PLASTEINS – A REVIEW*

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The preparation, properties and application of plastein in nutrition are reviewed from the point of view of utilization of the underutilized proteins. The supplementation of specific amino acids into the plastein during synthesis is also discussed. Different enzymes are compared for the production of plasteins as well as different protein sources. The advantages of plastein synthesis are seen namely in the utilization of underutilized proteins including waste proteins, corrections of the amino acids composition of food proteins and preparation of proteinaceous material having special properties.

food proteins; plant proteins; animal proteins; plastein; enzymes; proteinases; microbial proteinase; plant proteinase; animal proteinase; plasteins preparation; functional properties; physical properties; structure

INTRODUCTION

Plastein preparation is based on the old finding that protein hydrolysate of high concentration becomes an opaque solution in the presence of gastric juice and, after a time, is changed into the gel. This process was described first by Danilewski at the end of the 19th century. The term "plastein" for the product and "plastein reaction" for the process is used from the late fourties. Before the detailed study of proteosynthesis, the plastein reaction was thought to be the way of synthesis of the proteins in living organism. However, findings in 1957 of amino acid-activating enzymes and aminoacyl-RNA's gave a clue to the modern concept of peptide chain elongation *in vivo*, and the study of plastein synthesis ceased. The interest in this field reappeared later in seventies, when the plastein production was recognized as a possible way to modified proteins, convenient for food products.

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The plastein reaction is characterized as a complex process, which involves the ill-defined mixture of peptides as a substrate (protein hydrolysate). For this reason the description of the process must necessarily be more or less speculative. Known data indicate that in the plastein synthesis the condensation and transpeptidation reactions take place with the resulting mixture of high-molecular plastein and a low-molecular fraction as a by-product. If the incorporation of foreign amino acids is involved, the amino acids must be supplied as esters to be more accessible for the transpeptidation.

The specificity of proteases in plastein synthesis was found at least in some cases.

Plastein synthesis is applicable for constructing the material with new more convenient composition (e.g. having extremely low content of phenylalanine, preparing methionine enriched products, or obtaining products with better nutritional quality from blood or algal proteins), with improved solubility or with removed unwanted components like bad flavor, color, bitterness, fat etc.

The underutilized food proteins like blood, slaughter-house waste, soy and cereal proteins, whey proteins, and algal proteins can be improved by the plastein synthesis.

Flavoring agents can be obtained during the plastein reaction as well as carrying-out the reaction in the presence of saccharides.

PLASTEIN REACTION

The background of plastein synthesis, the plastein reaction, was discussed many times. The main question is whether a covalent bond is formed during the plastein synthesis, or whether only physical (probably hydrophobic) interactions take place. As early as in 1978 Arai and Fujimaki have published theoretical study concerning the plastein reaction in which the true covalent binding is shown to take place under the appropriate conditions. The plastein reaction includes transesterification, transamidation and transpeptidation reactions. In the peptidase reaction a peptide acts as a nucleophile, and attacks an already formed peptidyl-enzyme intermediate. This results in formation of a product with newly born peptide bond. When the terminal carboxyl group of a substrate reacts with an enzyme to form a peptidyl-enzyme intermediate, its attack on another peptide gives rise to a higher-molecular peptide. This is recognized as a peptide-peptide condensation reaction. Although the overall process is relatively unfavorable in a thermodynamic sense, several data accumulated up to now indicate that well-controlled reaction conditions would give condensation products of various kinds, depending on the properties of the substrate and the enzyme and, of course, on the

conditions of the reaction. The pH and substrate concentration are the most important factors. Terminal group analysis of the plastein prepared from zein hydrolysate with pepsin indicated the condensation reaction taking place during plastein formation as early as 1951 (Virtanen et al., 1951). Two plastein-forming peptides were isolated from peptone, and their plastein reaction was demonstrated in the presence of pepsin (Wieland et al., 1960). Later model experiment with synthetic peptides (Tyr-Leu-Gly-Glu-Phe) leads to polycondensation products (degree of polymerization 2, 3, 4, and 5) by incubating this monomeric substrate with pepsin at pH 4 (Determann, 1965). The appearance of new peptide bonds was manifested by the formation of a new peaks in gel permeation chromatograms using Sephadex G-25. Molecular weight determined by this way certified the synthesis of dimer to pentamer of the source peptide. Yamashita et al. (1972) have observed that ^{18}O-exchange does occur when a protein hydrolysate whose every carboxyl group has been labeled awith ^{18}O is subjected to the plastein reaction with α-chymotrypsin at pH 5.3. In the same time a decrease of the ninhydrin response of the reaction system occurs in the initial process. After initial decrease of ninhydrin response gradual increase take place indicating that transpeptidation reaction is involved (Yamashita et al., 1974).

Gololobov et al. (1986), on the other hand, shows in wide range of experimental conditions that the transpeptidation is the main reaction involved in plastein synthesis. This finding was supported by the estimation of the number-average molecular weights of the reaction mixture and by the measurements of the quantity of trichloroacetic acid-precipitated fractions. According these authors the plastein synthesis proceeds in two steps. In the fast first step the transpeptidation occurs, in the second one a non-covalent interaction of the polypeptides formed during the first step takes place. The first step lasts a few minutes, the second a couple of hours. Scanning microscope studies show that in plastein reaction the aggregation of peptides takes place which resulted in the formation of sponge-like product.

The plastein reaction is always accompanied by the formation of a water-insoluble product that causes turbidity of the reaction system. Water-insoluble product accounts for approximately half the total amount of plastein (trichloroacetic acid /TCA/ – insoluble product). The turbid solution is more-or-less clarified with urea, guanidine hydrochloride, sodium dodecylsulfate, or some alcohols. The turbid solution showed a high affinity for a hydrocarbon (n-heptan). Compared with the substrate, the water insoluble product causing turbidity was found to contain smaller amounts of hydrophilic and larger amounts of hydrophobic amino acid residues. Based on these results Aso et al. (1974) concluded that hydrophobic forces are a major factor in plastein chain assembly.

The same conclusion has drawn Lorenzen et al. (1996) in the characterization study of pancreatic casein plastein. In the course of this reaction hydrophobic peptides concentrate mainly in the aggregates (plasteins), whilst hydrophilic peptides remain in solution (supernatant). These authors then concluded, that plasteins have shown to be aggregation of short-chain peptides and free amino acids, which is brought by non-covalent, hydrophobic and ionogenic interactions. In the supernatants resulting from the plastein reaction acaseinophosphopeptide sequences, in particular from α-s-casein, were determined (Lorenzen et al., 1996). This finding is in accordance with the older study of plastein synthesis with egg albumin as substrate, and pepsin, chymotrypsin and papain as enzymes (Edwards et al., 1991). In this case the electrophoresis of plastein products in the presence of dodecyl sulfate indicated that no high molecular weight, protein-like material was produced by the plastein reaction. It is suggested that plastein reactions lead to the formation of insoluble peptide aggregates, which are thought to be held together by noncovalent bonds rather than by covalent peptide bonds.

Hofsten and Lalasidis (1976) have shown that the driving force of the plastein reaction is the formation of precipitate and gel matrices in which relatively small peptides are associated by means of a noncovalent bond. Nevertheless, the presence of proteolytic enzymes catalyzes the reaction, probably by the way of bringing the appropriate part of the peptide molecules into contact (Hofsten, Lalasidis, 1976). No plastein is produced in the absence of the enzyme (Harnett, Satterlee, 1990). NMR study indicated no new peptide bond formation during plastein synthesis (Stevenson et al., 1998).

Despite the prevailing data indicating the non-covalent interaction during the plastein formation, some data exist showing true covalent bond formation (Sang Joon Lee et al., 1992).

The conclusion can be drawn from existing data, that the main reaction in plastein synthesis is the non-covalent hydrophobic interaction in which protease must be present to bring the appropriate part of peptides into contact. Less important is ionic interaction taking place in the reaction catalyzed with some proteases active against charged amino acids. Transpeptidation take place in lower extent, but could have an importance in special cases.

Despite these facts, the plastein synthesis can serve as an efficient method for the protein modification as shown by the successful methionine introduction into plastein in the presence of methionine ethyl ester (Guevara et al., 1991).

Nevertheless, the product of higher nutritive value can be obtained, the unconventional protein sources can be used, the undesirable flavor can be removed, and improved protein solubility can be reached using plastein synthesis (Pallavicini et al., 1979).

ENZYME AND PROCESS

The enzyme most frequently used for primary hydrolysis in the plastein preparation is pepsin (see e.g. Kim et al., 1988b; Golobov et al., 1986; Lorenyen, 1994). Papain is one of the enzymes most frequently used for plastein synthesis from peptic digest of proteins (see e.g. Golobov et al., 1986; Edwards, Shipe, 1978). Second most frequently used enzyme for plastein synthesis is chymotrypsin (see e.g. Kim et al., 1988b; Fujimaki, 1970). Some data are summarized in Table I.

The other enzymes, beside that included in the table, are used as well, but in lower extent.

The protocol used for the process differs for different source proteins and, in lower extent, different enzymes. The kind of enzyme and the conditions do influence the yield of plastein and its properties (Edwards, Shipe, 1978). Egg white albumin as 1% solution was hydrolyzed at pH 1.7 with pepsin in the enzyme : substrate ratio as 1 : 100 under stirring. After appropriate deal of time the pH was adjusted to 7.0, and the mixture was held for 1 hour, followed with the decrease of pH to 5.0. The freeze dried product was then used for plastein synthesis with the use of pepsin, chymotrypsin and

I. The enzymes most frequently used for protein digestion and plastein synthesis

Enzyme used for		References
Digestion	plastein synthesis	
Pepsin	pepsin	Edwards, Shipe (1978)
Pepsin	papain	Arai et al. (1974, 1975a); Edwards, Shipe (1978); Golobov et al. (1982, 1986)
Pepsin	chymotrypsin	Fujimaki et al. (1970); Edwards, Shipe (1978); Dalaly, Brunner (1979); Golobov et al. (1982, 1986); Kim et al. (1988b)
Pepsin	pancreatin	Krause et al. (1984); Lorenzen (1994)
Pepsin	Bioprase 54 (bacterial proteinase)	Fujimaki et al. (1973)
Pronase	bromelain	Hyung Joo Suh et al. (1992)
Rhozyme (fungal proteinase)	rhozyme	Gabr et al. (1984)
Aspergillus satoi proteinase	papain	Kim et al. (1988a)
Chymotrypsin	chymotrypsin	Lozano et al. (1994)

papain at substrate 40% at 37 °C and appropriate pH for 24 hours. The maximum plastein yield was obtained after 18 hours in all the cases (Edwards, Shipe, 1978).

Animal blood was modified by the way of plastein preparation using thermitase with the enrichment in glutamic acid and sucrose to avoid the bitter taste of the product. The plastein yield was 10 to 40% of the theoretical amount. The plastein synthesis was done after decolorization of the blood hydrolyzate (Krause et al., 1986).

Similarly, faba bean protein was partially hydrolyzed and resynthesized to plastein with the production of emulsifying hydrophobic plastein products, depending on the enzyme used (Ludwig, Ludwig, 1985).

The plastein reaction has potential for recovery of proteins from unconventional resources. Fish silage, an easily prepared hydrolysate of fish wastes obtained by autolysis, was investigated as a raw material for plastein synthesis. Plastein reactive fish silage was prepared by ensilation of fish viscera at pH less than 2.5. Optimum plastein synthesis from fish silage occurred between 65 and 70% degree of hydrolysis, which was slightly lower than the max. degree of hydrolysis attainable. Pepsin, papain and chymotrypsin synthesized plasteins from fish silage optimally at pH 5.0, 6–7 and 8.0, resp., pepsin being the most productive enzyme. Plastein yield was highest when trichloroacetic acid was used as a precipitant, but higher gross yields were obtained with alcohol. Concentration of fish silage, by evaporation in an open pan at 60 °C instead of the conventional freeze drying, did not affect plastein yield (Raghunath, McCurdy, 1991). High temperature cooking conditions for 60% hydrolysis of fish protein extracts were optimized using response surface methodology; moreover, partial hydrolysates from fish were used as a substrate for determining optimum plastein product formation. Fish protein extracts were prepared from loach (*Misgurnus anguillicaudatus*), crucian carp (*Carassius carassius*), bastard halibut (*Paralichthys olivaceus*) and jacopever (*Sebastes inermis*). 4 models were proposed with regard to the effects of time, temperature and water/fish ratio on the amount of 0.3M trichloroacetic acid soluble fractions. Model coefficient ranged from P less than 0.0001 for jacopever to P less than 0.043 for bastard halibut. Cooking conditions for 60% hydrolysis were optimized at: a temperature of 140 °C, except for crucian carp (136.7 °C); a cooking time of 10.08, 7.25, 9.85 and 9.38 hours for loach, crucian carp, bastard halibut and jacopever, respectively; and a water/fish ratio of 1 : 1 for all fish except crucian carp (1.1 : 1). When protein hydrolysates were used for plastein product synthesis, optimum plastein reaction conditions were: pH 9.0 with chymotrypsin for loach and crucian carp hydrolysates; pH 9.0 with papain for bastard halibut hydrolysate; and pH 11.0 with trypsin for jacopever hydrolysate. Plastein reactions could

be carried out in water at a concentration of 20% (water/fish hydrolyzate) (Keun Tai Lee et al., 1997).

Influence of different alkali halides (LiF, LiCl, LiBr, NaF, NaCl, NaBr, KF, KCl and KBr) on α-chymotrypsin-catalyzed plastein synthesis was studied. Results showed an enhancing effect on the plastein synthesis by the presence of salt, proportional to salt concentration, which was decreased when the substrate concentration was increased. These ions can be classified according to their activation power, which was in agreement with the interpretation of the Hofmeister lyotropic series (Lozano, Combes, 1993).

The addition of hydroxylated compounds to the plastein reaction mixture enhances the plastein yield. The increase of polyol concentration and the number of hydroxylic groups per molecule increase the yield. The effect is higher in the lower concentration of substrate. It was concluded that the presence of additives in the reaction media, as a reducing agents, increases the condensation pathway of alpha-chymotrypsin action on plastein synthesis (Lozano, Combes, 1992).

There are two main processes: an enzymatic hydrolysis followed by solvent extraction to remove the impurities, e.g. odorants and fat, from a crude protein preparation; and an enzymatic synthesis of an acceptable protein-like product (plastein). This represents an original attempt in the field of food processing. Although some basic information was obtained from the experiments with the well known proteases such as pepsin and chymotrypsin, it was demonstrated that various microbial proteases could also be used to improve the acceptability of the food (Fujimaki et al., 1971).

According to all the experimental data obtained, the plastein reaction requires that three conditions are present: i) a low molecular weight peptidic substrate; ii) a substrate concentration between 20 and 40% (w/v); iii) an optimum pH value for the „synthetic" activity of the protease.

APPLICATION

The plastein reaction is an efficient way for debittering of protein hydrolyzate. Bitter taste is done by the presence of hydrophobic amino acids on the non-terminal position. The problem is frequently solved by the way of exo-protease application, but higher molecular weight peptides are usually necessary for some food application. In such cases the most convenient way of debittering is the plastein preparation (see e.g. Baldini et al., 1983).

Stevenson et al. (1998) utilize the protease-catalyzed plastein synthesis as a technique for removing bitter peptides from protein hydrolyzate using synthetic peptides of varying hydrophobicity. Alcalase 2.4 L (Subtilisin Carlsberg), Neutrase 0.5 L (*Bacillus subtilis* proteinase; both from Novo

Nordisk, Bagsvaerd, Denmark), Corolase PP (a pancreatic proteinase from Rohm GmbH, Darmstadt, Germany) and papain were used for the conversion of hydrophobic dipeptides into an insoluble precipitate by all 4 enzymes, but differences in enzyme efficiency were observed. Oligomeric products up to and including pentamers (decapeptides) were produced, and a decrease in bitterness accompanied product formation. Peptide bond formation was monitored by NMR and MS using ^{13}C-labelled peptides. It is suggested that product precipitation is a primary driving force for the condensation reaction, with the precipitated products forming unreactive, plastein-like precipitates. Use of hydrophilic dipeptides did not result in detectable condensation or precipitation. It is concluded that this proteinase-based condensation reaction could be used commercially to remove bitter peptides from protein hydrolysates.

The amino acid composition can be easily changed by the plastein synthesis. Namely the content of methionine (see Arai et al., 1974, 1979), tryptophan, lysine, threonine (Arai et al., 1975a) or glutamic acid (Arai et al., 1975a, b) was increased by this way. A soybean acid-precipitated protein isolate was dissolved in highly alkaline media and allowed to stand at room temperature for appropriate of time. After them, the solution was acidified to pH 1.6 and pepsin was added. The mixture was incubated for longer time at elevated temperature in the presence of L-methionine ethyl ester and L-cysteine. Then, pH was readjusted to 5.3 and papain was added for the synthesis of plastein. An odorless, tasteless and colorless plastein was obtained as a final product This process is expected to be employed for the alteration of amino acid composition and functionalization of cereal proteins (Arai et al., 1974).

Undesirable phenylalanine, on the other hand, can be removed by the proper application of plastein reaction (Fujimaki, 1978).

The protein produced by the cultivation of yeast on alkanes can be utilized by the way of hydrolysis, followed with the removal of remaining alkanes and their derivatives and plastein synthesis (Fujimaki et al., 1970). Undesirable components are easily avoided with the application of plastein reaction (Fujimaki et al., 1972). The algal and other plant proteins, as a consequence, can be utilized by this way (see e.g. Baraniak, 1992).

Plastein prepared from proteins, namely in the presence of other compounds (Ruttloff, 1982)), could serve as flavor potentiating factor (e.g. Fujimaki et al., 1974). A pronase hydrolysate of food protein concentrate was fractionated and a low mol. weight acid fraction with flavour-potentiating activity obtained. Oligopeptides isolated from this fraction contained high molar ratios of glutamic acid residues. These peptides, when treated again with pronase in the presence of other protein hydrolyzates (produced a more effective flavor-potentiator without concommitant bitterness (2 : 1) (Fujimaki et al., 1974).

Plastein reaction is used frequently also for the correction of the technological properties of proteins (from a number of works see e.g. Mozaffar, Haque, 1992; Sikorski, 1981). The proteins, one dispersible in water (97% w/w of total casein) and other indispersible in water, were, for instance, synthesized by treating casein hydrolysate with papain. Aggregates were dissociated by sodium-dodecylsulphate, indicating non-covalent interactions. Oil holding capacity of both peptide preparations was significantly improved compared to casein or its hydrolysate. The dispersible peptide preparation was highly soluble at neutral pH and was more thermostable than casein. Emulsion activity and stability of both preparations were decreased (Mozaffar, Haque, 1992).

Important were also the possibilities of utilizing the waste from food processing for the preparation of valuable product – the plastein (Raghunath, McCurdy, 1991).

CONCLUSION

The plastein reaction has become one of the main areas of research in food protein hydrolysis due to the striking ability of this phenomenon to improve the process in many senses. The plastein could be used for debittering of protein hydrolyzate, but also for the improvement of the amino acid composition, technological properties and many others.

Gel-like products from a concentrated protein hydrolyzate is formed during plastein reaction. Some undesired components can be easily removed with the extraction or selective precipitation. The remarkable advantage of plastein reaction is also the possibility of the valorization of proteinaceous waste.

The further investigation and technological development are necessary for the utilization of all the possibilities for plastein production and utilization.

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Plasteiny — přehledná studie.

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Příprava plasteinů spočívá ve starém poznatku, že koncentrované roztoky hydrolyzátů bílkovin se časem v přítomnosti proteáz zakalí, až se nakonec změní v gel. Tato skutečnost byla známá a popsaná již na konci 19. století. Pro vzniklý gel se ujalo označení plastein a pro proces plasteinová reakce.

Plasteinová reakce je charakterizována jako složitý proces sestávající z mnoha dílčích procesů, jako je transpeptidace a kondenzace, ale i nekovalentní hydrofobní interakce a další blíže nedefinované procesy. Příprava plasteinu pak umožňuje vznik nových produktů s výhodnějšími vlastnostmi, než měla surovina (např. velice nízký obsah fenylalaninu, zvýšený obsah methioninu apod.). Tvorbou plasteinu mohou být také odstraněny některé nepříznivé vlastnosti výchozích bílkovinných hydrolyzátů (hořká chuť, nežádoucí příchutě, zabarvení a jiné). Naopak zlepšení chuti a zavedení nových příchutí je možné při vedení plasteinové reakce ve specifickém prostředí, např. v přítomnosti sacharidů.

Dlouhé spory byly vedeny o tom, vznikají-li plasteinovou reakcí nové kovalentní vazby, nebo jedná-li se o čistě fyzikální interakce. Tento spor nebyl až dosud vyřešen. Plasteinová reakce byla modelově demonstrována na příkladu dvou peptidů, kde docházelo prokazatelně k polykondenzaci za vzniku delších peptidů. Tyto modelové studie byly později potvrzeny s použitím syntetických peptidů. Stupeň polymerace v obou případech byl až pět, s velkou variací od dvou do pěti. Také izotopové studie ukázaly, že dochází k tvorbě nových peptidových vazeb. Nepochybná je i účast transpeptidace během plasteinové reakce. Většinou se předpokládá, že plasteinová reakce

probíhá ve dvou stupních. První, velmi rychlý stupeň je provázen vznikem nových kovalentních vazeb, zatímco druhý, pomalý stupeň vede ke vzniku fyzikálních interakcí. První stupeň trvá několik minut, druhý stupeň několik hodin. V obou stupních je důležitá účast proteáz, které v prvním stupni katalyzují reakci svou peptidázovou aktivitou, ve druhém stupni se účastní vazebná místa proteáz, která uvádějí jednotlivé hydrofobní aminokyseliny do optimálních pozic.

Nejčastěji používanými proteázami jsou pepsin a chymotrypsin, často se však uplatňují i rostlinné (nejčastěji papain) a mikrobiální (hlavně subtilisin) proteázy. Volba enzymu má alespoň částečně vliv na kvalitu produktu, avšak hlavní vliv má druh použitého hydrolyzátu. Hydrolyzovat lze i několik různých bílkovin a jejich mísením upravovat aminokyselinové složení produktu.

Plasteinovou reakcí lze tedy upravit vlastnosti bílkovin nebo připravit produkt se zlepšeným aminokyselinovým složením. Typickou aplikací je příprava plasteinu se zvýšeným obsahem methioninu nebo lyzinu, plastein se zvýšenou šlehatelností, případně produkt neobsahující fenylalanin. Příprava plasteinu je také vhodnou cestou k využití a zhodnocení odpadních bílkovin.

potravinářské bílkoviny; rostlinné bílkoviny; živočišné bílkoviny; plastein; enzymy; proteázy; mikrobiální proteázy, rostlinné proteázy; živočišné proteázy; příprava plasteinu; funkční vlastnosti; fyzikální vlastnosti; struktura

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