**Contratto di ricerca tra DII-UNIPR e Natura Nuova s.r.l. sul tema:**
**"Semilavorati testurizzati per nuovi prodotti a base di soia e di frutta" (marzo 2011-2013)**
Responsabile scientifico Prof. Roberto Massini

RELAZIONE SULLE ATTIVITÀ DI RICERCA
EFFETTUATE NEL PERIODO: MARZO 2011 – DICEMBRE 2012

Parte A. Semilavorati Testurizzati per Nuovi Prodotti a Base di Soia

# Allegato A.3 – Trattamenti enzimatici applicati alla soia

sintesi della bibliografia richiamata

S. K. Goel, B. J. B. Wood (1978) Technical note: **Cellulase and exo-amylase in experimental soy sauce fermentations.** *International Journal of Food Science & Technology, 13(3):243–247* (Allegato A.3.1)

The present report deals with the participation of cellulase and exo-amylase in soy sauce fermentation. Production of both of these types of enzyme by members of the genus *Aspergillus* including *A. oryzae* has been long been known, but, so far as we have been able to ascertain, no evidence relating to the participation of cellulase in the soy sauce fermentation has been published. The disintegration of the initially intact beans during the Moromi fermentation suggested to us that considerable degradation of the cell walls must be taking place, and this would suggest cellulase involvement. In the case of exc-amylase, many writers on the soy sauce fermentation infer that this type of activity participates in the extracellular hydrolyses effected by the mould, and the levels of reducing sugar present in the latter stages of the Koji fermentation, can only be explained at all readily through the participation of exo-amylase.

A. Rosenthal, et al. (2001) **Combined effect of operational variables and enzyme activity on aqueous enzymatic extraction of oil and protein from soybean**. *Enzyme and Microbial Technology, 28:499–509* (Allegato A.3.2)

The individual effect of two different enzymes - protease and cellulase - on oil and protein extraction yields combined with other process parameters - enzyme concentration, time of hydrolysis, particle size and solid-to-liquid ratio - was evaluated by Response Surface Methodology. The selection of the enzymes for the study was based on preliminary experiments that showed higher increments in the extraction yield with the use of the two enzymes when compared to hemicellulase and pectinase. The use of protease resulted in significantly higher yields over the control (protein yield increased from 27.8 to 66.2%, oil yield increased from 41.8 to 58.7%) only when heat treated flour was used, or when non-heat treated flour with large particle sizes was used in the extraction. The yields of protein and oil from non-heat treated material in general decreased slightly with the use of enzymes.

N. Kasai, et al. (2003) **Extraction of Soybean Oil from Single Cells**. *J. Agric. Food Chem., 51:6217-6222* (Allegato A.3.3)

Single cells prepared from autoclaved soybeans and cellulase treatment of the cells were effective in digesting the cell walls of and extracting the oil from soybeans. The first cell wall of the soybean single cell was completely removed using cellulases; the thin and transparent second cell wall of the cell was swollen. Oil in the cell formed spherical or hemispherical oil drops, and oil leaking from the oil bodies was observed. The oil was almost retained within the second cell wall. Water-extractable substances were obtained at ≈ >60% of the weight. Flotation of oil drops by centrifugation was easily done. Ambient n-hexane extraction was also possible; however, residual oil remained in the oil bodies. Protease or peptidase digested the structure of the oil bodies; however, separation of the oil and the hydrolysates was impossible. The oil from the oil bodies was obtained effectively (>85%) by pressing the single cells and/or cellulase-treated single cells.

S. Jung, et al. (2006) **Functionality of soy protein produced by enzyme-assisted extraction**. *Journal of the American Oil Chemists' Society, 83(1):71-78* (Allegato A.3.4)

This study investigated the potential of enzymes to increase soy protein extractability without causing protein degradation. The aqueous extraction of protein was performed from defatted soy flakes on a laboratory-and pilot-plant scale. Yields of protein and reducing sugars were determined

in the alkali extracts obtained with cellulases and pectinase, added alone or as cocktails. Using 5% (wt/g of protein) Multifect pectinase resulted in the best improvement of protein yields, which were 50 and 17% greater than the controls in laboratory- and pilot-plant-scale trials, respectively. This enhanced protein extraction was accompanied by an increased reducing sugar content in the aqueous extract compared with the control. Under the conditions tested, no enzyme cocktail markedly increased the protein yield compared with the use of single enzymes. The solubility curve for Multifect pectinase-treated soy protein isolate (SPI) was typical of SPI at pH 2–10. Its foam stability significantly improved, but the emulsification properties declined. Multifect pectinase markedly reduced the viscosity of SPI. SDS-PAGE showed that the α' and α subunits of β-conglycinin were modified, and glycoprotein staining showed that these modifications were probably due to a protease secondary activity in the pectinase preparation. One cellulase and one pectinase were identified as effective in modifying the protein and reducing sugar extractablity from the defatted soy flakes.

B.P. Lamsal, et al. (2006) **Flaking and Extrusion as Mechanical Treatments for Enzyme-Assisted Aqueous Extraction of Oil from Soybeans.** *JAOCS, 83(11):973-979* (Allegato A.3.5)

Flaking and extruding dehulled soybeans were evaluated as a means of enhancing oil extraction efficiency during enzyme-assisted aqueous processing of soybeans. Cellulase, protease, and their combination were evaluated for effectiveness in achieving high oil extraction recovery from extruded flakes. Aqueous extraction of extruded full-fat soy flakes gave 68% recovery of the total available oil without using enzymes. A 0.5% wt/wt protease treatment after flaking and extruding dehulled soybeans increased oil extraction recovery to 88% of the total available oil. Flaking and extruding enhanced protease hydrolysis of proteins freeing more oil. Treating extruded flakes with cellulase, however, did not enhance oil extraction either alone or in combination with protease. Discrepancies in oil extraction recoveries were encountered when merely considering crude free fat because some oil became bound to denatured protein during extrusion and/or sample drying. Bound fat was unavailable for determination by using the hexane extraction method, but was accounted for by using the acid hydrolysis method for total oil determination. Oil extraction recovery from extruded soybean flakes was affected by oil determination methods, which was not the case for unextruded full-fat soy flour.

S. Jung , A. A. Mahfuz (2009) **Low temperature dry extrusion and high-pressure processing prior to enzyme-assisted aqueous extraction of full fat soybean flakes**. *Food Chemistry, 114:947–954* (Allegato A.3.6)

Oil, protein and solid extraction yields obtained during aqueous extraction processing (AEP) of full fat soybean flakes (FFSF), FFSF extruded at a die temperature of 100 °C and FFSF pressurised at 200 and 500 MPa for 15 min at 25 °C, were compared to those obtained during enzyme-assisted aqueous extraction processing (EAEP) using 0.5% of protease Protex 7L. Without enzyme addition, pretreatment of the FFSF with extrusion and 500 MPa increased and decreased, respectively, the oil extraction yield while protein extraction yield was significantly decreased after both treatments. The best treatment in terms of oil and protein recovery was EAEP of extruded flakes with 90% and 82% of oil and protein extraction yield, respectively, and 17% of free oil. Addition of protease during extraction significantly decreased the yield of isolated soy protein (ISP) due to an increased solubility of the proteins at pH 4.5. ISP from extruded EAEP had higher solubility at pH 7.0 and better functionality. The DSC results, combined with the protein extraction yields, showed that a proportion of the proteins became insoluble after extrusion and 500 MPa treatment, while only those extracted from 500 MPa FFSF had a reduced native state.

B. Karki (2011) **Comparison and optimization of enzymatic saccharification of soybean fibers recovered from aqueous extractions**. *Bioresource Technology, 102:1228–1233* (Allegato A.3.7)

Soybean insoluble fractions recovered from aqueous extraction processing (AEP) and enzyme-assisted AEP (EAEP) of full-fat soybean flakes (FFSF) and extruded FFSF were evaluated as a feedstock for the production of fermentable sugars using enzymes. Among the four insoluble fractions (AEP FFSF, EAEP FFSF, AEP extruded FFSF and EAEP extruded FFSF), the composition analysis revealed that the one recovered from EAEP of extruded FFSF had the highest glucan content, 16% [dry basis (db)], as compared to about 10% (db) for the other fractions. Thirty-three percent of the initial glucan of the insoluble recovered from AEP and EAEP of FFSF were converted into glucose using 33 FPU of Accellerase 1000/g-glucan. This saccharification yield was increased to 44% with extruded fibers. The higher saccharification yield of 49% was obtained at 45 °C, 1% glucan loading, and 101 FPU/g-glucan enzymes loading after 27 h of hydrolysis.

I. Suppavorasatit, et al. (2011) **Optimization of the Enzymatic Deamidation of Soy Protein by Protein-Glutaminase and Its Effect on the Functional Properties of the Protein**. *J. Agric. Food Chem. 59:11621–11628* (Allegato A.3.8)

The effects of enzymatic deamidation by protein-glutaminase (PG) on the functional properties of soy protein isolate (SPI) were studied. Conditions for the deamidation were evaluated by means of response surface methodology (RSM). Optimal conditions based on achieving a high degree of deamidation (DD) with a concurrently low degree of hydrolysis (DH) were 44 °C, enzyme:substrate ratio (E/S) of 40 U/g protein and pH 7.0. Under optimal conditions, both DD and DH increased over time. SDS-PAGE results indicated that lower molecular mass subunits were produced with increasing DD. Far-UV circular dichroism spectra revealed that the α-helix structure decreased with higher DD, while the β-sheet structure increased until 15 min of deamidation (32.9% DD), but then decreased at higher DD. The solubility of deamidated SPI was enhanced under both acidic and neutral conditions. SPI with higher DD showed better emulsifying properties and greater foaming capacity than SPI, while foaming stability was decreased. It is possible to modify and potentially improve the functional properties of SPI by enzymatic deamidation using PG.

WEI Lu-ning, et al. (2012) **Effect of Glycine Betaine and Complex Cellulase on the Fermentation of Soy Sauce.** *Food Science*, in Cinese (Allegato A.3.9)

The microorganisms and inherent or exogenous enzymes have very low activity because of high osmotic pressure during the traditional fermentation of high-salt liquid state soy sauce, which results in the low conversion rate of raw materials and the long term fermentation period. Glycine betaine is one of the compatible solutes synthesized by microorganisms under high osmotic pressure environment which protects the microorganisms and enzymes. Glycine betaine was used in different periods (0d or 30d) in the fermentation of high-salt liquid state soy sauce to protect microorganisms and external complex cellulase. The experimental results showed that adding 0.20% or 0.30% glycine betaine with 0.15% complex cellulose (105 EGu/100g sauce mash) simultaneously at 0d or 30d later of soy sauce fermentation could obviously improve protein conversion rate by 2.26% ~ 7.92%. Glycine betaine could improve the quality of soy sauce effectively, and shorten the fermentation period by 15ds. In contrast with the group that was added with glycine betaine and complex cellulase simultaneously at 0d, adding at 30d later could improve protein conversion rate by about 3.17%.

R.l Opazo, et al. (2012) **Reduction of Soybean Meal Non-Starch Polysaccharides and α-Galactosides by Solid-State Fermentation Using Cellulolytic Bacteria Obtained from Different Environments.** *PLOS ONE, 7(9):e44783* (Allegato A.3.10)

Soybean meal (SBM) is an important protein source in animal feed. However, the levels of SBM inclusion are restricted in some animal species by the presence of antinutritional factors (ANFs), including non-starch polysaccharides (NSPs) and agalactosides (GOSs). The aim of this study was to reduce the soybean meal NSPs and GOSs by solid-state fermentation (SSF) using a combination of cellulolytic bacteria isolated from different environments (termites, earthworms, corn silage and bovine ruminal content). To analyse the key enzymatic activities, the isolates were grown in minimal media containing NSPs extracted from SBM. The selected bacterial strains belonged to the genera Streptomyces, Cohnella and Cellulosimicrobium. SSF resulted in a reduction of nearly 24% in the total NSPs, 83% of stachyose and 69% of raffinose and an increase in the protein content. These results suggest that cellulolytic bacteria-based SSF processing facilitates SBM nutritional improvement. In addition, the use of fermented SBM in animal diets can be recommended.

A. Wakameda, et al. (1990) **Process for the production of transglutaminase-containing food having improved texture.** *US Patent No 4,917,904* (Allegato A.3.11)

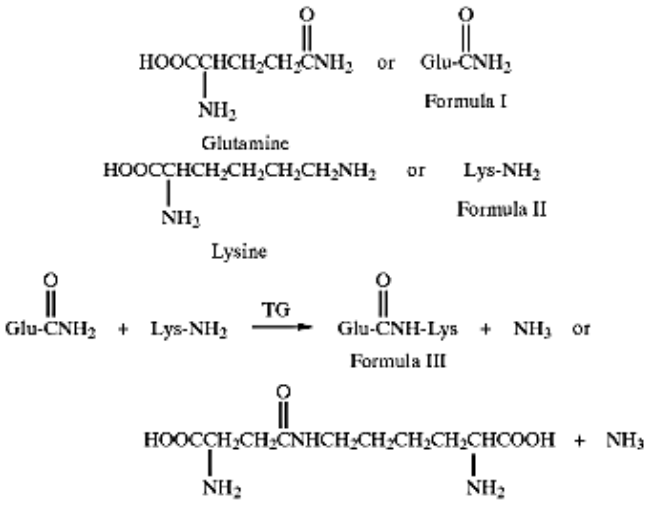
When transglutaminase is added to a food material containing soybean protein, the water retentivity of the food material is enhanced and the texture thereof becomes hard and smooth. When the amount of the transglutaminase is smaller than 0.001 part by weight, only a small amount of the transglutaminase would bind to the proteins in the food material, which results in only a limited effect. When it exceeds 5 parts by weight, on the other hand, the effect is achieved within a short period of time, which makes the processing of the food material difficult. In the present invention, a calcium salt may be added together with the transglutaminase to the food material, if required, to thereby further enhance the improving effect. Examples of the calcium salt include calcium chloride, calcium carbonate, calcium sulfate and calcium phosphate. It is preferable that the calcium salt is added in an amount of 0.001 to 2 parts by weight, still preferably 0.005 to 0.1 part by weight, to 100 parts by weight of the food material. When the amount of the calcium salt is smaller than 0.001 part by weight, only a limited effect is achieved. When it exceeds 2 parts by weight, on the other hand, it imparts an undesirable taste to the food.

M. Nonaka, et al. (1994) **Changes caused by microbial transglutaminase on physical properties of thermally induced soy protein gels.** *Food Hydrocolloids, 8(1):1–8*

Two types of soy protein product, soy protein isolate (SPI) and soy protein concentrate (SPC), were incubated with a $Ca^{2+}$-independent microbial transglutaminase to prepare thermally induced gels. Polymerization of soy protein molecules in the gels were shown on SDS-polyacrylamide gel electrophoresis. In the appropriate range of enzyme concentration several physical properties of the thermally induced gels were substantially enhanced. The impairment of physical properties due to the addition of NaCl as well as the reduction of solid content were well compensated by the enzyme treatment. Thus, the transglutaminase treatment was very useful for the improvement of heat induced gel of soy proteins.

Der Chyan Hwang (2005) **Transglutaminase soy fish and meat products and analog thereof**. *US Patent No 6,908,634* (Allegato A.3.12)

This invention is directed to a transglutaminase-cross-linkcd vegetable protein composition that has a controlled salt sensitivity and a process for preparing the composition. The composition has utility in fish and meat products or in a meat analog product. These products all contain salt and it thus becomes important lo have a transglutaminase cross-linked vegetable protein composition with an acceptable or controlled salt sensitivity. The transglutaminase cross-linking is the bringing together of the glutaminc and lysine residue in protein as per the following equation, wherein TG signifies transglutaminase:



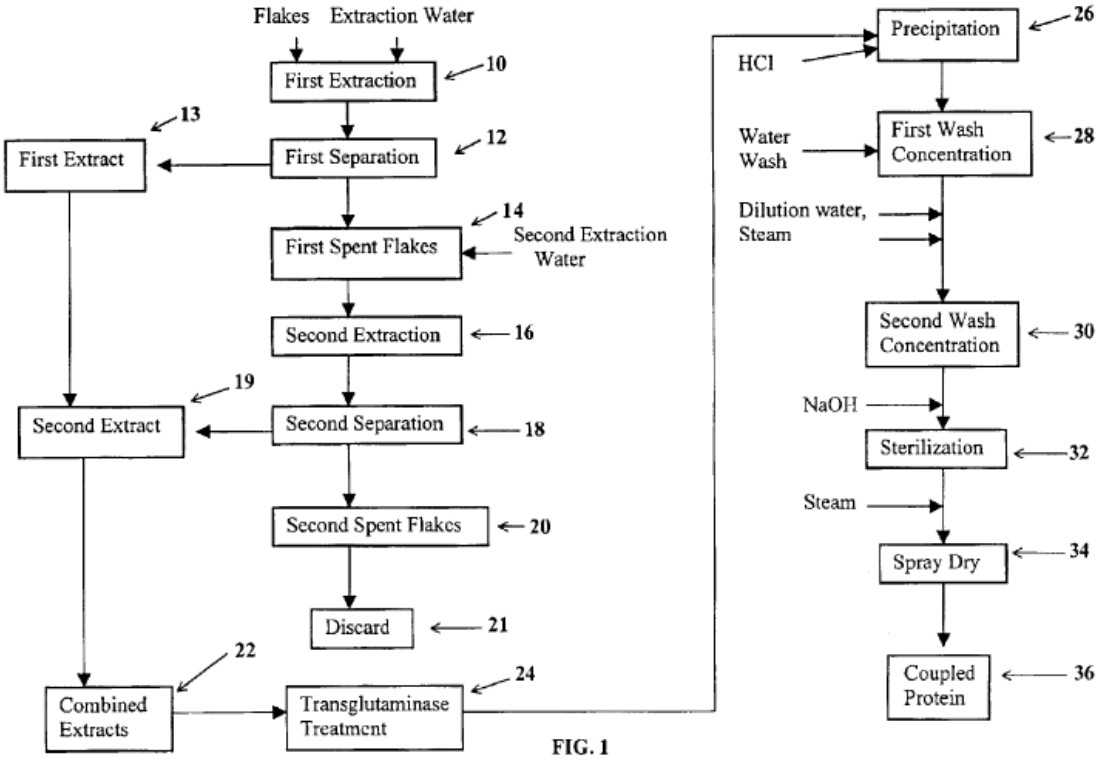




FIG. 1

Masahiko Nonaka, et al. (1996) **Retort-resistant tofu prepared by incubation with microbial transglutaminase.** *Food Hydrocolloids, 10(1):41–44*

Addition of $Ca^{2+}$-independent microbial transglutaminase with glucono-$\delta$-lactone before a coagulation step of packed tofu manufacturing process made the tofu retort-resistant. The enzyme treatment effectively suppressed retort-induced water-release and hardening of the tofu sample pieces packed with water in a retort-pouch. The occurrence of polymerized soybean proteins in the enzyme-treated tofu was found.

Akinori Mizuno, et al. (2000) **Effect of Transglutaminase Treatment on the Glass Transition of Soy Protein.** *J. Agric. Food Chem., 48(8):3286–3291* (Allegato A.3.13)

The effect of microbial transglutaminase (MTG) treatment on the glass transition temperature ($T_g$) of two fractions which were isolated from a soy protein sample was studied. The $T_g$ of each fraction measured by differential scanning calorimetry was lowered by the MTG treatment, which generated cross-links in the samples, and this result agreed with the result of dynamic mechanical analysis. From the $^1H$ NMR measurement, the line width of the $^1H$ signal of the MTG-treated sample was observed to be greater than that of the MTG-nontreated sample at similar water content, which implied that there was relatively more immobilized water in the MTG-treated sample. The MTG treatment seemed to cause the increment in immobilized water, which might affect the $T_g$ of the soy protein sample.

E. E. Babiker (2000) **Effect of transglutaminase treatment on the functional properties of native and chymotrypsin-digested soy protein.** *Food Chemistry, /0(2):139–145* (Allegato A.3.14)

Native and chymotrypsin-digested soy protein were polymerized by transglutaminase (TGase). SDS-PAGE pattern of the polymerized proteins showed that TGase treatment resulted in proteins of higher molecular mass above the stacking gel. The free amino groups of the polymerized proteins were greatly reduced. The solubility of the protein polymer was greatly improved at pH 2 and pH 8–12 whereas, at pH 4 and 6, it was least soluble. The solubility of the digest polymer was significantly improved at all pH levels, except pH 4. The protein polymer started to coagulate when the heating temperature exceeded 50°C. The digest polymer, on the other hand, resisted heat-induced aggregation up to 60°C; thereafter, its solubility declined slightly. The emulsifying and foaming properties of the digest polymer were greatly improved compared to the protein polymer. The protein polymer was observed to form a harder gel than the digest polymer.

J.C Ramírez-Suárez, Y.L Xiong (2003) **Effect of transglutaminase-induced cross-linking on gelation of myofibrillar/soy protein mixtures.** *Meat Science, 65(2):899–907* (Allegato A.3.15)

Microbial transglutaminase (MTGase)-catalyzed interaction and gelation of mixed myofibrillar (MPI)/soy (SPI) protein isolates were investigated at varying ionic strengths and MPI:SPI ratios, with or without SPI being preheated (80°C). MTGase treatments in deionized water converted myosin heavy chain and actin into lower molecular-weight polypeptides, which gradually diminished as the ionic strength increased up to 0.6 M NaCl. A reduced intensity in the electrophoretic bands of soy proteins (7S and 11S except the basic subunits) was observed in all treatments, suggesting cross-linking with MPI. The enzyme treatment slightly increased the thermal transition (denaturation) temperatures of MPI/SPI but greatly enhanced ($P<0.05$) the elasticity of the mixed protein gels when compared with untreated samples, independent of incubation time.

D.J. Walsh, et al. (2003) **Modification of the nitrogen solubility properties of soy protein isolate following proteolysis and transglutaminase cross-linking.** *Food Research International, 36(7):677–683* (Allegato A.3.16)

The effect of (a) limited hydrolysis [0.5–2.0% degree of hydrolysis (DH)] with Alcalase™, (b) cross-linking with transglutaminase (TGase) and (c) combinations of these modifications on the nitrogen solubility (pH 3–8) of soy protein isolate (SPI) was investigated. Between pH 3.0 and 5.0, SPI hydrolysates, hydrolysates of cross-linked SPI and the cross-linked products of SPI hydrolysates displayed significant ($P$<0.05) increases in solubility compared to unmodified SPI. Cross-linking pre- or post hydrolysis did not alter the overall trend of increased ($P$<0.05) solubility relative to the unmodified control at low pH. At 2% DH, cross-linking pre- or post-hydrolysis resulted in greater solubility ($P$<0.05) than that observed in hydrolysates per se at low pH. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) indicated that the 22 kDa 11S basic polypeptide was relatively resistant to Alcalase hydrolysis and that the 18 and 22 kDa 11S basic polypeptides were not susceptible to TGase cross-linking. The results demonstrate that a combination of enzymatic treatments and the order in which they are applied may have potential for creating novel food ingredients with improved functional properties, especially those properties that are dependant on high solubility at low pH.

K. Yokoyama, et al. (2004) **Properties and applications of microbial transglutaminase**. *Applied Microbiology and Biotechnology, 64(4):447-454* (Allegato A.3.17)

Some properties and applications of the transglutaminase (TGase) referred to as microbial TGase (MTGase), derived from a variant of *Streptomyces mobaraensis* (formerly classified as *Streptoverticillium mobaraense*), are described. MTGase cross-linked most food proteins, such as caseins, soybean globulins, gluten, actin, myosins, and egg proteins, as efficiently as mammalian TGases by forming an ε-(γ-glutamyl)lysine bond. However, unlike many other TGases, MTGase is calcium-independent and has a relatively low molecular weight. Both of these properties are of advantage in industrial applications; a number of studies have illustrated the potential of MTGase in food processing and other areas.

Chuan-He Tang, et al- (2006) **Effects of transglutaminase treatment on the thermal properties of soy protein isolates.** *Food Research International, 39(6):704–711* (Allegato A.3.18)

The effects of covalent cross-linking of microbial transglutaminase (MTGase) on the thermal properties of soy protein isolates (SPI), including the thermal denaturation and glass transition were investigated by conventional and modulated differential scanning calorimetry (DSC). The MTGase treatment significantly increased the thermal denaturation temperatures (including the on-set temperature of denaturation, $T_m$ and the peak temperature of denaturation, $T_d$) of glycinin and β-conglycinin components of SPI ($P \leq 0.05$), and the thermal pretreatment of SPI further increased the extent of this improvement. The MTGase treatment also improved the ability of SPI to resist the urea-induced denaturation. Modulated DSC analysis showed that there were two glass transition temperatures ($T_g$) in the reversible heat flow signals of native SPI (about 5% moisture content), approximately corresponding to 45 and 180°C, respectively. These $T_g$ values of SPI were significantly decreased by the MTGase treatment (at 37°C for more than 2 h) ($P \leq 0.05$). The improvement in the hydration ability of protein and the formation of high molecular biopolymers may account for the changes of thermal properties of soy proteins caused by the MTGase cross-linking.

Chuan-He Tang, et al- (2006) **Influence of transglutaminase-induced cross-linking on in vitro digestibility of soy protein isolate**. Journal of Food Biochemistry, 30:718–731 (Allegato A.3.19)

The influence of covalent cross-linking by microbial transglutaminase (MTGase) on the sequential in vitro pepsin and trypsin digestion process and the digestibility of soy protein isolate (SPI), was investigated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and nitrogen release analyses. Various subunits of □-conglycinin and acidic subunits of glycinin were cross-linked by MTGase to form high molecular weight (MW) biopolymers, while basic subunits of glycinin were unaffected. These results suggest that the cross-linking by means of transglutaminase may negatively affect the nutritional properties of food proteins.

Chuan-He Tang (2006) **Formation and properties of glycinin-rich and β-conglycinin-rich soy protein isolate gels induced by microbial transglutaminase.** *Food Research International, 39(1):87–97* (Allegato A.3.20 da scaricare)

The gelation and gel properties of glycinin-rich and β-conglycinin-rich soy protein isolates (SPIs) induced by microbial transglutaminase (MTGase) were investigated. At the same enzyme and protein substrate concentrations, the on-set of gelation of native SPI and the viscoelasticity development of correspondingly formed gels depended upon the relative ratio of glycinin to β-conglycinin. The turbidity analysis showed that the glycinin components also contributed to the increase in the turbidity of SPI solutions incubated with MTGase (at 37°C). Textural profile analysis indicated that the glycinin components of SPIs principally contributed to the hardness, fracturability, gumminess and chewiness values of corresponding gels, while the cohesiveness and springness were mainly associated with the β-conglycinin components. The strength of MTGase-induced gels of various kinds of SPIs could be significantly improved by the thermal treatment. The protein solubility analyses of MTGase-induced gels, indicated that besides the covalent cross-links, hydrophobic and H-bondings and disulfide bonds were involved in the formation and maintenance of the glycinin-rich SPI gels, while in β-conglycinin-rich SPI case, the hydrophobic and H-bondings were the principal forces responsible for the maintenance of the gel structure. The results suggested that various kinds of SPI gels with different properties could be induced by MTGase, through controlling the glycinin to β-conglycinin ratio.

C-H. Tag, et al. (2006) **Coagulation and gelation of soy protein isolates induced by microbial transglutaminase**. *Journal of Food Biochemistry, 30(1):35–55* (Allegato A.3.21)

The reaction process and corresponding mechanism of coagulation and gelation of native soy protein isolates (SPIs) induced by microbial transglutaminase (MTGase) were investigated. The protein constituents of SPIs, including a majority of subunits of □-conglycinin and acidic subunits of glycinin, could be polymerized by MTGase to form high weight molecular (WM) biopolymers. Both the coagulation and gelation reactions of native SPI solutions induced by MTGase were dependent upon the initial protein substrate concentration ($[C]_0$). In the coagulating reactions, the turbidity of SPI solutions continually increased with increasing $[C]_0$ in the range from 0.25 to 3.0%. As for the gelation reactions, with the concentration increasing from 3 to 8% (w/v), the onset time of gelation of native SPIs induced by 0.8 units/mL of MTGase at 37C shortened by ~5-fold, and the storage modulus (G') of finally formed gels (after 4 h) increased from ~1 to 1300 Pa. Both the coagulation and gelation reactions of SPI solutions were promoted remarkably by increasing the enzyme concentration.

Mark Dube, et al. (2007) **Texturisation and modification of vegetable proteins for food applications using microbial transglutaminase**. *European Food Research and Technology, 225(2):287-299* (Allegato A.3.22)

Microbial transglutaminase (MTG) isolated from *Streptomyces mobaraensis* has been available on a commercial scale for several years. MTG generates inter- and intramolecular cross-links between γ-carboxylamide groups of glutamine residues and ε-amino groups of lysine residues in proteins. Due to its great potential to improve various functional properties of proteins, MTG is mainly used to enhance texture, stability, and water binding. Application of MTG for the production of plant protein-based foodstuffs such as tofu, noodles, bread and bakery products, is still limited to raw materials from soybean and wheat. However, with the increasing demand for vegetarian foods, the utilisation of novel proteins as functional ingredients, e.g. from peas, lupins, sesame, and sunflower, seems promising. To open new horizons for MTG application, this review aims at demonstrating the actual potential of MTG in processing foodstuffs based on vegetable proteins. Particular focus was laid on novel plant protein sources suitable for cross-linking with MTG. Furthermore, strategies for improving texture and nutritive value of the proteins are discussed.

Chuan-He Tang (2007) **Effect of thermal pretreatment of raw soymilk on the gel strength and microstructure of tofu induced by microbial transglutaminase.** *LWT - Food Science and Technology, **40(8):**1403–1409* (Allegato A.3.23)

The influence of thermal pretreatment of raw soymilk on the gel hardness and microstructure of tofu, induced by microbial transglutaminase (MTGase), was investigated in this paper. Modulated differential scanning calorimetry analysis showed that individual proteins in soymilk were to a various extent denatured by different thermal pretreatments. The viscosity of the soymilk and the gel hardness of MTGase-induced tofu were more highly related with the heating rate (up to 90°C) than the mode of heating. At any enzyme concentration of MTGase, the tofus prepared from soymilk heated at 75°C for 10 or 30 min showed highest gel hardness among all tested ones ($P \leq 0.05$). Scanning electron microscopy analysis indicated that the microstructure of the tofu from soymilk heated at 75°C for 30 min had a unique coral-like structure, much more continuous and homogenous than that from soymilk at 95°C for 5 min. These results confirmed that the appropriate heat pretreatment (e.g. in the present, at 75°C for 10–30 min) remarkably improved the gel strength of tofu by means of MTGase, and strengthened the tofu gel structure.

S. Bin Md Yasir, et al. (2007) **The impact of transglutaminase on soy proteins and tofu texture.** *Food Chemistry, 104(4):1491–1501* (Allegato A.3.24)

The enzyme transglutaminase was investigated for its cross-linking effect on the soy proteins of tofu. In vitro incubations confirmed that **soy proteins are excellent substrates for transglutaminase, especially when denatured**. The macroscopic effects resulting from the addition of transglutaminase were compared to changes at the microstructural and molecular level. Treatment produced a firmer tofu, with a significantly increased fracture force. Examination by SEM showed a change in the matrix structure, with transglutaminase resulting in a finer-stranded, uniform network that accounted for the increase in fracture force. At the molecular level, little, if any, cross-linking occurred within the tofu matrix in situ. This suggests that the change in functional properties afforded by addition of transglutaminase to tofu is due to a side reaction of the enzyme, for example hydrolysis of glutamine residues, rather than its cross-linking activity. These ideas are further explored in the accompanying paper.

Chuan-He Tang, et al. (2007) **Formation and rheological properties of 'cold-set' tofu induced by microbial transglutaminase.** *LWT - Food Science and Technology, **40(4):**579–586* (Allegato A.3.25)

Microbial transglutaminase (MTGase) has been shown to effectively induce soymilk with a certain level of solid content to form filled tofu. The gel formation of this kind of tofu and the influence of reaction parameters on the properties of formed tofu were investigated by dynamic oscillatory and/or large-strain rheological measurements. The gelation process and the development of the mechanical moduli (especially the storage modulus, *G'*) of this kind of tofu were highly dependent upon the incubation temperature. Textural property analysis (TPA) results showed that many TPA parameters of this kind of tofu, including gel hardness, gumminess, springiness and cohesiveness, were also affected by the applied enzyme amount, the pH of soymilk and the presence of NaCl, especially for gel hardness. In addition, the additional heating and cooling treatment could significantly improve the gel strength of tofu, induced by MTGase at lower temperatures (e.g. 25 and 37°C). These results suggested that a new kind of tofu with good quality could be produced using the enzymatic cross-linking technique, by the combination with the thermal treatment.

Chee-Yuen Gan, et al. (2008) **Physicochemical properties and microstructures of soy protein isolate gels produced using combined cross-linking treatments of microbial transglutaminase and Maillard cross-linking.** *Food Research International*, *41(6):600-605* (Allegato A.3.26)

The relationship between macroscopic (elasticity, swelling), microstructural and molecular properties was investigated in soy protein hydrogels formed by cross-linking to different degrees with a model Maillard type cross-linking agent (glutaraldehyde) in the absence or presence of 120 mM NaCl. Hydrogel macroscopic properties were not influenced by protein secondary structure. In contrast, rheological properties were strongly influenced by degree of cross-linking and microstructure. Cross-linking soy protein in the absence of salt led to the formation of a more porous network consisting of bigger flocs and/or bigger particles and to weaker gels. Gel swelling did not appear to depend on microstructural properties. The influence of cross-linking and salt on swelling would be connected to their respective impacts on elastic and ionic contributions to swelling.

Chee-Yuen Gan, et al. (2009) **Gelling of microbial transglutaminase cross-linked soy protein in the presence of ribose and sucrose**. *Food Research International, 42(10):1373–1380* (Allegato A.3.27)

Soy protein isolate (SPI) was incubated with microbial transglutaminase (MTGase) enzyme for 5 (SPI/MTG(5)) or 24 (SPI/MTG(24)) h at 40 °C and the cross-linked SPI obtained was freeze-dried, and heated with 2% (w/v) ribose (R) for 2 h at 95°C to produce combined-treated gels. Longer incubation period resulted in more compact and less swollen SPI particle shape when reconstituted with sugar solution. Thus, this MTGase treatment affected samples in terms of flow behaviour and gelling capacity. Rheological study showed different gelling profiles with the cross-linking treatments and combined cross-linked SPI gave a higher G' value compared to single treated samples. These are due to the formation of additional ε-(χ-glutamyl)lysine bonds and ''Maillard cross-links" within the SPI protein network during the MTGase incubation and heating in the presence of ribose (i.e. reducing sugar). Network/non-network protein analysis found that network protein increased with cross-linking treatment, which also resulted in different SDS–PAGE profiles.

Shu-Juan Jiang, Xin-Huai Zhao (2010) **Transglutaminase-induced cross-linking and glucosamine conjugation in soybean protein isolates and its impacts on some functional properties of the products**. *European Food Research and Technology, 231(5):679-689* (Allegato A.3.28)

In the presented work, we exploited microbial transglutaminase as a biocatalyst and glucosamine as an acyl acceptor to modify soybean protein isolates (SPI) by cross-linking and glucosamine conjugation and evaluated some functional properties of the modified product prepared. Electrophoretic studies revealed that transglutaminase-induced cross-linking and glucosamine conjugation occurred simultaneously during modification reaction, and some polymers of glycoproteins with higher molecular weights were formed in the modified product. HPLC analysis demonstrated that about 3.3 mol of glucosamine could be conjugated to 1 mol of SPI, under the preparation conditions as following: SPI concentration of 3% (w/v), acyl donor in SPI/glucosamine acceptor molar ratio of 1:3, transglutaminase addition level of 10 U $g^{-1}$ proteins, reaction temperature of 37°C, and reaction time of 6 h. Compared to SPI and transglutaminase-induced cross-linked SPI, the modified product with glucosamine conjugation about 3.3 mol $mol^{-1}$ SPI clearly exhibited lower surface hydrophobicity, better interfacial properties (especially in emulsion and foaming stability), markedly increased apparent viscosity in the prepared dispersion, and higher enzymatic digestibility in vitro. Our results showed that this modification technique might have the potential as an effective approach to improve the functional properties of SPI.

Sung-Il Joo, et al. (2011) **Physicochemical properties of whole soybean curd prepared by microbial transglutaminase**. *Food Science and Biotechnology, 20(2):437-444*

Whole soybean curd (WSC) was manufactured using micronized full-fat soybean (MFS) powder and microbial transglutaminase (TGase). The WSC prepared with 15% MFS had typical soybean curd texture with a hardness of 513 dyne/$cm^2$. It was confirmed that 7S and 11S protein fractions as major soy proteins disappeared in SDS-PAGE. Also, WSC prepared with 15% MFS and 10% TGase had excellent textural properties with a hardness of 645 dyne/$cm^2$ and springiness of 0.98. Addition of 0.5% gelatin in WSC prepared with 15% MFS and 5% TGase resulted in higher hardness (708 dyne/$cm^2$) and springiness (0.98), as well as the highest values of G′ and G″. The surface properties of WSC were observed using a SEM, indicating the interrelationship of higher hardness and compact protein network filled with small cells. It was concluded that WSC prepared after heat treatment of 15% MFS at 95°C for 5 min, followed by an enzyme reaction with 10% TGase for 1 h, had more enhanced hardness and springiness than commercial WSC. Despite the addition of 5% TGase, WSC with improved textural properties can be manufactured by the fortification of 0.5% gelatin.

Chuan-He Tang, et al. (2011) **Mechanical and Water-Holding Properties and Microstructures of Soy Protein Isolate Emulsion Gels Induced by $CaCl_2$, Glucono-δ-lactone (GDL), and Transglutaminase: Influence of Thermal Treatments before and/or after Emulsification.** *J. Agric. Food Chem. 59(8):4071–4077* (Allegato A.3.29)

The mechanical properties, water-holding capacities (WHC), and microstructures of emulsion gels, induced by glucono-δ-lactone (GDL), $CaCl_2$, and microbial transglutaminase (MTGase) from unheated and heated soy protein isolate (SPI)-stabilized emulsions (at protein concentration 5%, w/v; oil volume fraction, 20%, w/v), were investigated and compared. The influence of thermal pretreatments (at 90 °Cfor 5 min) before and/or after emulsification was evaluated. Considerable differences in mechanical, water-holding, and microstructural properties were observed among various emulsion gels. The thermal pretreatment after emulsification increased the strength of the emulsion gels induced by GDL and $CaCl_2$, whereas in the case of MTGase, thermal pretreatments

before and/or after emulsification on the contrary greatly inhibited gel network formation. The application of the enzyme coagulant exhibited much higher potential to form SPI-stabilized emulsion gels with higher mechanical strength than that of the other two coagulants. The WHC of the emulsion gels seemed to be not directly related to their gel network strength. Confocal laser scanning microscope analyses indicated that the network microstructure of the formed emulsion gels, mainly composed of aggregated protein-stabilized oil droplets and protein aggregate clumps, varied with the type of applied coagulants and emulsions. The differences in microstructure were basically consistent with the differences in mechanical properties of the gels. These results could provide valuable information for the formation of cold-set soy protein-stabilized emulsion gels.

Mao Yang, et al. (2011) **Properties and microstructure of transglutaminase-set soy protein-stabilized emulsion gels**. *Food Research International (on line)* (Allegato A.3.30)

The development of cold-set protein-stabilized emulsion gels has attracted increasing interests, due to their potential to be applied as a kind of release-controlled carriers, especially for labile lipid-soluble bioactive compounds. This work aimed to elucidate the importance of changing oil volume fraction ($\phi = 0.2–0.6$) for the formation and properties of cold-set soy protein isolate (SPI)-stabilized emulsion gels, induced by microbial transglutaminase (MTGase). The gelation process, mechanical properties, water-holding capacity (WHC) and microstructure of the correspondingly formed gels at various ø values were evaluated. The results indicated that increasing ø progressively increased the storage modulus (gel strength) and WHC, from about 200 to 8000 Pa and 52 to 88%, respectively. The high ø dependence of the gel strength and WHC was closely related to the microstructure of the formed gels. The network of the emulsion gels at high ø values (e.g. 0.6) was particulate in nature with coarse strands mainly composed of compact 'aggregated' oil droplets, while that at low ø values was filamentous or fine-stranded with oil droplets incorporated. The enzymatic treatment resulted in a progressive increase in amount of the proteins entrapped within the network, with highest extent observed at $\phi = 0.5$. The entrapped proteins involved the biopolymers of $\beta$-conglycinin subunits or glycinin acidic polypeptides, covalently cross-linked by the enzyme, together with glycinin basic polypeptides. The gelling mechanism at high ø values could be largely related to aggregation or coagulation of protein-coated oil droplets, while that at low ø values, the gels was more like enzyme-set protein gels. These results would be of great help for the understanding of the gelling mechanism and the development of enzyme-set protein-stabilized emulsion gels.