SUPERIOR BENEFITS OF BIOCATALYSTS' ENZYMES IN THE PRODUCTION OF PLANT PROTEIN HYDROLYSATES

Traditionally, animal-derived proteins such as meat, eggs, gelatin and milk proteins have been widely used as ingredients in the food industry. Animal proteins have excellent nutritional properties and are regarded as 'high quality' proteins as they contain all the essential amino acids required for human growth and development. In recent years, plant proteins have emerged as a popular alternative for meeting the protein requirements of the growing population. Consumer trends towards healthy living, veganism, weight management, protection of the environment and saving money have resulted in the overall plant protein market being predicted to reach $16.3 billion by the end of 2025.[1]

BIOCATALYSTS LTD: EXPERTISE TO HELP YOU ADD VALUE TO PLANT PROTEINS

Biocatalysts Ltd has over 35 years' experience of working with customers to create different types of protein hydrolysates. Over this time, we have gained a wealth of knowledge on how enzymes can be used to add value to different types of animal and vegetable proteins. Over 20 years ago, we developed the Soy Cascade System which has been used to successfully hydrolyse soy protein to improve solubility and create a savoury flavoured soy hydrolysate. In more recent years, we have created different protease products for our customers to help improve the functionality of wheat gluten, pea protein, potato protein, rice protein and corn.

Biocatalysts work with its customers to develop new plant protein hydrolysates with one or more functional benefits. We use our expertise, toolkit of enzyme products, and knowledge of plant protein chemistry to rationally and rapidly select the most suitable enzyme product for a customer's specific application. If a suitable enzyme is not available in our off-the-shelf range, we offer our in-house enzyme discovery platform, MetXtra®, to identify suitable enzymes and produce a panel of novel enzymes for you to evaluate. Following testing, any chosen enzymes can be developed, scaled-up and manufactured in commercial quantities.

SELECTING THE OPTIMAL ENZYMES CAN IMPROVE PLANT PROTEIN FUNCTIONALITY

Many plant proteins have poor solubility in water which limits their use as a food ingredient. Proteases can be used to partially hydrolyse these plant proteins thus improving the solubility and application potential of plant proteins. In addition to improving plant protein solubility, enzymes can also be used to create different flavours, improve foaming, emulsification, texture and bioactive properties of plant proteins as outlined in Figure 1.

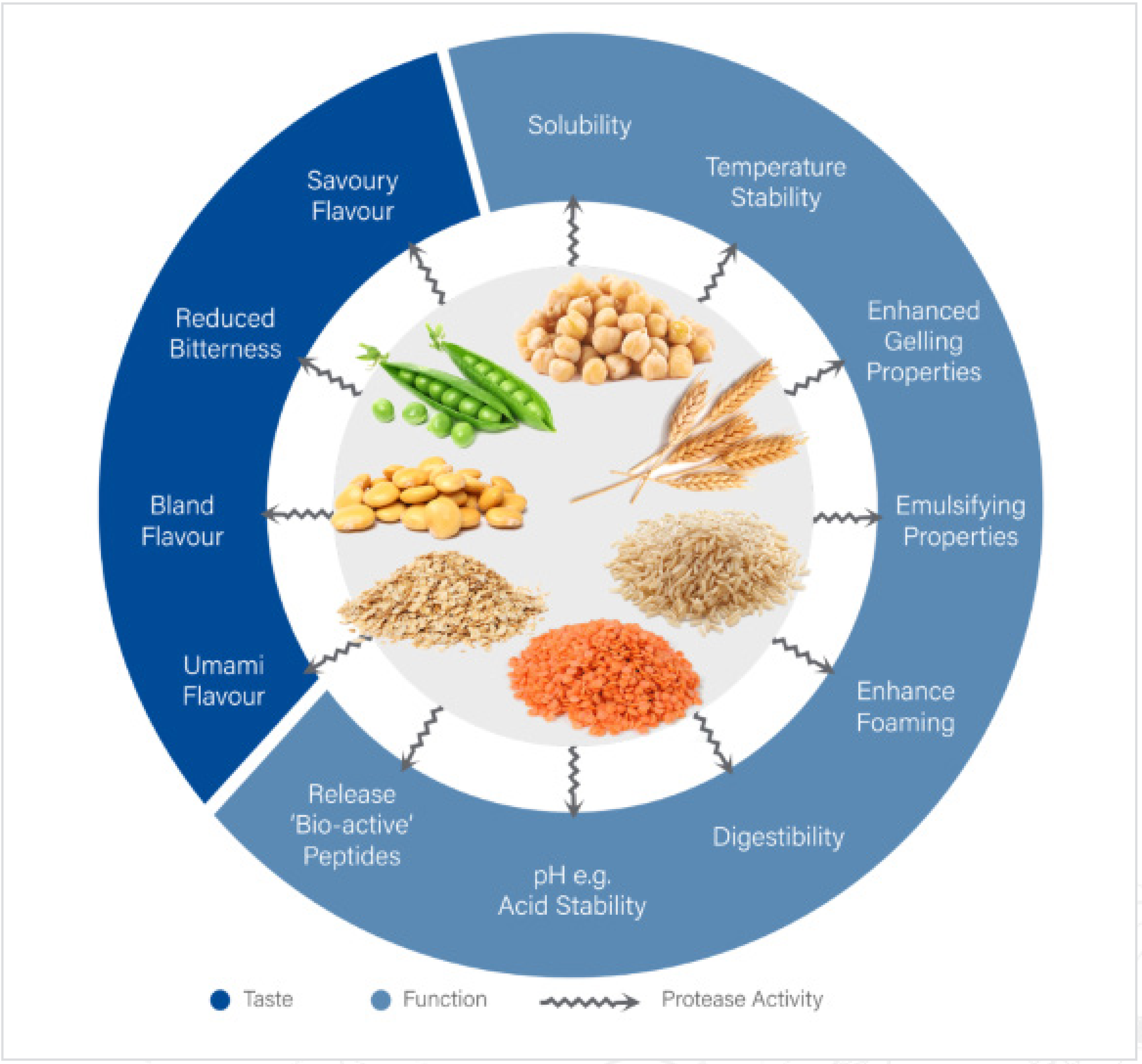
Solubility
Temperature Stability
Enhanced Gelling Properties
Emulsifying Properties
Enhance Foaming
Digestibility
pH e.g. Acid Stability
Release 'Bio-active' Peptides
Umami Flavour
Bland Flavour
Reduced Bitterness
Savoury Flavour
Taste
Function
Protease Activity

Figure 1: Benefits of using proteases to create plant protein hydrolysates.

Proteases are enzymes that catalyse the hydrolysis of peptide bonds in proteins and are derived from a variety of different sources; animal (porcine trypsin), microbial (subtilisin) and plant (papain). The extent of protein hydrolysis using proteases is generally quantified by measuring the degree of hydrolysis DH (the percentage number of peptide bonds hydrolysed within the protein).

Proteases are subdivided into two main groups, endopeptidases and exopeptidases, depending on their site of action. Endopeptidases catalyse cleavage of internal peptide bonds in polypeptide chains and are further classified based on their catalytic mechanism and the nature of their catalytic site (serine endopeptidases, cysteine endopeptidases,). In contrast, exopeptidases will cleave one or a few amino acids from either the amino or carboxyl termini of a polypeptide/peptide chain and are known as aminopeptidases and carboxypeptidases respectively (Figure 2).

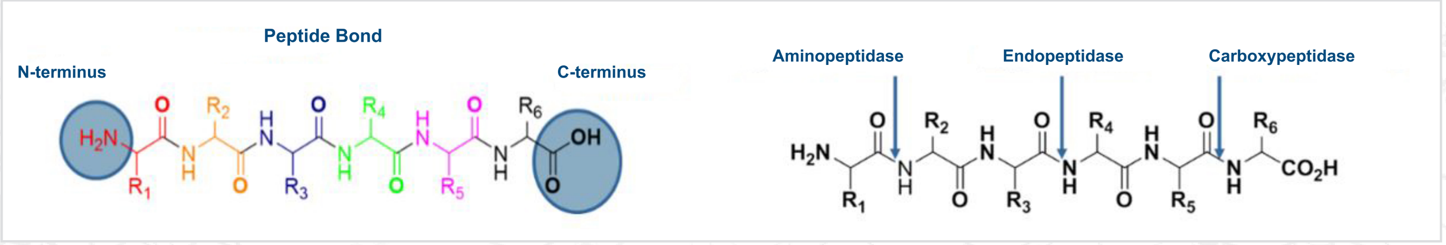

Figure 2: Mechanism of Action of Endopeptidases and Exopeptidases on Proteins.

Endopeptidases are widely used in the food industry for efficient hydrolysis of different plant-based proteins to improve protein solubility, lower viscosity and reduce the molecular weight of the protein. Exopeptidases can be used to help control and enhance the flavour of a plant protein hydrolysate.

Commercially available proteases will differ widely with respect to purity, specificity, pH and working temperature. Specificity is an important feature of a protease as it represents the amino acid sites where the protease can hydrolyse within the protein substrate. For example, porcine trypsin hydrolyses only at the carboxy terminal side of arginine or lysine. That said, most commercial preparations of porcine trypsin are contaminated with some chymotrypsin which selectively catalyses the hydrolysis of peptide bonds on the carboxy terminal side of tyrosine, phenylalanine, tryptophan and leucine; this "additional specificity" can be a benefit or disadvantage depending on the protein modification application. Proteases with different specificities will produce protein hydrolysates containing different types of peptides and/or amino acids that will in turn have different functional properties.

The functional properties of a plant protein hydrolysate will vary depending on the protein type, enzyme type, enzyme dose, pH, temperature and enzyme hydrolysis time. Whether you are trying to produce a neutral tasting pea protein hydrolysate for a sport's nutritional product or a savoury tasting wheat gluten hydrolysate as a food flavouring ingredient, Biocatalysts can recommend the most suitable protease product from our Promod® and Flavorpro™ range. It is our extensive knowledge base that allows us to translate customers' required features and benefits for protein hydrolysates into the best enzyme recommendations to alter the protein chemistry to meet very specific requirements. In addition to technical requirements, cost-in-use and regulatory aspects are always essential in the enzyme selection process.

ENZYMES FOR HYDROLYSING WHEAT GLUTEN

Wheat gluten is the protein derived from wheat or wheat flour and contains a high content of glutamic acid and proline and has a good flavour profile. The expanding utilization of wheat gluten in food and non-food industries has been limited due to the poor solubility of wheat gluten. Enzymatic hydrolysis of wheat gluten has been shown to improve the functional and flavour properties of this protein.

Biocatalysts has developed two protease products that are suitable for hydrolysing wheat to create superior flavouring ingredients for foods; Flavorpro® 766MDP and Flavorpro® 795MDP. These enzymes hydrolyse the wheat gluten using carefully designed combination of specific endo- and exopeptidases to produce a wheat gluten hydrolysate with good solubility and high levels of free amino acids (see Figure 3). As the endopeptidases hydrolyse the wheat gluten, the degree of hydrolysis and solubility of the wheat gluten increases. As the degree of hydrolysis of the protein increases (>10% DH) there is an increased risk of producing a bitter-tasting protein hydrolysate. Specific exopeptidases contained in these products hydrolyse amino acids from the ends of bitter peptides resulting in the production of a non-bitter tasting protein hydrolysate. Flavorpro® 795MDP also has a glutaminase side activity which enhances the savoury flavour of wheat gluten hydrolysates as this enzyme converts the amino acid glutamine to glutamate/glutamic acid. Wheat gluten hydrolysates may be used as flavouring agents in savoury food products.

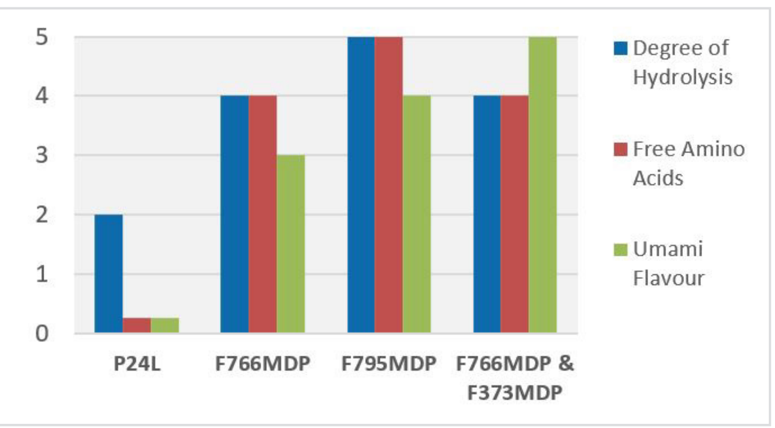

Figure 3: Summary of the performance of Biocatalysts Ltd enzymes for wheat gluten hydrolysis (pH 5.0, 50oC for 12 hours).

Gluten is also used as a nutrient for feeding calves and the low solubility of this protein can make it difficult to handle. Promod® 24L can increase gluten solubility and digestibility for this application. This highly cost-effective enzyme is based on a bacterial neutral endopeptidase which produces a hydrolysate with a bland neutral flavour profile

ENZYMES FOR THE PRODUCTION OF SOY PROTEIN HYDROLYSATES

Traditionally, hydrolysed soy protein has been used as a savoury flavour in the form of soy sauce. The soy proteins are digested by the endogenous extracellular proteases of the starter culture (e.g. Aspergillus oryzae) to produce a sauce that is highly flavoursome and nutritious because of its high amino acid and peptide content. This fermentation process usually takes between 3-12 months.

In the 1990s, Biocatalysts developed the Soy Cascade Enzyme System. This enzyme system hydrolysed soy in a 3-stage process. Stage 1 used a specific endopeptidase to generate peptides, stage 2 and 3 used exopeptidases to create high levels of free amino acids. The Soy Cascade Enzyme System hydrolysed soy protein in a controlled manner to produce a savoury tasting, enzyme hydrolysed vegetable protein without the undesirable by-products of a chemical process.

More recently, Biocatalysts Ltd developed two new products, Flavorpro® UMAMI 852MDP (F852MDP) and Flavorpro® 795MDP for production of soy protein hydrolysates with an enhanced savoury-type flavour. These products contain endopeptidase, exopeptidase and glutaminase activities that synergistically work together for efficient, cost-effective flavour generation in a single enzyme hydrolysis step. The resulting soy hydrolysate will contain a high level of free amino acids, including glutamic acid which imparts a strong umami flavour. Umami enhances the savoury flavour profile which helps increase the apparent saltiness of the soy hydrolysate, thus allowing for less salt to be added to the hydrolysate for production of a natural low sodium savoury flavour enhancer.

If an enzyme is required to hydrolyse soy protein at low pH (pH2.0-4.0), Promod® 987MDP, an endopeptidase, can be used to mildly hydrolyse the soy protein. The resulting hydrolysate has a low degree of hydrolysis, reduced viscosity and bland flavour, making it suitable for use as a protein ingredient in the acid beverage market.

IMPROVING THE FUNCTIONAL PROPERTIES OF PEA PROTEIN USING BIOCATALYSTS ENZYMES

Over the last 5-10 years, pea protein ingredients have surged in popularity and are expected to continue to grow over the next 5 years.[2,3] The yellow pea is composed of approximately 50% starch, 25% protein, 15% fibre, 8% ash and sugars, and 2% fat.[4,5] The protein is extracted using physical separation methods to produce pea protein isolates and concentrates. The protein extracted from yellow pea has numerous benefits for use as a protein ingredient in foods including; naturally sourced, GMO-free, gluten-free, clean label, low in calories and fat, low allergenicity, well balanced amino acid profile and suitable for vegans.

Pea protein isolates have a stable fibrous texture, along with good water and fat holding capacity, making them suitable to use as a plant-based meat substitute in meat-free burgers, nuggets, sausages and dips. One disadvantage of pea protein isolate is that it has a poor solubility particularly in the pH range of 3.5-5.5 (Figure 4) and it needs to be blended with pectin for use in acidic beverages. The solubility of pea protein can be improved by treatment with proteases to reduce the molecular weight of the protein and increase the content of hydrophilic peptides, which improve protein solubility. Promod® 987MDP is an endopeptidase which can be used to hydrolyse pea protein at low pH (pH 3.5), improving protein solubility and producing a pea protein hydrolysate with excellent clarity.

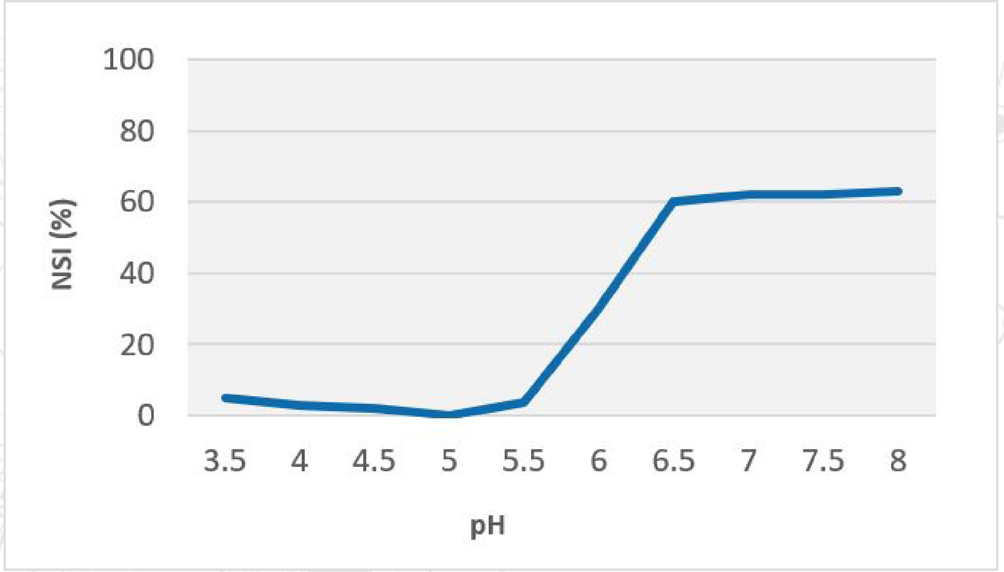

Figure 4: Solubility of pea protein (3% in water).

Biocatalysts also has a range of protease products which can be used to hydrolyse pea protein isolate at neutral pH to help lower the viscosity and improve the solubility and digestibility of the pea protein (Figure 5). Promod® 184MDP is an endopeptidase that can be used to mildly hydrolyse pea protein isolate to a low degree of hydrolysis (<5% DH) and improve solubility and foaming properties. Whereas, Flavorpro® 750MDP and Flavorpro® 766MDP can be used to hydrolyse pea protein isolate to a higher degree of hydrolysis (10-20% DH) and further improve the pea protein solubility and digestibility for the production of pea protein hydrolysates for sports nutrition, elderly nutrition and infant formula.

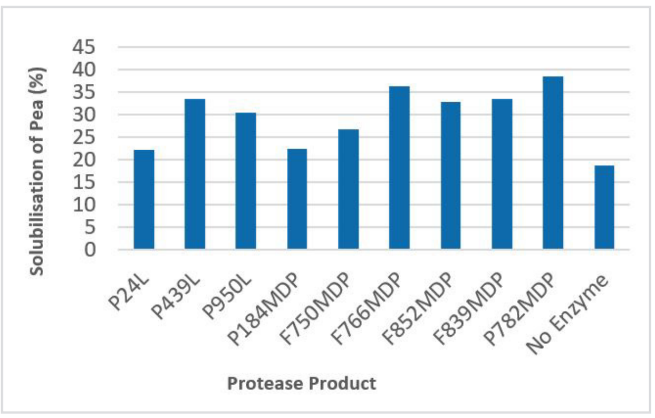

Figure 5: Summary of Pea Solubility achieved with 9 different Biocatalysts Ltd Protease Products dosed at 1% w/w on protein at pH 7, 50oC for 6 hours.

Flavorpro® F766MDP contains endopeptidases and exopeptidases which can hydrolyse pea protein isolate to produce a hydrolysate with smaller, more digestible peptides, and high content of free amino acids which include; arginine, leucine, isoleucine and valine. This pea protein hydrolysate can be used to help maintain muscle mass for sporting athletes and the ageing population.

PRODUCTION OF EXTENSIVELY HYDROLYSED POTATO PROTEIN HYDROLYSATES USING PROTEASES

Potato is a major world crop with over 300 million tonnes produced annually. Fresh potato contains approximately 17-21% starch and 0.5-1.2% proteins. During the production of potato starch, waste streams are produced which contain potato proteins. Traditionally, these proteins were not extracted from these waste streams, but nowadays cold processing and advanced absorption technology are being employed to quickly extract the potato proteins to maximise protein yields. These potato proteins can be used in sports drinks, beverages, whipped cream and ice cream sorbets.

Potato proteins can also be hydrolysed using proteases, adding further value. They produce water soluble hydrolysates with little odour, making them acceptable for use in the cosmetic industry. Historically, animal protein hydrolysates e.g. collagen and keratin hydrolysates have been used in the cosmetic industry, but in recent years plant protein hydrolysates have started to be used due to their different amino acid profiles and consumer demand. Enzymatic hydrolysis is preferred to chemical hydrolysis as it uses milder conditions and it doesn't destroy amino acids.

Biocatalysts have two protease products, Promod® 439L and Promod® 782MDP, which can be used in combination and have been shown to efficiently hydrolyse a potato protein to produce a highly water-soluble potato protein hydrolysate in which > 97% of the peptides have a molecular weight of < 1.0kDa (Figure 6). Potato protein hydrolysates contain a high level of small peptides and free amino acids which can bind water and are used in shampoos, conditioners and hair styling products to help moisturise hair and prevent damage to hair fibres.

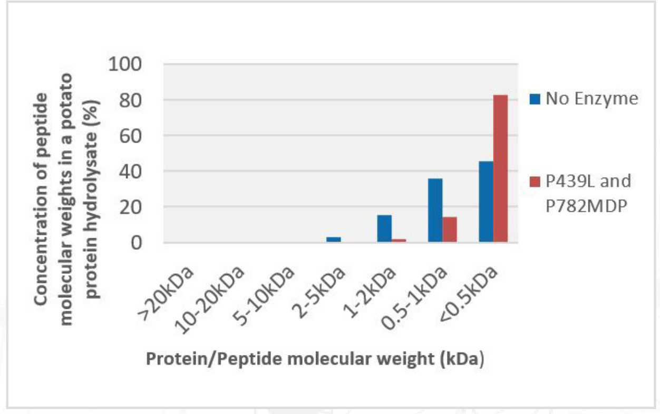

Figure 6: Gel Filtration Profile of a Potato Protein Hydrolysate made with P439L and P782MDP (1% w/w on protein at pH 7.0, 50oC for 24 hours).

PLANT PROTEINS HYDROLYSATES: SUMMARY AND OUTLOOK

The trend for using plant proteins and their hydrolysates across varied applications continues to increase exponentially. The functional, nutritional and flavour properties, together with the availability and affordability of plant proteins influences their suitability for use as future ingredients in food applications. Sources of plant proteins are diverse including; cereals (wheat, corn, rice, oat, rye and barley), legumes (soybean, pea, lentil, chickpea, beans and lupin), oilseeds (canola, flaxseed, sunflower and hemp), ancient grains (quinoa, chia, teff, millet and sorghum) and other sources (potato, algae, nuts, insects and leaves), giving customers a wide range of options to choose from. In this technical bulletin we have discussed how Biocatalysts has developed proteases that add value and improve functionality of wheat gluten, soy, pea and potato proteins.

It is certain the plant protein market will increase and become more diverse in the coming years. We understand that food manufacturers ability to react to these market changes are critical to their business and that enzymes have a key role in facilitating this. At Biocatalysts we can support our customers with in depth knowledge of how specific enzyme activity can change the functional properties of plant proteins (i.e. reduce salt content but keep the umami flavour of a product) to meet these consumer demands.

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If you would like to discuss how we can help with your enzymatic hydrolysis or find out more about our unique enzyme development & manufacturing service, please get in contact. Alternatively, sign up to our newsletter to keep up to date with all Biocatalysts articles.

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