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Plant-Based Hydrolysates for Alternative Protein Applications: Technology, Uses and Challenges

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Significance

Plant-based hydrolysates are a processed form of plant-biomass in which the molecular chains (biopolymers) are broken into smaller fragments. [1] Hydrolysates are known to improve nutritional properties and, in some cases, improve organoleptic properties as well. [2] This has various implications of value across multiple applications and market segments. [3] Here, we explore the potential implications for alternative proteins.

Technology overview

Enzymatic hydrolysis of plant biomass has been researched by scientists for a number of decades and was selected as a key area for biomass technology development in the 1980s by the US department of energy. [4] However, the project was deemed too risky since the cost of extracting the enzymes needed for hydrolysis was too high. [5] However massive cost reduction was since then achieved after the Cellulase enzyme was discovered in *Trichoderma reesei* mold, and the mold was later optimized at Rutgers University as an efficient enzyme production host using classical mutagenesis methods. [6] Enzymatic hydrolysis of cellulosic biomass is now widely recognized as a viable strategy for increasing yields and lowering the cost of fermentation reactions because the hydrolyzed biomass is more bioavailable than the unhydrolyzed biomass and is lower in cost than refined sugar feedstocks.

Cellulase and Peptidase are the most commonly used enzymes. The hydrolysates are produced in bioreactors at a controlled temperature of about 40-50C. An enzyme in combination with an acid or base is used to split biopolymers into smaller fragments **(Figure 1)**. [7] This can also be described as a nucleophilic attack of a water molecule on the covalent peptide bond between amino acids. Depending on the pH of the medium, the release of carboxyl and amino groups are in their dissociated or protonated forms which can affect the functionality of the resulting hydrolysate. [8] Hydrolysis of biopolymers is not exclusive to proteins; starches and fibers can be hydrolyzed as well. [9]

[1] "Modification of plant proteins for improved functionality: A review." 4 Jan. 2021, https://ift.onlinelibrary.wiley.com/doi/abs/10.1111/1541-4337.12688. Accessed 19 Dec. 2023.
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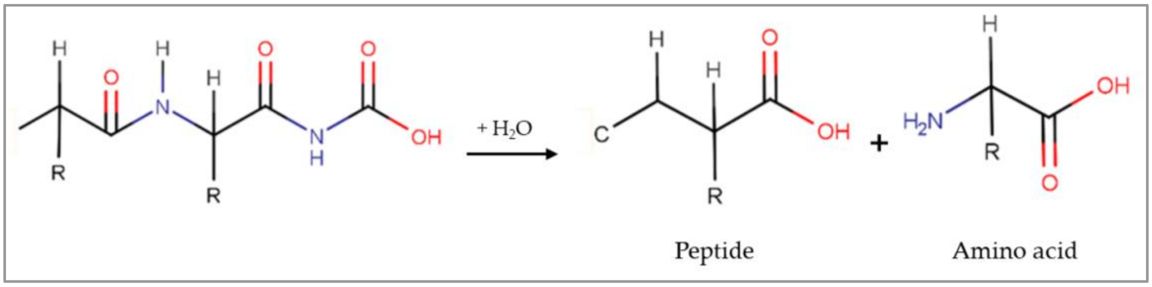

Figure 1. *Lewis representation of the main hydrolysis reaction. Adapted from Heredia-Leza et al. 2022.*

The hydrolysis reaction is continued until the desired level of hydrolysis is achieved. Stopping the reaction is most commonly achieved via heat inactivation of the enzyme. The level of hydrolysis can be measured using a number of methods but is most commonly measured using the Degree of hydrolysis (DH). Using this method, the percentage of peptide bonds which have been cleaved can be proxied via the amount of acid or base needed to maintain a constant pH during the hydrolysis reaction.

The functional benefit depends on the degree of hydrolysis performed and the pH at which it was performed and stored. This is mainly due to the effective density of free hydrophobic/hydrophilic regions which become exposed in the solution after being hydrolyzed **(Figure 2)**. Therefore, it is immensely clear that a thorough characterization of the substrate protein structure and the enzyme's specificity is needed in order to achieve the desired result.

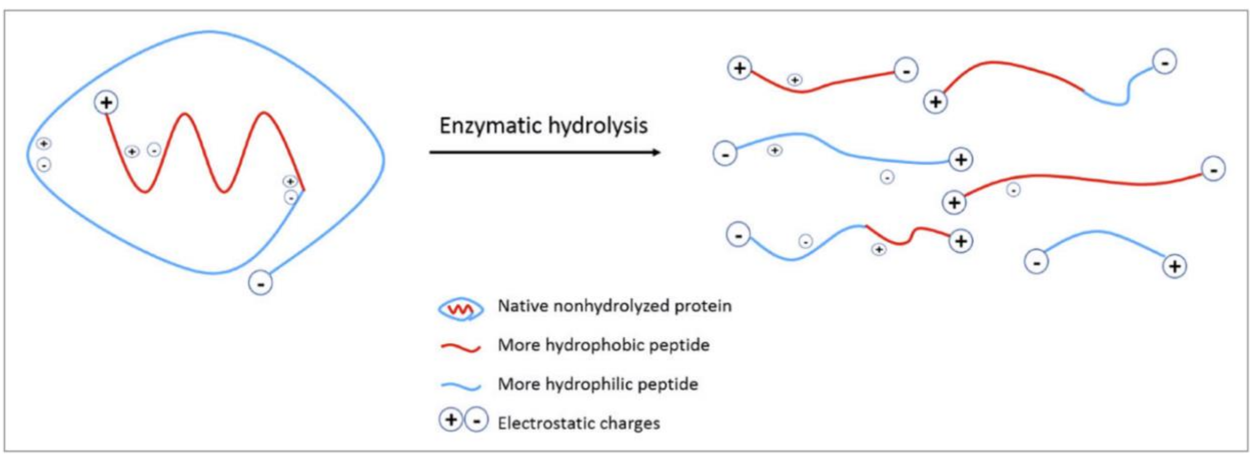

Figure 2. *Illustrative overview of the effect of enzymatic hydrolysis on the tertiary structure of proteins. The native structure, where more hydrophobic regions are shielded by hydrophilic regions (or vice versa) become exposed after being broken down into smaller peptides. Adapted from Wouters et al. 2016.*

Benefits and Uses

Historically, the main applications of hydrolysates in the food system were hydrolyzed dairy proteins for infant nutrition (reducing allergic reactions and improving nutrition) and hydrolyzed plant-protein for animal feed (improving nutrition). [10,11]

Within the traditional vegetarian and vegan food industry, hydrolyzed vegetable protein (HVP) or hydrolyzed plant protein (HPP) has been extensively used as a flavoring agent to give a meat-like flavor to processed food products. [12,13,14] In some products, this has been superseded by yeast extracts, due to the potential concerns around MSG and 3MCPD (see challenges section below for more detail). HVP/HPP has also been included in extrusion processes for increasing the protein content in snack items. [15] Hydrolyzed soy protein has also been used in soymilk for improved whippability and nutrition. [16,17] However, there may be additional applications for which HVP/HPP can be used in alternative proteins in addition to flavoring.

There is significant marketing around so-called "bioactive peptides" resulting from hydrolysates for improving human health and treatment of various diseases including diabetes. This is based on the concept that a pseudo-randomly hydrolyzed protein set will yield exposed motifs which mimic the valency required to interact with the body in a beneficial way. While the peptides are considered safe, it's not clear whether the benefits are significant or meaningfully large. [18,19,20,21] More research is needed to understand the health benefits and establish defined protocols for extraction of different peptide mixtures with stable and well characterized effects. [22,23]

For alternative protein products, the functional benefits of hydrolysates are mainly summarized as increased whippability, foam stability, solubility, and water absorption. [24,25] In some manufacturing of plant-based meat, hydrolyzed plant-protein is used for its increased solubility and cross-linking capacity. [26] Protein solubility is a balance of hydrophobic and hydrophilic interactions. The presence of sufficiently exposed hydrophilic residues after hydrolysis can tip the scale of interactions such that protein-water interactions are stronger than protein-protein interactions - thereby making the protein more soluble. Additionally, smaller proteins tend to be more

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[10] "Understanding infant formula - ScienceDirect.com." https://www.sciencedirect.com/science/article/abs/pii/S1751722219301362. Accessed 8 Jan. 2024.
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[23] "Bioactive peptides: A review | Food Quality and Safety." https://academic.oup.com/fqs/article/1/1/29/4791729. Accessed 24 Jan. 2024.
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[26] "Plant-based and cell-based approaches to meat production - Nature." 8 Dec. 2020, https://www.nature.com/articles/s41467-020-20061-y. Accessed 8 Jan. 2024.

soluble than larger ones because of the reduced complexity and need for tipping the hydrophobic/hydrophilic interaction scale (higher increase in entropy). [27] For both of these reasons, hydrolysis generally improves protein solubility profoundly. Protein solubility is extremely important as it is a prerequisite for most of the other functionalities relevant to alt protein. Water holding capacity and fat holding capacity are important for determining the juiciness, mouthfeel, and tenderness of food products. [28,29] The amount of water or fat which is physically bound to the proteins depends on the strength of those interactions which is influenced by the availability of exposed hydrophilic/hydrophobic or positive/negative charges as well. Therefore, hydrolysis can significantly increase both water and fat holding capacities. Gelation is also undoubtedly of high interest to alternative proteins for use in both plant-based fat, muscle, and egg applications. Gelation is a result of protein-protein interactions between denatured peptides. Although different interactions can be present such as covalent bonds, hydrophobic/hydrophilic, charge based, etc... Generally, hydrolysis negatively impacts gelation since the molecular mass of the proteins is reduced and therefore the average number of interactions per protein reduces. There are certain cases where partially hydrolyzed protein can increase gelation, although the conditions need to be very precisely tuned for this. [30] Foaming and emulsification are also important to alternative protein, mostly for dairy applications. These properties are a result of surface interactions and assembly of proteins at interfaces (oil-water or water-air or air-oil). Generally, hydrolysis increases these functionalities because the smaller protein fragments can diffuse, adsorb, and arrange themselves at interfaces. However, the effects of hydrolysis on foaming and emulsification are more complicated than that of solubility, as they can easily be negatively affected by the wrong conditions. [31]

HPP in combination with extrusion has been shown to produce an effective emulsifier out of soy protein. [32] Additionally, there is a white space around harnessing the ability for hydrolysis to modulate the texture of extruded meat analogues. HPP can be included prior to the extrusion or hydrolysis can be performed after the extrusion. One study performed hydrolysis after extruding soy protein at different temperatures and found that the hydrolysis increased the water holding capacity and increased the number of disulfide bonds, thereby modulating the overall texture. [33] Another study mixed hydrolyzed wheat gluten with soy protein concentrate prior to extrusion and also demonstrated the ability to modulate texture, with greater mimicry to chicken meat with respect to hardness, chewiness and toughness. [34] High protein digestibility and antioxidant content also alluded to improved nutrition.

[27] "Physical Chemistry of Foods | Pieter Walstra | Taylor & Francis eBooks." 8 Oct. 2002, https://www.taylorfrancis.com/books/mono/10.1201/9780203910436/physical-chemistry-foods-pieter-walstra. Accessed 8 Jan. 2024.
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Plant-based hydrolysates may also be used for alternative feedstocks for fermentation and cultivated meat applications, thereby lowering the cost. [35,36,37] This makes use of the coincidental bioactivity of peptide motifs which are pseudo-randomly generated during the hydrolysis process and can mimic the bioactivity of animal-based growth factors and supplements. While some scalable processes utilizing hydrolysates have been precedented, this technology still requires much more screening and validation. This serves as another white space in the academic sector since a significant portion of this research for alternative protein applications is considered intellectual property of companies in the industry.

Challenges

In terms of flavor, it was found that hydrolysis increases umami flavor, although further hydrolysis into very small peptides can cause bitterness due to the accumulation of hydrophobic residues. [38,39,40] However, another study found that the amount of bitterness and umami depends on the specific preparation of the enzyme with some preparations providing for low bitterness and high umami flavors. [41,42,43] People have also demonstrated several post processing techniques to de-bitter hydrolysate products, however this technology is only in its infancy. [44,45] This potential issue with hydrolyzed protein could warrant the need for alternative technologies, which can similarly break down or modify proteins and expose functional domains without imparting negative off-flavors.

In terms of safety, the hydrolysis of plant proteins was shown to generate 3-Monochloropropane-1,2-diol (aka 3-MCPD), a molecule which has a contentious safety record. The FDA considered 3-MCPD as a potential carcinogen and provides GRAS certification for use <1 ppm. [46,47] California specifically lists 3-MCPD under its proposition 65 for causing cancer. [48] However these conclusions were drawn based on rodent studies, which are not always reliable predictors of carcinogen discovery in humans. [49,50] To this date, no carcinogen studies

[35] "Market Trends Driving the Embrace of Plant-Based Cell-Culture ...." 6 Dec. 2023, https://www.pharmasalmanac.com/articles/market-trends-driving-the-embrace-of-plant-based-cell-culture-media-ingredients. Accessed 18 Dec. 2023.
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have been performed in humans. In 2018, EFSA released a revised safety statement which relied on an "updated scientific approach" stating that 3-MCPD is considered safe for most consumers but is a potential health concern for infants. [51] 3-MCPD is also found in various refined plant oils, soy sauce, and other processed foods and sauces which utilize high temperatures during production. Additionally, it is worth noting that the production of HVP/HPP does produce free glutamic acid (aka MSG). which is known to have controversial consumer perception despite the evidence to the contrary having little relevance to normal dietary human intake patterns. [52] The FDA considers MSG as GRAS. [53]

Conclusion

There is a large and underexplored potential for plant-based hydrolysates to serve important roles in alternative proteins. Currently, it is understood that the main functional benefits are increased solubility and water/fat holding capacity. However, there is a significantly large white space of matching the enzyme to the substrate for perhaps increased functionality in alternative protein applications. There are potential opportunities in extruded meat analogues and various dairy products which are yet to be explored. Additionally, the functional benefits of hydrolyzing plant starches and fibers is not studied in the literature and may be a white space for the alternative protein field as well. Further research on the nutritional benefits and the ability to lower the cost of fermentation and animal-based cultures should be executed. The potential negative off-flavors and consumer perceptions for safety should be considered when designing and using these products.

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