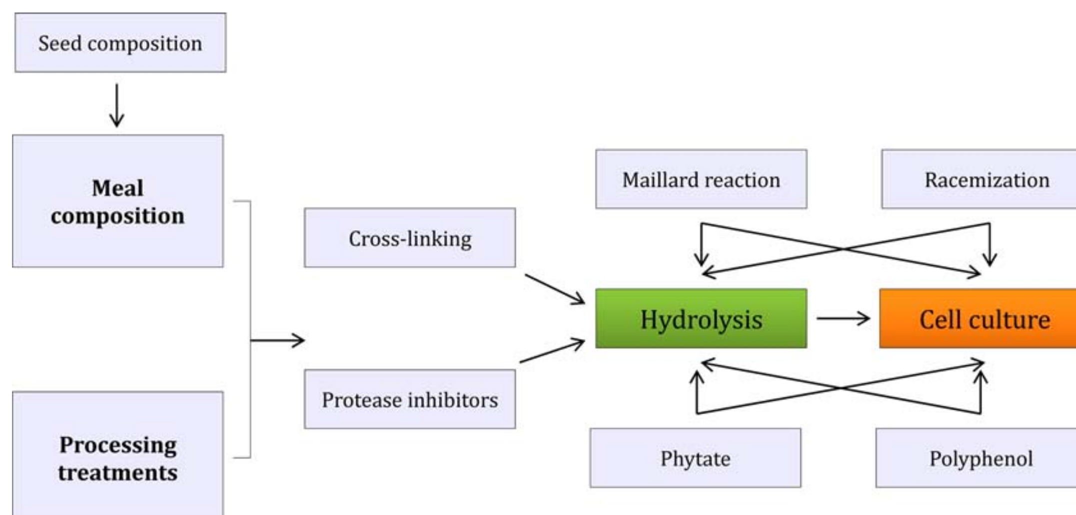

Figure 1. Schematic overview of chemical reactions and minor compounds that affect protein hydrolysis and functionality. The minor compounds are affected by meal composition and processing treatments.

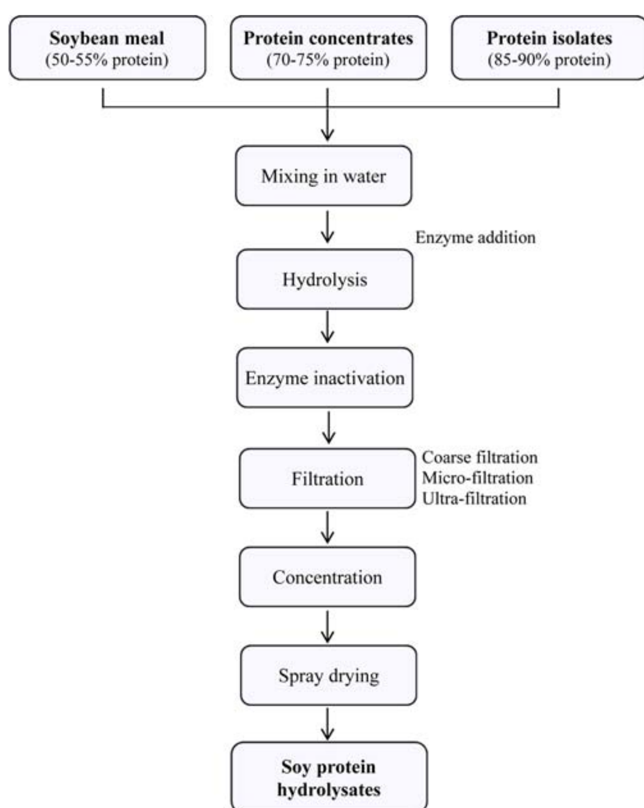

Figure 2. Schematic diagram for production of soy protein hydrolysates from soybean meal, protein concentrates, or isolates (based on Paspuleti et al.).[58]

are a major component of the cell wall membrane of most Gram-negative bacteria. The presence of endotoxin in hydrolysate adversely affects its functionality. In B-9 cells, the presence of 20 ng/mL endotoxins resulted in 30% reduced production of a recombinant human protein called Mullerian inhibiting substance.[62] After ultrafiltration, the hydrolysate is composed of only water-soluble compounds (including the minor compounds described in previous sections). Finally, the hydrolysate solution is concentrated and spray-dried. Conventionally, commercial soy protein hydrolysates are characterized for molecular weight distribution of peptides. Other analyses may include determination of protein, ash, moisture, and amino acids.[63]

Compounds Affecting Enzymatic Hydrolysis of the Soybean Meal. The enzymatic hydrolysis of the soybean meal is affected by the minor compounds following three mechanisms: first, due to processing treatments, the substrate protein may be modified (e.g., MRP and cross-linked proteins). Second, compounds present in the soybean meal (e.g., protease inhibitors) may inhibit the enzyme used for hydrolysis. Third, some compounds (e.g., polyphenols and phytates) can both modify the substrate and inhibit the enzyme (Figure 1). Because systematic data on soy protein hydrolysis in relation to these mechanisms are not available, references that describe effects on other types of proteins are included (Table 2). In addition, data on in vitro protein digestibility are not available and, therefore, results from in vivo animal nutrition studies are used to illustrate the effects of these mechanisms on protein digestibility. These effects are then used as an indication to state that similar effects can be expected in in vitro protein hydrolysis during hydrolysate production.

Specific Substrate Modification. *Racemization.* In several studies, racemization, that is, conversion of L-form to D-form, has been shown to occur in amino acids and peptides. In these studies, combinations of extreme experimental conditions (pH 2−12; temperature = 25−130 °C; heating time = 1−24 h) have been used to induce racemization.[64,65] However, these processing conditions never occur during hydrolysate production. The extent of racemization has not been investigated under representative conditions (neutral pH; temperature = 50−100 °C; heating time = 0.5−1 h). This is important, because the occurrence of even a very small extent of racemization in proteins and/or peptides greatly affects hydrolysis.[66] In vitro hydrolysis of synthetic racemized and nonracemized tripeptides (Ala-L-Glu-Ala, Ala-D-Glu-Ala, Ala-L-Asp-Ala, Ala-D-Asp-Ala, Val-L-Asp-Val, Val-D-Asp-Val, Ala-L-Phe-Leu, Ala-D-Phe-Leu, Ala-Met-Ala·HCl, Ala-D-Met-Ala·HCl, Val-Met-Phe·HCl, and Val-D-Met-Phe·HCl) using intestinal peptidases was investigated. Whereas tripeptides containing L-amino acid residues were completely hydrolyzed, the tripeptides containing D-amino acid residues were not hydrolyzed at all.[67,68] In another study, peptic hydrolysis of benzyloxycarbonyl-L-Ala-L-Phe-L-Tyr, benzyloxycarbonyl-L-Ala-L-Phe-L-Leu-L-Ala, benzyloxycarbonyl-L-Ala-L-Gly-L-Phe-L-Tyr and

Table 2. Influence of Chemical Reactions and Minor Compounds on the Enzymatic Hydrolysis on Protein Substrates and Enzymes

reaction	hydrolysis conditions	substrate	hydrolysis (%)[a]	reference
Maillard reaction	xylose added (1 and 3% soybean meal weight); heat treated (120, 160 °C) for 30 or 60 min; in situ hydrolysis; 48 h	soybean meal	−8 to −46	83
protein−polyphenol	BSA−quercetin (1:1 to 20:1 w/w); trypsin (E/S = 1:80); 35 °C;4 h	bovine serum albumin	−16 to −45	95
protein−phytate	pepsin (E/S 1:250); pH 2.0−4.5; phytate 2 mg/mL; 37 °C; 30 min	soybean 11S	−43	91
racemization	immobilized crude porcine mucosa intestinal peptidases; 37 °C; 24 h	synthetic peptides	−100	68
	swine pepsin; pH 1.5; enzyme = 0.02−0.5 mg/mL; 20 h; 40 °C	synthetic peptides	−93 to −99	69
cross-linking	wheat protein; 24 h; pH 11.5; 65 °C; in vivo hydrolysis	wheat protein	−17	73
saponins	1 mg/mL soy saponin; 1 mg/mL substrate; 0.1% α-chymotrypsin; 38 °C; pH 7.6; 3 h	glycinin	−57	94
		β-conglycinin	−27	94
protease inhibitors	crude soybean trypsin inhibitor type II S, 0−9.7 trypsin inhibitor activity (mg bovine trypsin inhibited per g feed); in vivo hydrolysis	salmon	−38 to −83	78
	crude soybean trypsin inhibitor type II S, 0−1.48% trypsin inhibitor; in vivo hydrolysis	trout	−70 to −93	80

[a]Hydrolysis (%) = ((chemically modified substrate protein hydrolyzed (in the presence of minor compounds))/(unmodified substrate protein hydrolyzed (in the absence of minor compounds))) × 100.

their corresponding D-isomers was studied. Whereas the rates of hydrolysis were 22, 95, and 584 nmol/min/mg pepsin for L-isomers, they were 1, 7, and 4 nmol/min/mg pepsin for D-isomers, respectively.[69]

Lysinoalanine (LAL) Formation. The processing treatments used during oil extraction from soybeans and production of the meal and hydrolysates can lead to formation of compounds such as LAL, lanthionine, dehydroalanine, and β-aminoalanine. The cross-linking reaction and formation of these compounds are well described in the literature.[70,71] Soy proteins (defatted soy flour precipitated at pH 4.5, washed with water and freeze-dried) were heat treated at 100−120 °C and pH 6.5 for 1−3 h. The LAL content in these heat-processed soy proteins was <40−130 μg/g protein.[72] In commercial soy protein isolates, the LAL content has been reported to vary from 0 to 370 μg/g protein.[72] Likewise, wheat protein processing (65 °C, 24 h, pH 11.5) resulted in conversion of 15% lysine to LAL, decreasing the protein digestibility from 93% (unprocessed) to 76% (processed) in miniature pigs.[73]

Specific Enzyme Inhibition. *Protease Inhibitors.* Protease inhibitors found in soybeans are Bowman−Birk and Kunitz inhibitors. The molecular size and structure of these inhibitors are well described by Rackis et al.[74] The specificity of these inhibitors is not only for trypsin and chymotrypsin but also for elastase and several other serine proteases.[38] Whereas the Kunitz inhibitor is heat-labile, Bowman−Birk is a heat-stable protease inhibitor.[75] In a starch matrix, the inactivation rate constants of the Kunitz and Bowman−Birk inhibitors were 12×10^4 and 6.9×10^4 s^{-1}, respectively.[76] Thus, a strong heat treatment, such as autoclaving (121 °C/10 min, 15 psi), is required to inactivate protease inhibitors.[75] This harsh processing initiates other reactions such as the Maillard reaction and racemization in the soybean meal. Generally, commercial defatted soybean meals retain some protease inhibitory activity (0−12.1 mg/g protein, Table 3). A wide variation in the protease inhibitory activity of soybean meals is reported in the literature. For instance, in three samples of heated soy flours, trypsin inhibitory activity (TIA) was 22, 11, and 4 mg trypsin inhibited/g sample.[77] The supplementation of Atlantic salmon (*Salmo salar*) diets with crude soybean trypsin inhibitor extract ranging from 0 to 9.7 TIA resulted in reduced protein digestibility from 83 to 38%.[78] The protein digestibility was calculated from the difference between total nitrogen of the fish diet and feces measured using the micro-Kjeldahl method.[79] In a similar study with rainbow trout, the in vivo protein digestibility of fish meal containing 0−1.5% soybean protease inhibitors ranged from 93 to 70%.[80] As protease inhibitors affect in vivo protein digestibility, it is expected that in vitro protein hydrolysis during hydrolysate production is also affected. This will affect the peptide composition of the hydrolysate and consequently affect the cell culture functionality. A direct effect of protease inhibitors on the cell culture functionality is not expected because they are removed during the ultrafiltration step in hydrolysate production.

Table 3. Prominent Compositional Differences between the Underprocessed, Adequately Processed, and Overprocessed Soybean Meals

	underprocessed	adequately processed	overprocessed
trypsin inhibitors,[105] trypsin inhibited/protein (mg/g)	12.1	1.77	0
protein solubility (%)[123]	>85	74−85	<74
lysine content (% dry matter)[124]	3.5	3.0	2.7
weight gain[105] (g/chick)	605	643	596
Hunterlab (+*a*) color values[104]	2.9	3.2	10.1
g LAL/100 g protein[125]	nd[a]	nd[a]	0.2
protein content (%)[104]	53	52.9	52.5
moisture content (%)[104]	11.2	10.9	7.5
ash content (%)[104]	6.2	6.3	6.1

[a]nd, not determined.

Free Fatty Acids. Free fatty acids such as oleic, linoleic, and linolenic acid in soybean meal can act as protease inhibitors. In the presence of oleic, linoleic, and linolenic acid (0.6 μmol/4 mL of assay solution), the chymotrypsin activity decreased by 30, 40, and 60%, respectively.[81] Whereas oleic and linoleic acid totally inhibited trypsin, linolenic acid reduced trypsin activity by 50%.[81]

Table 4. Composition of Soybeans, Meal, Protein Concentrates, and Isolates

	unit	soybeans	meal	concentrates	isolates
protein	%	31–48[126,127]	44–61[126,128]	62–69[129]	85–96[130]
oil	%	12–24[126,131]	0.5–9.0[126,128]	0.5–1.0[129]	0.5–1.0[129]
carbohydrates	%	33–44[132]	32–38[128]	17–25[128]	0.3–0.6[128]
ash	%	4–5[128]	6–7[128]	3.8–6.2[129]	2.5–6.3[130]
isoflavones	%	0.1–0.4[133]	0.2–0.3[53,134]	0.02–0.3[134]	0.1–0.3[27,134]
phytates	%	1.4–2.3[135]	1.3–4.1[128]	1.3–2.2[133]	1.0–1.7[133]
saponins	%	0.2–0.3[136]	0.7[137]	0.4[100]	0.8[133,137]
trypsin inhibitors	% of protein	3.5–12.2[133]	1.2–1.5[105,133]	0.8–1.1[133]	0.1–2.9[133]
moisture	%	7.7–10.1[138]	3.5–11.4[126]	4–6[129]	4–6[129]

Substrate Modification and Enzyme Inhibition. *Maillard Reaction.* The background of the Maillard reaction, factors affecting its rate of occurrence, and methods of analysis are well described in the literature.[82] The Maillard reaction in the soybean meal results in lysine modification, which is the site of action for enzymes such as trypsin. This hinders the enzymatic hydrolysis of the soybean meal. The effect on hydrolysis was studied using heat-treated soybean meal (120 and 150 °C; 30 and 60 min) in the presence of xylose (1 and 3%, soybean meal weight basis). The heat-treated samples and a nonheated control were enzymatically hydrolyzed in the rumen of a ram for 48 h. The protein degradability for heat-treated soybean meal in the presence of xylose was 47–85%, whereas it was 93% for untreated soybean meal.[83] Moreover, due to the Maillard reaction, protein polymerization can occur. Kato et al. studied protein polymerization in ovalbumin stored on 1:1 w/w ratio with maltose, cellobiose, isomaltose, lactose, and melibiose for 0–20 days at 50 °C and 65% relative humidity. After 7 days, the polymers formed in ovalbumin–disaccharide mixtures ranged from 8 to 23%.[84] After 15 days of storage, the proportion of polymers formed increased to 13–44%.[84] These cross-linked protein polymers have reduced susceptibility toward enzymatic hydrolysis.[85−87] Because the composition (protein/carbohydrates) used in this study is similar to that of defatted soybean meal (Table 4), it is expected that similar reactions could occur, even though the data for cross-linking due to the Maillard reaction in soybean meal have not been reported. In addition to substrate proteins, MRP affect enzyme activity. Carboxypeptidase A and aminopeptidase N were strongly inhibited by 0.5 and 0.25 mg/mL, respectively, of a low molecular weight fraction of MRP.[85,88] However, the specific MRP present in the low molecular weight fraction were neither identified nor quantified. Therefore, a quantitative relationship between the inhibition of hydrolysis and MRP could not be obtained.

Protein–Phytate Interactions. During processing of the soybean meal, complexes can be formed between proteins and phytate.[89] These complexes are insoluble and, as a result, they are hydrolyzed at a slower rate than the noncomplexed proteins.[90] In the presence of 1 mg/mL phytate, a 43% reduction in the peptic hydrolysis of soybean 11S proteins was observed.[91] Furthermore, phytate may directly inhibit enzyme. In soy flour, an increase in the phytate content by 2.1% resulted in decreased carboxypeptidase A activity by 14%.[92] In the presence of 10–90 mM phytate, 2.7–19.6% of trypsin activity was inhibited.[93]

Saponins. Soy saponins suppress protein hydrolysis by forming enzyme–saponin and/or protein–saponin complexes. Addition of 1 mg/mL soy saponins to 1 mg/mL oglycinin and 1 mg/mL β-conglycinin reduced the protein hydrolysis by approximately 57 and 27%, respectively.[94] However, such high concentrations of saponins do not occur in soybean meal, soy protein concentrates, or soy protein isolates (Table 4).

Protein–Polyphenol Complex. Isoflavones are the major polyphenols present in defatted soybean meal (0.2–0.3 g/100 g, Table 4). These isoflavones form complexes with proteins during harvesting, storage, and seed/meal processing. This complex formation reduces the susceptibility of proteins to enzymatic hydrolysis. The covalent binding of bovine serum albumin (BSA)–quercetin derivatives in 20:1, 10:1, 7:1, 5:1, and 2:1 (w/w) ratios lowered the hydrolysis by 16, 26, 30, 37, and 45%, respectively, as compared to the noncomplexed BSA (100%).[95] In addition to food proteins, complex formation between enzymes and polyphenols has been reported. More than 40% enzymatic activity was lost when chymotrypsin was complexed with chlorogenic acid.[96] The mechanism of protein–polyphenol complex formation and its influence on the protein hydrolysis are well described by Kroll et al.[97]

The concentration of the above-mentioned minor compounds in hydrolysates is determined not only by the hydrolysate production itself but also by the meal-processing conditions. The variation in the seed composition influences the concentration of these minor compounds as well.

SOYBEAN MEAL PRODUCTION

The harvested mature whole soybeans are cracked, dehulled, steam-heated, and pressed to obtain full-fat flakes. Subsequently, oil is extracted from the flakes to obtain defatted soybean meal (Figure 3). Oil is extracted from the flakes using processes such as extruder-expeller, continuous screw press, and solvent extraction. After oil extraction, solvent is removed from the residual meal by direct heating or steam. Subsequently, the defatted meal is toasted to inactivate protease inhibitors. A conventional and economical method of toasting is to use direct steam injection at 120 °C for 30 min under atmospheric or pressurized conditions.[98] After toasting, the meal is ground to the desired particle size. The defatted soybean meal contains 44–61% protein, 32–38% carbohydrates, 6–7% ash, and 0.5–9% lipids (Table 4). The composition of the soybean meal is primarily affected by two factors, those being processing treatments and seed composition. The influence of these two factors on the meal composition is discussed further.

Influence of Processing Treatments. Processing treatments such as desolventization and toasting cause *qualitative changes* (e.g., protein denaturation and aggregation) in the soybean meal. As a result of these processes, the nitrogen solubility index (% NSI, eq 1) of the soybean meal decreases significantly.

$$\text{nitrogen solubility index (\% NSI)} = \frac{\text{water-soluble N}}{\text{total N}} \times 100 \quad (1)$$

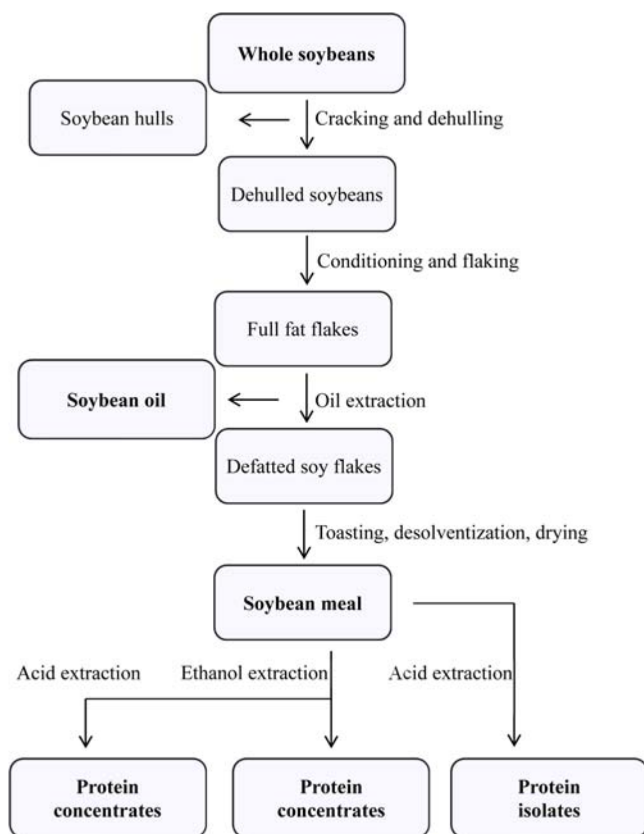

Figure 3. Schematic diagram for processing of soybeans to defatted soybean meal, soy protein concentrates, and isolates (based on Alden et al.).[118]

Whereas in one study a 70% decrease in NSI was reported,[99] in another study an 18% decrease was reported.[100] This shows that the NSI of defatted soybean meal strongly depends on the way it is processed. A direct link between NSI and the hydrolysate composition has not been made, but the variation in NSI is an indication of different (effects of) heat treatments in the production of the defatted soybean meal. In addition to differences in solubility, also the protease inhibition and occurrence of the Maillard reaction will result from such heat treatments. For instance, during toasting, under the warm and humid conditions, several amino acids such as lysine, glycine, arginine, cysteine, and methionine, react with reducing sugars, each with their own reaction rate, and undergo the Maillard reaction.[101,102] In soybean meals after autoclaving for 4 h at 15 psig pressure, 41% lysine, 35% arginine, 17% histidine, and 16% tryptophan underwent the Maillard reaction.[103] In many studies, the "redness" of samples has been used as an indicator to determine the extent of the Maillard reaction. For low-Maillard-reacted to high-Maillard-reacted soybean meals, the Hunterlab values ranged from 2.9 to 10.1 +*a* (redness).[104] Another approach to measure MRP is by binding free amino acid groups to binding agents such as ortho-phthalaldehyde, 2,4,6-trinitrobenzenesulfonic acid, and fluorodinitrobenzene.[82] The MRP formed in later stages, such as furosine and hydroxymethylfurfural, can be determined using chromatographic methods.

Other reactions that simultaneously occur with the Maillard reaction include racemization and cross-linking, resulting in the formation of cross-linked and racemized peptides. As described previously, these compounds affect the functionality and influence the enzymatic hydrolysis of soybean meal.

Depending on the processing conditions, soybean meal varies in quality, that is, underprocessed, adequately processed, and overprocessed (Table 3). Conventionally, different qualities of meals are characterized on the basis of urease units, trypsin inhibitory activity, and protein solubility index. Whereas underprocessed meal scores high on these parameters, overprocessed meal scores very low (Table 3). The overprocessed meals have high concentrations of minor compounds (e.g., MRP and LAL) formed due to overprocessing, whereas these compounds are either absent or present in trace amounts in underprocessed and adequately processed meals. Despite these differences, the gross compositions of different meal qualities are similar. The protein contents of underprocessed, adequately processed, and overprocessed meals were 53, 52.9, and 52.5%, respectively (Table 3). Despite their "compositional equivalence", significant differences in their in vivo protein digestibility are reported, which is attributed to the presence/absence of minor compounds. In animal trials, the nutritional values of underprocessed, adequately processed, and overprocessed meal, as evaluated by the weight gain of animals upon meal consumption for a test period, were 605, 643, and 596 g/chick, respectively.[105]

Influence of Seed Composition. Soybeans are naturally variable from crop to crop because of genetic and environmental factors (e.g., temperature and rainfall). This natural variation of soybeans is carried over to the meals produced from them, thereby resulting in meals of different qualities from batch to batch. The influence of compositional variability of soybeans on the variability of meal composition has been demonstrated in several studies. For instance, soybeans were collected from Argentina, Brazil, the United States, India (high quality and low quality), and China, and soybean meals were prepared from them using a common process. For six types of soybeans and soybean meals, the coefficient of variation (% CV, eq 2) in protein, total essential amino acids, total nonessential amino acids, and fat content was calculated.

$$\text{coefficient of variation (\% CV)} = \frac{\text{standard deviation}}{\text{mean}} \times 100 \qquad (2)$$

The CVs for protein, total essential amino acids, and total nonessential amino acids of the soybeans (10.4, 9.9, and 10.9%) were comparable to those of the respective soybean meals (7.5, 5.7, and 7.6%).[106] On the other hand, the CVs in fat content of the soybeans and soybean meals were 6.5 and 20.1%, respectively.[106] Likewise, in another study, the lysine/sucrose ratio ranged from 0.4 to 0.8 for 10 varieties of soybeans and from 0.5 to 0.9 for the soybean meals prepared from them.[107] This shows that the compositional differences in the soybeans are reflected in the composition of defatted soybean meals produced from them. Furthermore, when processing treatments are well-controlled, the seed processing does not induce changes in the gross composition (i.e., protein content). However, as described in the previous sections, processing treatments do induce changes that are not reflected in the conventional gross compositional analysis. For instance, variation in processing conditions, such as temperature and pH, significantly influences the rate of Maillard reaction, whereas the gross composition may be unaffected.[108]

In the next section, factors that influence the seed composition are described. Additionally, the resultant changes in the seed composition due to these factors are discussed.

■ COMPOSITIONAL VARIABILITY OF SOYBEANS

The general composition of the soybeans is 31−48% protein, 33−44% carbohydrates, 12−24% lipids, and 4−5% ash (Table 4). In addition, other compounds, such as phytates, isoflavones, and saponins, together contribute <1% of the dry matter of the seed. These compounds are not typically included in standard analysis. However, they could be important, because, for instance, it has been shown that saponin fractions protected CHO cells (AS52) against DNA damage and cytotoxicity.[109]

Genotype and Environment. The seed composition is affected not only by genotype but also by environmental factors such as temperature, rainfall, and geography. In the literature, there are a limited number of studies available that describe the influence of a specific factor on the seed composition. To show differences between studies, %CV per study was calculated. Additionally, an overall effect was indicated by calculating overall %CV, where results from all of the studies were combined together (Table 5). The compositional variation between different soybean varieties was higher due to genotype than due to environmental factors. The genotypic differences between soybean varieties resulted in CV of 4−21% for proteins and 4−11% for lipids. Due to environmental factors, the CVs for these compounds were 3−13 and 3−11%, respectively. Smaller variation was observed for the carbohydrate content due to genotype (5−8%) and environment (2%), whereas large variations were observed for saponin (10−35%) and isoflavone (4−113%) content due to all of the factors. This is also apparent when variation in compound concentration (overall CV) is considered including all of the factors. Saponins and isoflavones showed an overall CV of 33 and 84%, respectively, whereas proteins, lipids, and carbohydrates had CVs of 8, 9, and 10%, respectively. This suggests that due to genotype and environment, saponins and isoflavones are affected to a greater extent than proteins, lipids, and carbohydrates.

Postharvest Storage. In addition to genotypic and environmental factors that lead to gross compositional variation (e.g., protein content), postharvest storage may lead to qualitative changes (e.g., lipolysis) in the seed composition.

Lipids. Nakayama et al. studied changes in the lipid composition of soybeans stored at 35 °C for 6 months. At the end of storage, 45% of total phospholipids were enzymatically hydrolyzed to phosphatidic acid and lysophosphatidylcholine.[110] Moreover, an increase in acid value of lipids from 0.13 to 0.90 mL (0.1 N KOH to titrate 1 g of oil extracted from soybeans) was reported when soybeans were stored at 30 °C and 80% relative humidity for 10 months.[111] The lipolysis also resulted in an increase in the hydroperoxide content. These hydroperoxides can react with seed proteins and result in protein aggregation and formation of protein−lipid and protein−protein cross-links.[112,113] The adverse effects of these cross-links on the peptide composition and hydrolysate functionality are described in earlier sections.

Proteins. Increase in the acid value of seed lipids during storage affects protein solubility. In soybeans stored at 25 °C and 85% relative humidity (S1) and at 35 °C and 85% relative humidity (S2) for 6 months, 20% (S1) and 45% (S2) reductions in NSI were observed.[114] This decrease in NSI is mainly due to precipitation of 11S proteins (isoelectric pH 6.4).[115] Additionally, protein−lipid and protein−protein interactions occur due to lipolysis in soybeans stored at higher temperature and relative humidity.[116]

Table 5. Compositional Variability of Soybeans Due to Genotype and Environmental Factors[a]

	moisture	protein	lipids	saponins	isoflavones	carbohydrates
			Genotype			
species	*n* = 12[138] 7.7−10.1 (15.2)[b]	*n* = 4[127] 31.3−48.3 (20.7)	*n* = 24[138] 20.9−25.1 (4.4)	*n* = 4[136,137,140] 0.2−0.5 (35.3)	*n* = 15[54] 0.3−0.9 (32.3)	*n* = 9[55] 33.4−43.9 (8.4)
		n = 24[138] 36.2−41.4 (3.8)	*n* = 6[139] 19.8−26.7 (10.9)		*n* = 6[50] 0.1−0.2 (12.1)	*n* = 8[132] 31.1−35.5 (4.7)
			Environmental Factors			
temperature	nd[c]	*n* = 4[141] 37.3−41.5 (4.1)	*n* = 4[141] 23.5−25.4 (2.9)	nd	*n* = 6 (Lee)[55] 0.32−0.34 (3.6)	nd
					n = 7 (Fukuyataka)[55] 0.02−0.2 (112.5)	
rainfall	nd	*n* = 4 (JS335)[142] 37.1−39.7 (3.0)	*n* = 4 (JS335)[142] 18.5−20.1 (3.7)	nd	*n* = 7 (Kairyoshirome)[55] 0.06−0.2 (51.3)	nd
		n = 4 (KHSb2)[142] 32.2−41.6 (13.2)	*n* = 4 (KHSb2)[142] 16.5−21.2 (10.6)		*n* = 7 (Lee)[55] 0.02−0.1 (106.4)	
location	*n* = 3 (Clark)[143] 7.1−8.4 (8.2)	*n* = 4 (Reyna52)[139] 38.8−42.3 (4.0)	*n* = 4 (Rojas INTA)[139] 20.7−26.7 (11.3)	*n* = 5 (PI437654)[136] 0.4−0.6 (18.1)	*n* = 4 (Hardin)[53] 0.05−0.2 (52.1)	*n* = 9[128] 33.9−41.3 (1.9)
	n = 3 (Woodworth)[143] 7.4−8.5 (7.6)	*n* = 4 (Conesita INTA)[139] 32.9−42.7 (11.4)	*n* = 4 (Reyna52)[139] 20.7−22.2 (3.3)	*n* = 5 (Magellan)[136] 0.2−0.3 (10.1)	*n* = 4 (Corsoy79)[53] 0.08−0.2 (34.1)	*n* = 4[128] 29.3−34.8 (2.1)
overall CV	9.5	7.6	9.4	32.6	83.9	9.7

[a]All values are in percentages. [b]Values in parentheses are % coefficient of variation. [c]nd, not determined.

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Carbohydrates. During storage, a 30-fold increase in reducing sugar content was observed when soybeans were stored at 35 °C and 82% relative humidity for 6 months.[116] This increase in reducing sugars was, however, species-dependent. Whereas the reducing sugar content increased from 0.2 to 8% in MD 27-51 cultivar, it remained constant at <0.2% in SJ-4 and Palmetto cultivars over 9 months of storage.[117]

Although, due to genotype and environmental factors, there is variation in the gross composition, the functionality of soy protein hydrolysates is largely affected by variations in processing conditions. These conditions result in the formation of minor compounds, such as Maillard reaction products and cross-linked peptides. In pure systems, these compounds have a profound effect on the cell culture functionality. This suggests that the presence of these compounds in soy protein hydrolysates also affect cell growth and IgG production. Because these compounds are not included in the gross compositional analysis, a more detailed compositional analysis including minor compounds is needed to understand and control variability in the functionality of soy protein hydrolysates.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

IgG, immunoglobulins; CHO, Chinese hamster ovary; LAL, lysinoalanine; MRP, Maillard reaction products; TIA, trypsin inhibitory activity; BSA, bovine serum albumin; NSI, nitrogen solubility index; CV, coefficient of variation

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