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Dairy Protein Hydrolysates

THE USE OF ENZYMES IN THE PRODUCTION OF DAIRY PROTEIN HYDROLYSATES

Whey and casein protein hydrolysates are valuable ingredients with nutritional and functional benefits. On the downside, hydrolysis of these two dairy proteins very often have an undesirable taste or texture, meaning the blander the protein hydrolysate, the better. A simple yet effective way to improve your dairy protein hydrolysate and set your product apart is by using enzymes. Whether you want to improve its taste or texture, reduce its bitterness, enhance its digestibility or something else, Biocatalysts Ltd can help. This technical bulletin will introduce you to the use of enzymes for improving dairy protein hydrolysates with detailed application information.

The demand for whey protein continues to soar as a result of its rising popularity as a healthy ingredient, delivering high quality protein in a digestible form. Casein protein on the other hand is slowly emerging in the protein market as its nutritional and functional benefits are becoming more widely understood. The protein market, as a whole, has expanded rapidly and is forecasted to continue to do so. With this in mind, R&D departments continue to find novel ways of improving or introducing new dairy protein products to keep up with current trends. Enzymes have an essential role to play in the development and manufacture of valuable and innovative dairy-based ingredients.

The enzymes covered in this technical bulletin are from our current range. If you find that these are not suitable, Biocatalysts has the enzyme development & manufacturing capabilities to create a completely unique enzyme for you. In turn this allows you to create a truly innovative product, adding to your competitive advantages. For more information on this process contact use directly at: enquiries@biocats.com

INTRODUCTION TO WHEY PROTEIN

Milk contains two primary protein groups, casein, and whey protein. During the manufacture of cheese, the liquid whey separates from the 'curd' or coagulated casein. Generally, the types of whey fall into two main categories; acid whey produced during the manufacture of cottage cheese, ricotta cheese or casein and sweet whey produced during the manufacture of hard cheeses, such as cheddar and mozzarella. Whey protein is a valuable by-product from the manufacture of cheese with important nutritional and functional properties. It may be processed using various technologies to produce whey protein powders, whey protein concentrates (WPC), whey protein isolates (WPI) or whey protein hydrolysates (WPH). The most abundant protein in whey is beta-lactoglobulin and the second most abundant protein is alpha-lactalbumin, both offer benefits in the development of healthy alternative protein products.

The use of whey protein in food products began in the early 1990s and was initially used for sports nutrition products. Whey protein was a popular choice for these products because it is a high-quality protein, which is efficiently absorbed into the body and contains a rich source of branched chain amino acids (isoleucine, leucine and valine). Whey is also known to provide various health benefits including reduction in cholesterol, boosting immune function and weight management control. Currently, usage of whey protein is also significant within the mainstream food market where it can be used to improve the appearance, flavour, texture and aroma of foods. The neutral flavour of whey protein allows it to be added to a wide variety of food and beverages to increase protein content without affecting taste. Whey proteins are now used in yogurts, fresh and processed cheeses, beverages, baked goods, confectionery, dietetic and sports nutrition products.

BENEFITS OF WHEY PROTEIN HYDROLYSATES

Whey protein manufacturers can use protease enzymes to hydrolyse whey proteins into peptides and amino acids generating a whey protein hydrolysate product. Whey protein hydrolysates may be used in a wide variety of applications as they provide a number of benefits compared to non-hydrolysed whey protein concentrates and isolates. The benefits of using whey protein hydrolysates include:

- Improvement in heat stability. Whey protein hydrolysates are more heat stable than whey protein isolates and whey protein concentrates at certain concentrations, pH and temperatures.
- WPHs are less viscous than WPIs or WPCs and may be used to improve the texture of beverages, yogurts, and smoothies.
- WPHs offer the benefits of enhanced absorption and digestibility because they are pre-digested.
- WPHs may be added to protein bars to improve shelf life and texture.
- Improvement of foaming and emulsification properties.
- The allergenicity of whey protein may be reduced by the enzymatic hydrolysis of whey protein allergens.
- WPHs can contain biologically active peptides that may provide health benefits.
- WPHs with a bitter taste may be used to replace coffee or chocolate flavourings in ready to drink beverages.

APPLICATIONS OF WHEY PROTEIN HYDROLYSATES

A number of commercial whey protein hydrolysates are available with a degree of hydrolysis between 4-30%. Degree of hydrolysis is a term used to describe the extent to which a protein has been hydrolysed by a protease(s). Limited enzymatic hydrolysis of whey proteins is utilised to improve the functional properties of whey e.g. foaming, emulsification and gelation properties. Generally, whey protein hydrolysates designed for nutritional applications such as sports nutritional drinks, nutritional bars, enteral formulas (tube feeding) and hypoallergenic infant formulas have a moderate-high degree of hydrolysis. In order to achieve these whey protein hydrolysates the whey protein is generally quite extensively hydrolysed with a protease system. It is essential to carefully control the degree of hydrolysis using a suitable protease system to avoid the production of bitter-tasting peptides which may limit their application in foods.

The excellent nutritional and functional properties of whey protein hydrolysates, allows them to be used in a wide range of applications including:

- Acid beverages/fruit juices.
- High protein nutritional bars.
- Ready to drink (RTD) beverages.
- Tablets and supplements.
- Pet food.
- Yogurt drinks and smoothies.
- Sports nutritional products.
- Infant formulas.
- Convalescence foods.
- Meal replacement products.
- Weight management products.

APPLICATIONS OF WHEY PROTEIN HYDROLYSATES

Whey protein is a high quality 'complete' protein containing all the essential amino acids in amounts needed to support the growth of human beings. Whey contains high levels of branched chain amino acids (BCAAs); isoleucine, leucine and valine which play an important role in muscle metabolism. These amino acids are required for stimulating a high rate of protein synthesis in muscle and help protect against muscle breakdown during heavy exercise. In comparison to other dietary sources of protein, whey proteins are the richest known source of naturally occurring BCAAs containing up to 26% BCAAs. In addition, whey is a good source of the amino acid cysteine, which is essential to the preservation of muscle, particularly during exercise.

Whey proteins are absorbed more rapidly than other dietary proteins and therefore deliver more amino acids to tissues and stimulate a higher rate of protein synthesis that results in a higher net gain of protein in the body. Therefore, whey is the ideal protein to consume before, during or after periods of exercise.

Whey proteins provide a number of unique benefits to athletes:

- Rapidly digested and stimulate a higher rate of protein synthesis compared to other dietary proteins.
- Richest known source of BCAAs.
- Provides a rich source of cysteine that boosts antioxidant capacity and improves exercise performance.
- Promotes higher glycogen levels within the liver, an important storage form of energy for exercise.
- Provides greater strength gains during resistance training and improved muscle size increases during body building exercise.
- Provides a source of bioavailable calcium to help maintain bone health and prevent stress fractures that many athletes experience during training.
- Whey protein hydrolysates are ideal for use in sport beverages (or meal replacement products) for consumption before, during and 30-60 minutes after exercise.
- Directly enhances the immune system and protects against illness and infection.

The use of whey protein hydrolysates in sports nutrition products such as: beverages, high protein bars, powders, gels, pastes and tablets is increasing in popularity. Biocatalysts has developed two unique protease products called Flavorpro® 750MDP (F750MDP) and Promod® 782MDP (P782MDP) which can be used on their own or together to hydrolyse whey proteins to produce a whey protein hydrolysate with a clean, non-bitter flavour profile. These enzyme products contain carefully selected endopeptidases and exopeptidases with specific substrate specificities that can be used to produce a whey protein hydrolysate with a higher DH and good palatability. Therefore, this eliminates the need to add bitter-masking agents which are costly and can alter the composition and functionality of the end product. Biocatalysts Ltd commissioned an external flavour analyst to evaluate the flavour profiles of whey protein hydrolysate made with F750MDP and P782MDP as well as a commercially available whey protein hydrolysate. Comparison of the sensory attributes of the two whey protein hydrolysates (see Fig. 1) revealed that the WPH produced with F750MDP and P782MDP had an extremely low bitterness score whereas the commercial WPH had a much higher bitter intensity score. In addition, the aroma intensity of the WPH produced with F750MDP was lower than the aroma intensity of the commercial WPH. Sulphur/eggy and brothy/potato attributes that were detected in the commercial WPH were absent in the WPH produced with F750MDP and P782MDP.

Whey protein hydrolysates produced with Flavorpro® 750MDP and Promod® 782MDP are absorbed faster than non-hydrolysed WPI or WPC because the whey protein has been hydrolysed by the proteases into smaller sized peptides and amino acids. Therefore, athletes' recovery times can be reduced significantly from days to hours when whey protein hydrolysates are consumed compared to non-hydrolysed whey protein.

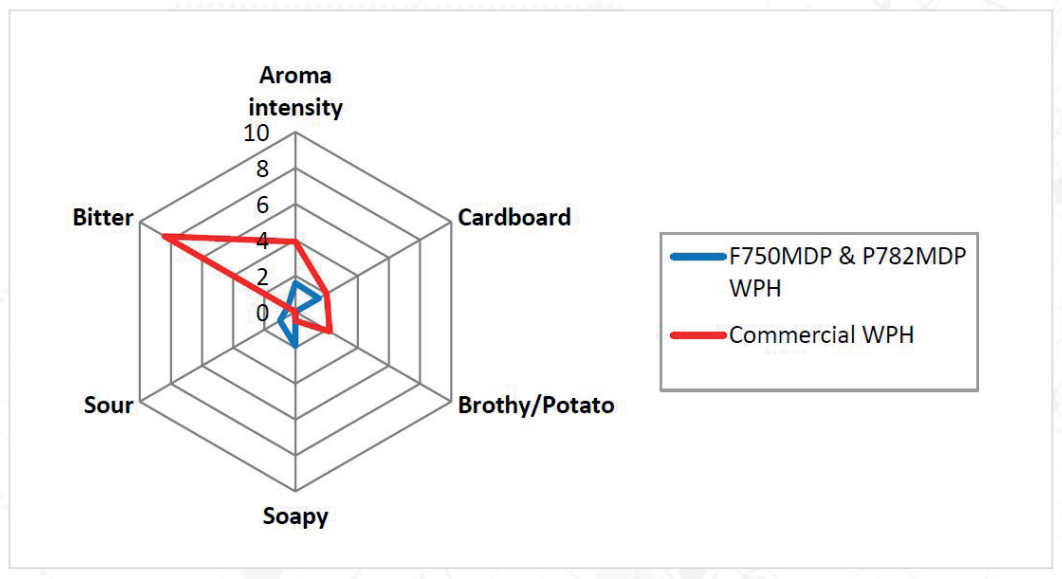


Figure 1: Sensory spider diagrams for a WPH produced with Flavorpro® 750MDP and Promod® 782MDP and a commercially available WPH (results produced by an external flavour analyst).

High protein nutritional bars are available with up to 50% protein. It is a challenge for formulators to deliver a high level of protein in the bar with a good taste and texture. WPHs with good palatability can be added to protein bars to fortify the protein level and minimise bar hardening, which often occurs in high protein bars. Hydrolysed whey proteins do not tend to draw moisture away from the other ingredients in the bar; adding WPH will help improve the shelf life of the bar by maintaining a softer texture.

Sports nutritional gel products are increasing in popularity as they are easy to carry and consume. These gel products are a high source of energy and the whey proteins in these products have been shown to help minimise muscle damage, improve endurance and aid in recovery after exercise.

UTILISATION OF WHEY PROTEIN HYDROLYSATES FOR INFANT FORMULA

It is well recognised that the ideal food for new-born infants is mother's milk, as it enhances immune functions and is hypoallergenic. However, in certain cases this is not possible and infant formula is used. Infant formula that contains unmodified cow's milk proteins may produce an early onset of allergic diseases in some infants. While the incidence of allergies to cow's milk protein is low, the symptoms are severe and, in some cases, life-threatening. The symptoms include vomiting, diarrhoea, gastrointestinal disturbances, excessive crying, eczema, loss of weight and even anaphylactic shock. These symptoms generally resolve themselves by 3 years of age. However, they may be a precursor to other food allergies, asthma, respiratory allergies etc. later on in life. The main allergen in cow's milk protein is beta-lactoglobulin. Beta-lactoglobulin is the most abundant protein in whey accounting for 50% of the total protein.

Infant formula manufacturers are increasingly adding whey proteins to infant formula. Hydrolysed whey protein can be used to reduce allergenicity. The concentration of hydrolysed whey protein can range from 48-100% of total protein in infant formulas.

The main method used to reduce the allergenicity of beta-lactoglobulin is enzymatic hydrolysis of the protein using endopeptidases and exopeptidases which display specificity for hydrolysis of beta-lactoglobulin. Whey protein hydrolysates utilised in infant formula are generally either extensively hydrolysed or partially hydrolysed.

Extensively hydrolysed infant formula contains peptides with molecular weights <3,000Da and are essentially allergen free. Partially hydrolysed infant formula offers advantages over extensively hydrolysed infant formula in that they are more cost effective to produce and more palatable. However, partially hydrolysed infant formulas contain proteins/peptides in the molecular weight range of 3,000-10,000Da and can therefore contain higher levels of allergens.

Traditionally, animal-derived enzymes such as trypsin, chymotrypsin and pancreatin have been used to produce hypoallergenic whey protein hydrolysates for use in infant formula. However, due to customers' demands for Kosher and Halal infant formula, Biocatalysts manufacture a number of non-animal proteases which are suitable for use in the production of whey protein hydrolysates with reduced allergenicity. Flavorpro® 766MDP is a non-animal derived protease system with both endopeptidase and exopeptidase activities. This product can be used to hydrolyse whey protein to produce a highly digestible whey protein hydrolysate, with a high degree of hydrolysis. The enzyme will efficiently hydrolyse beta-lactoglobulin and reduce the allergenicity of this protein. Comparison of the flavour profiles of a whey protein hydrolysate produced with Flavorpro® 766MDP (F766MDP) and a whey protein hydrolysate produced with porcine trypsin revealed that the whey protein hydrolysate produced with F766MDP had a significantly lower bitterness score as measured by an external flavour analyst (see Fig. 2).

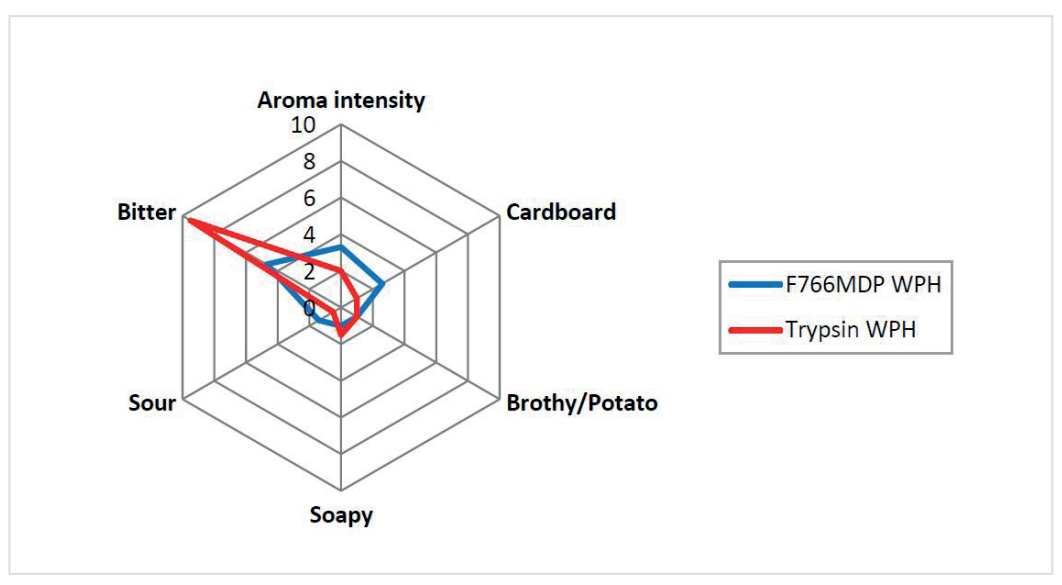


Figure 2: Sensory spider diagram for a WPH produced with Flavorpro® 766MDP and Porcine Trypsin.

TECHNICAL CAPABILITIES IN PRODUCTION OF ENZYMES FOR WHEY PROTEIN HYDROLYSIS

The development of new enzymes for hydrolysis of whey proteins involves both a scientific approach and sensory analysis. The extent to which specific proteases can hydrolyse whey proteins over time can effectively be monitored by measuring the degree of hydrolysis (see Fig. 3).

Gel filtration chromatography can be used to assess the molecular weight profile of the whey protein hydrolysate produced with a specific protease(s) (see Fig. 4 and 5).

The enzyme development work can also include the sensory analysis of whey protein hydrolysate samples by external sensory analyst experts to provide an exact description of the aroma and flavours generated by specific proteases.

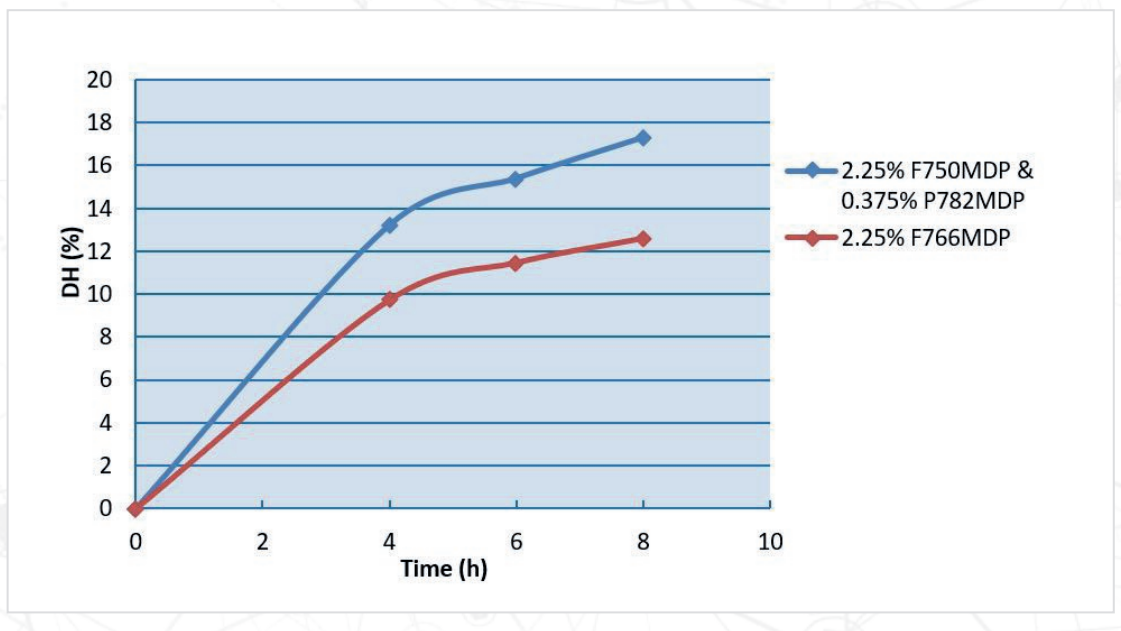


Figure 3: Hydrolysis of WPI with 2 different protease systems at 50°C over 8h.

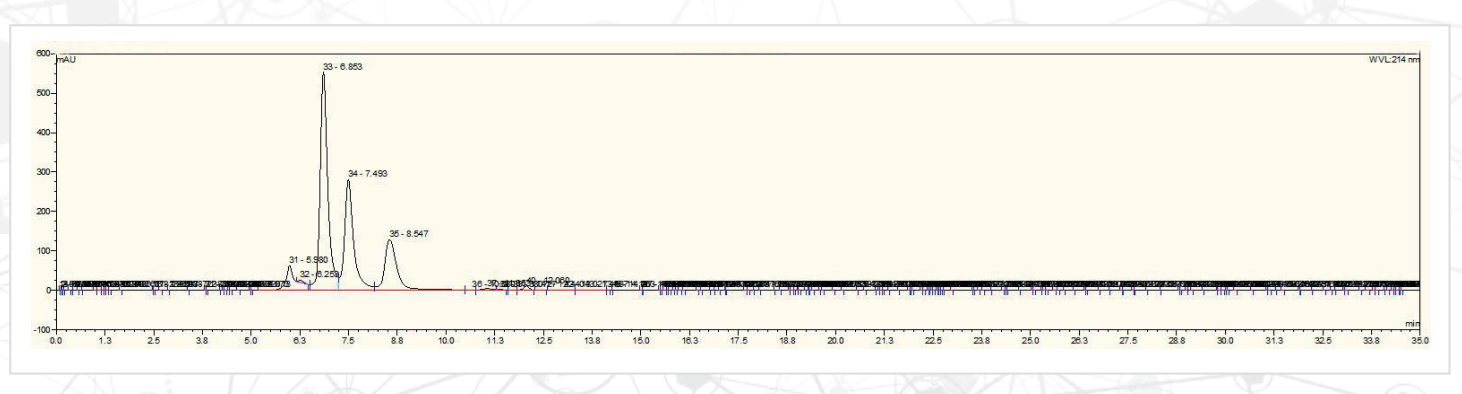


Figure 4: Gel filtration chromatogram of non-hydrolysed WPI (T0h).

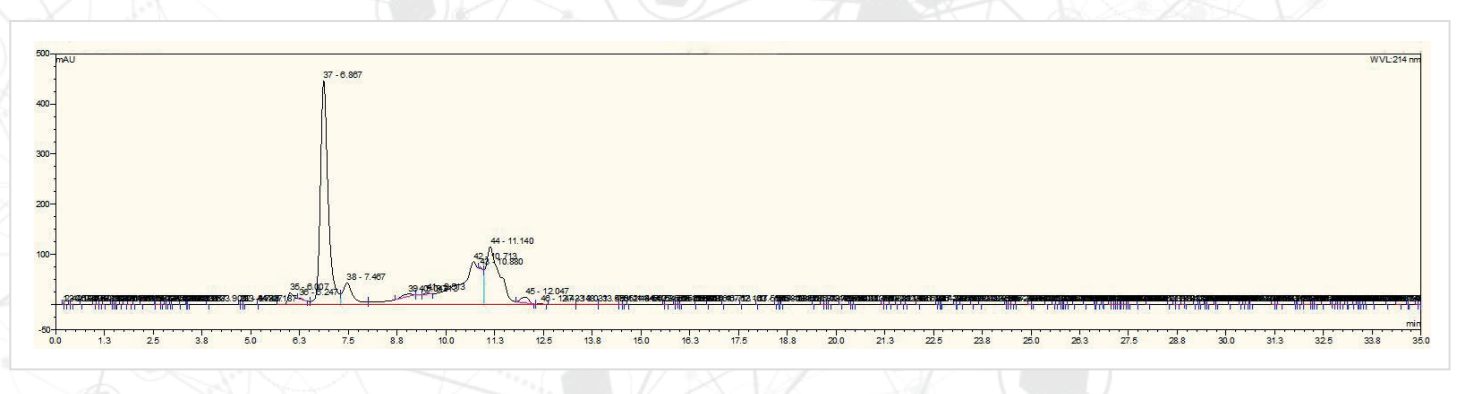


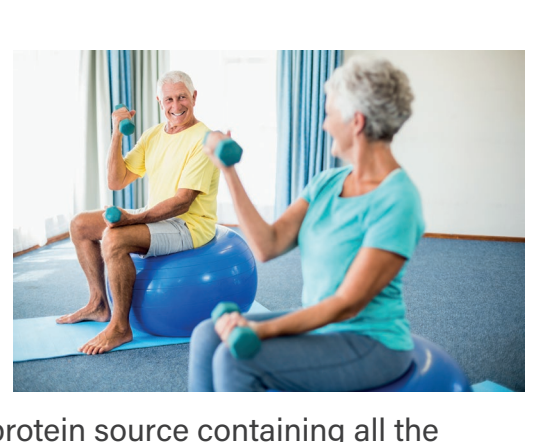
Figure 5: Gel filtration chromatogram of WPI hydrolysed with F750MDP and P782MDP for 6h at 50oC.

INTRODUCTION TO CASEIN PROTEIN

Casein is the most abundant protein in cow's milk, comprising approximately 80%. The significant growth of the whey protein market has led to an abundance of casein, and like whey, casein can also provide many nutritional benefits. As companies look to add value to casein it is important to note where casein can add value to compliment the benefits of whey protein.

Unlike whey, casein is a slow-digesting protein and therefore its role in nutritional products differs. As casein is broken down it releases the amino acids much more slowly and therefore the benefits are achieved over a longer period of time. The increased levels of amino acids in the blood can last between 4-5 hours compared to approximately 90 minutes with whey protein.

The difference in amino acid absorption rate of casein and whey protein, makes casein more suited to different applications and nutritional products. As casein is a much slower-digesting protein than whey, it is a valuable protein source to be consumed in the evening before going to bed to assist with recovery and reduce muscle breakdown whilst asleep.



Casein protein is rich in branched chain amino acids (BCAAs), and with its lower absorption rate it makes it a significant protein for elderly nutritional products. The branched chain amino acid composition of casein is particularly important for maintaining muscle strength and preventing sarcopenia, or age-related loss of muscle mass. The slow release of amino acids means casein protein can play a significant role in elderly nutrition in combating and reducing the effects of sarcopenia during the night whilst fasting. Like whey protein, casein is also a complete protein source containing all the essential amino acids a body requires for growth and repair.

The benefits of casein's slow-releasing protein can complement those also seen in whey protein to develop an all-round nutritional diet regime to rebuild muscle following exercise. Sports nutrition is one of the largest markets for whey protein. With the awareness and understanding of the benefits and nutritional value of casein, casein protein is becoming increasingly added to sports nutrition products, the slow release of amino acids over several hours supports muscle protein synthesis as well as reducing the breakdown of muscle protein through exercise.

UTILISATION OF CASEIN PROTEIN HYDROLYSATES FOR INFANT FORMULA

Dairy protein is a complete protein containing all essential amino acids, thus making it a recognised ingredient in infant formula products, as it is the closest to a mother's milk with many similar benefits. Whey protein hydrolysates are widely used in infant formula products, and more recently infant formula manufacturers are increasingly looking to add value to casein protein adding it to infant formulas. However, like whey, casein also contains allergenic proteins and can therefore also be a source of dairy intolerance in babies. Sensitivity towards casein is more common from new-born babies through to children aged up to five. The allergens present in casein protein (αS1-Casein, αS2-Casein, β-Casein and κ-Casein) can be reduced through extensive hydrolysis using enzymes. This means that casein can be either partially or extensively hydrolysed which will produce formulas with differing levels of allergenicity. Proteases can be used to break down (hydrolyse) the casein protein into small peptides and amino acids, this enzymatic treatment of casein can improve its digestibility and improve tolerance for casein protein formulas. It also improves its functional properties increasing solubility and dispersibility making it a more versatile ingredient and easier to process and incorporate into food products.

Benefits of Casein Protein Hydrolysates in Infant formula:

- Keeps babies fuller for longer as it takes longer to digest,
- Bolsters the immune system
- Supports muscle growth and development
- Boost the development of antioxidant function
- Promotes healthy weight and metabolism
- Contributes to the maintenance of healthy triglyceride levels

PRODUCTION OF CASEIN PROTEIN HYDROLYSATES FOR FOODS FOR SPECIAL MEDICAL PURPOSES

Dairy protein is a valuable food ingredient not only in early nutrition, but across all ages. Whey is currently the most common of the two dairy proteins incorporated into nutritional food products, due to its availability from the cheese-making process. However, casein offers different benefits to whey and as these become more widely understood companies are looking at including casein in their products, in particular into Foods for Special Medical Purposes (FSMPs). Foods for Special Medical Purposes are specialist food products developed specifically for individuals with medical conditions that negatively impact their ability to achieve the right nutritional balance through a normal diet. As a slower release protein, casein hydrolysates offer greater nutritional benefits to those individuals with limited digestion and nutrient absorption.

For individuals with limited nutrition due to a medical condition, foods with 'available' protein becomes more important, and in these instances, focus is on the quality rather than quantity of protein present. The Digestible Indispensable Amino Acid Score (DIAAS) is a method of measuring the quality of protein to determine which protein sources deliver the greatest nutritional benefit based on how the human digestive system digests and absorbs the amino acids. As complete proteins, casein and milk protein concentrates rank among the highest in terms of DIAAS. The difference in speed of amino acid absorption, especially in casein, contributes to its effectiveness as a protein achieving greater absorption and therefore a higher DIAAS, making it an ideal ingredient in FSMPs.

Changing the functionality of casein can make it more easily formulated into specialist medical food products. Enzymes, such as Promod® 517MDP can be used to hydrolyse casein, breaking the protein down into smaller peptides and amino acids. This hydrolysis of casein using enzymes pre-digests the protein enhancing the absorption of essential amino acids, improving the overall digestibility of casein protein, and increasing its solubility making it easier to formulate into medical food products.

TECHNICAL CAPABILITIES OF PROMOD® 517MDP FOR CASEIN PROTEIN HYDROLYSIS

The extent to which proteases hydrolyse casein protein is determined by measuring the degree of hydrolysis. The degree of hydrolysis of a protein is defined as the percentage of peptide bonds cleaved in the protein. For every peptide bond hydrolysed a free α-amino group is formed. The free α-amino groups react with OPA (O-Phthaldialdehyde) and form a yellowish complex. The optical density is measured spectrophotometrically at 340nm.

Promod® 517MDP is a highly active exopeptidase that is capable of achieving >40% degree of hydrolysis of casein protein. Figure 6 demonstrates the degree of hydrolysis that can be achieved using between 1-3% dosage rates of Promod® 517MDP on sodium caseinate over 6 hours. To determine the levels of hydrolysis that can be achieved, a 10% sodium caseinate solution and the required dosage of Promod® 517MDP was incubated with agitation at pH 7 and 60°C.

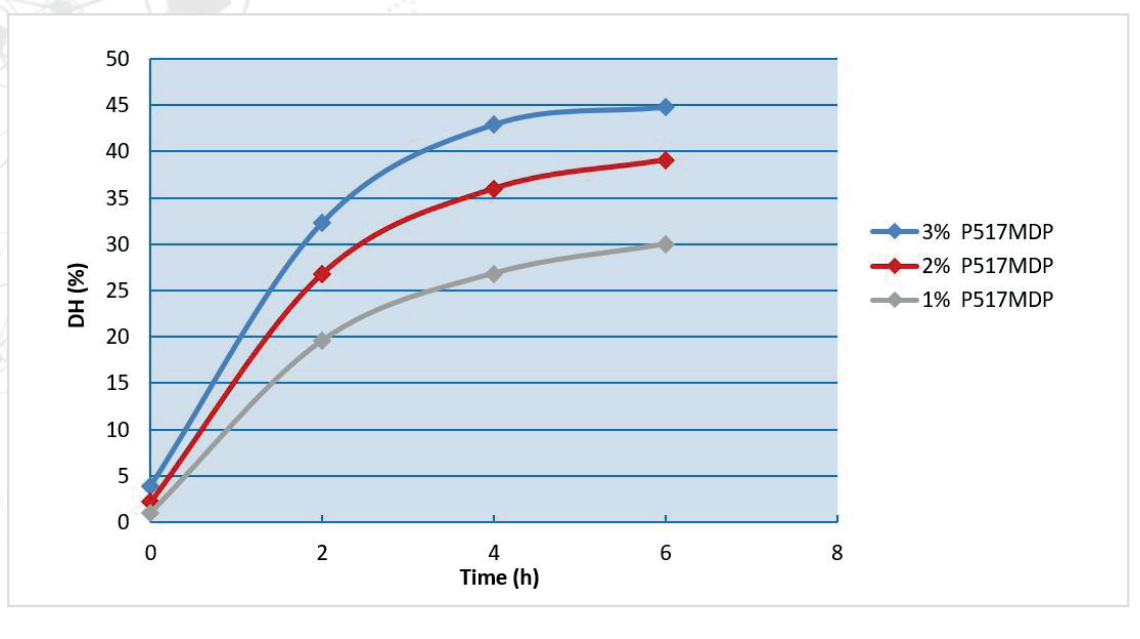


Figure 6: Hydrolysis of sodium caseinate with Promod® 517MDP over 6h.

Promod® 517MDP has also been developed to have a high concentration of Leucine aminopeptidase (LAP) activity. It catalyses the hydrolysis of amino acid residues from polypeptide chains, especially from leucine containing peptides. Leucine is one of the essential amino acids we need to survive and is responsible for stimulating the body to synthesise protein and build muscle. Caseinates contain approximately 8% leucine, when hydrolysed using a protease with a high Leucine aminopeptidase activity they can produce a casein protein hydrolysate with a rich source of leucine enhancing its use as an ingredient in infant formula.

HEAT STABILITY

When producing dairy protein hydrolysates, once the desired reaction and degree of hydrolysis of the protein has been achieved, the solution must be heated at 80-90°C for 30 minutes to inactivate the enzyme. It is common in whey protein hydrolysis to observe the whey hydrolysate to gel when it is heated at temperatures required to completely inactivate protease activity. This can be due to a number of different reasons: the enzyme specificity, the degree of hydrolysis achieved and also the whey substrate itself. The thermal deactivation of the enzyme can therefore negatively affect the structural integrity of the whey protein hydrolysate.

Casein hydrolysate produced with Promod® 517MDP with a DH in the range of approximately 35-45% displayed good clarity and heat stability under the conditions required to inactivate Promod® 517MDP activity (85°C for 30 minutes).

TABLE 1: CHOOSING THE RIGHT ENZYME FOR YOUR APPLICATION

Enzyme Product(s)	Dairy Protein	Application of WPH	Benefit of WPH
Flavorpro® F750MDP & Promod® 782MDP	Whey Protein	High Protein Nutritional Bars	• Maintenance of a soft texture & improvement in shelf life. • Enhanced absorption & digestibility. • Protein fortification. • Bland, non-bitter flavour profile.
Flavorpro® F750MDP & Promod® 782MDP	Whey Protein	Powdered Mixes	• Excellent dispersibility & solubility. • Bland flavour at high concentrations. • High digestibility. • Abundant source of BCAAs.
Flavorpro® F750MDP & Promod® 782MDP	Whey Protein	Tablets & Supplements	• Good flowability & compressibility. • Moderate-extensively hydrolysed WPHs are suitable. • Abundant source of BCAAs. • Good taste (non-bitter).
Promod® 523MDP Flavorpro® 766MDP	Whey Protein	Infant Formula	• Low allergenicity. • WPHs with molecular weights <3kDa (extensively hydrolysed infant formula). • WPHs with molecular weights between 3-10kDa (partially hydrolysed infant formula). • WPHs with low/no free amino acids. • Acceptable taste.
Promod® 517MDP	Casein Protein	Infant Formula	• High performance exopeptidase for extensive hydrolysis. • High concentration of LAP activity. • Achieve high DH of casein protein. • Improving solubility • Reduces allergenic casein proteins.

TABLE 2: COMMON PROBLEMS ENCOUNTERED WHEN USING ENZYMES IN THE PRODUCTION OF DAIRY PROTEIN HYDROLYSATES

Problem	Enzyme Solution
Whey protein hydrolysate gels on heating.	Increase the enzyme dose to improve the hydrolysis of the whey proteins.
Whey protein hydrolysate has a bitter taste.	Reduce the enzyme dose to decrease the degree of hydrolysis to approximately 10%.
Whey protein hydrolysate has slight savoury or brothy flavours.	Reduce the enzyme dose to decrease the amount of free amino acids produced.

TABLE 3: BIOCATALYST'S RANGE OF ENZYMES FOR DAIRY PROTEIN HYDROLYSIS

Product	Activity	Optimum pH	Optimum Temperature	Specificity
Flavorpro® 750MDP	55 Casein Protease U/g	5.5 - 7.5	45 - 55°C	Leu, Phe, Lys, Met, Glu, Val, Thr, Cys
Flavorpro® 766MDP	204 Leucine Aminopeptidase U/g	5.0 - 7.5	45 - 55°C	Arg, Lys, Ala, Tyr, Gly,Thr, Met, Cys, Phe, Leu, Glu, Val
Promod® 144MDP	Multiple strengths available: 100 & 300TU/mg	5.0 - 7.0	50 - 60°C	Arg, Gln, Ser, Thr, Ala, Lys, Phe, Leu
Promod® 184MDP	400 Casein Protease U/g	6.0 - 7.5	50 - 55°C	Arg, Lys, Ala, Tyr, Gly
Promod® 523MDP	1,200 GDU/g	6.0 - 7.5	50 - 55°C	Arg, Lys, Ala, Tyr, Gly
Promod® 782MDP	1,720 Anson Protease U/g	6.0 - 8.0	45 - 55°C	Arg, Gln, Ser, Thr, Met, Ala, Phe, Leu
Promod® 517MDP	700 Leucine Aminopeptidase U/g	6.0 - 8.0	45 - 55°C	Leu, Phe, Lys, Met, Glu, Val, Thr, Cys