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Application of Biotechnologycal Methods for Removal of Phenylalanıne from Different Protein Sources

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Authors' contributions

This work was carried out in collaboration between all authors. Authors SKU and MÇ designed the study. Author HKY managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Inborn errors of amino acid metabolism include inherited biochemical disorders in which a specific enzyme defect interferes with the normal metabolism of protein. In these disorders as a result of diminished or absent enzyme activity, certain compounds accumulate in the body to toxic levels. Metabolic disturbances can lead to severe cognitive impairment and even death. Phenylketonuria, is the most common amino acid disorder. Dietary restriction prevent the accumulation of a substrate to toxic levels to a certain extend but application of biotechnological methods could provided more promising tools. The main topics discussed are: removal of phenylalanine by activated carbon, by tailored enzymatic hydrolyses of cheese whey, by modified corn cobs as adsorbents and by using enzymatic membrane reactor.

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1. INTRODUCTION

1.1 Inborn Errors of Metabolism

Inborn errors of metabolism (IEM) include inherited biochemical disorders in which a specific enzyme defect interferes with the normal metabolism of protein, fat or carbohydrate. As a result of diminished or absent enzyme activity certain compounds accumulate in the body to toxic levels. IEM are chronic diseases that can develop from infancy through adulthood and affect the entire family system [1]. Clinical presentations and symptom profiles are varied and include episodes of metabolic decompensation, neurological symptoms, multi-system organ involvement, developmental delays, learning disabilities, and behavioral problems [2,3]. Through early identification and initiation of treatment, many of the adverse outcomes of IEM can be mitigated or prevented. For many IEM, treatment strategies rely on the provision of specialized medical foods and dietary supplements [4].

1.2 Aminoacidopathies

In phenylketonuria, homocystinuria and maple syrup urine diseases, specific amino acids accumulate to toxic levels. Moreover, in all three disorders, the concentrations of the relevant amino acid (s) remain unacceptably high, even on the minimum safe intake of natural protein. Management, therefore, requires a diet extremely low in natural protein, and supplements of all the other amino acids [5].

1.2.1 Phenylketonuria

Phenylketonuria (PKU) is the protein metabolism disorder caused by an inherited mutation in the gene encoding phenylalanine hydroxylase which is an enzyme involved in the metabolism of phenylalanine (Phe). As a result of mutations in the phenylalanine hydroxylase enzyme it's activity may be lost fully or partially. The result of this, Phe and metabolites formed with it's transamination (phenyl pyruvic acid, phenyl lactic acid, phenylacetic acid) accumulate in the patients' blood, urine, other body fluids and brain and caused serious damages in brain functions of human. These metabolic abnormalities lead to toxic levels of brain Phe; inadequate neurotransmitter synthesis; intellectual disability; and abnormal motor, neurocognitive, and behavioral outcomes.

Nutritional treatment for PKU was first used successfully in 1951, prior to the implementation of state public-health newborn screening programs since that time, nutritional treatment for PKU has been refined. When initiated within the first weeks of life and maintained throughout life, an appropriately designed nutritional treatment regimen can enable individuals with PKU to achieve and maintain normal intellectual development. Phe is an essential amino acid, meaning that it can't be made in the human body and must be obtained from food sources. Phe's essential nature has allowed for the development of nutritional treatment for PKU that remains the standard of practice today. Dietary Phe from intact protein sources can be restricted to the amount that allows for normal growth and development while preventing excessive build-up of Phe in the blood [6,7,8,9].

1.3 Dietary Modifications

Up to know many inborn errors of metabolism are treated by dietary modification. There are three main strategies. Some disorders lead to deficiency of a crucial product, which can be supplied by a special diet. In other disorders, dietary restriction can prevent the accumulation of a substrate to toxic levels. Example include the use of a low-phenylalanine diet in PKU. In last cases, catabolism during minor illnesses can lead to acute problems [5,10,11,12].

2. APPLICATION OF BIOTECHNOLOGYCAL METHODS FOR REMOVAL OF PHENYLALANINE

The main treatment of PKU used today is using of Phe -restricted diet. Generally, the recommended Phe levels in blood is 2-6 mg / dL. Diets that are high in Phe content such as meat, milk , cheese, eggs, fish and cereals are not recommended. Foods low in Phe content such as fruits, vegetables, grains given in appropriate quantities. In order to keep the blood Phe content as required these patients (90% of babies and 50% of children) must consume diet with special composition of amino acids. Since the proteins used to support the amino acid mixtures have unpleasant taste and smell, cause difficulties in diet applications. Both dietary compliance as well as the advancements in science leads to new strategies in the treatment of PKU. Among these are using of neutral amino acid tablets along with diet, using of BH4 (Tetrahydrobiopterin) in patients with BH4 deficiency, enzyme replacement therapy, gene therapy. All alternative treatment options have some advantages and disadvantages. Given the current progress only tetrahydrobiopterin therapy (only 1-2% of patients) it seems likely that the complete release of the diet. Other than that, all options are carried out in combination with diet therapy.

Most of the methods used for amino acids removal from protein hydrolysates are based on the principle that a sufficient amount of amino acid is liberated by enzymatic hydrolysis and the free aminoa acids is, then, removed by gel filtration, adsorption by activated carbon or resins [13,14,15,16]. Protein hydrolysis by using specific proteases may provide changing nutritional, bioactive and functional properties of food proteins, improve digestibility, modified sensory quality (such as texture or taste), improve antioxidant capability or reduce allergenic compounds of foods. Protease applications in industrial processes are advantageous compared to chemical processes due to hydrolysis specificity, product preservation, purity and reducing environmental impact [17,18,19,20].

2.1 Using of Active Carbon

In most of studied Phe is removed from protein hydrolisates by enzymatic hydrolyses and then liberated Phe is separated by gel filtration, adsorption by active carbon or resins. Different protein sources required different conditions (different agents / mixing ratio / temperature) of mentioned methods. In a study evaluating the effects of different conditions for removal of Phe as the protein source was investigated skimmed milk powder. Enzymatic hydrolyses were done by six different proteases from *Aspergillus oryzae* isolated or in association with papain and the best concentration of active carbon / casein ratios were determined. In the same study was stated that the removal of Phe (98%) was higher comparing with previous studies even using of one enzyme for protein hydrolyses with duration of 5 hours [15].

2.2 Using of Tailored Enzymatic Hydrolyses of Cheese Whey

One of the ways for using of whey as a product with low Phe is it's modifications with enzymes. One of the advantages of enzymatic proteolysis is to produce tailor-made peptides that could be used with different purposes. Additionally, such sequential specific enzymatic reactions allowed being produced products with desired properties such as lower bitterness. Considering these privileges, sequential hydrolyses of cheese whey by trypsin, chymotrypsin and carboxypeptidase A (CPA) immobilized on agarose - glyoxyl matrix, which facilitates using of biocatalyst for many times were evaluated. During hydrolysis were employed different enzyme/substrate ratios. After hydrolyses Phe was removed by the action of CPA. The best results were obtained by using directly chymotrypsin for whey protein hydrolyses at 55ºC pH= 8.0. The phenylalanine (98%) released by CPA (45ºC, pH = 7.0) was separated by membrane filtration. Developed empirical model for the apparent reaction rate allowed the prediction of the concentration of ''hydrolyzed peptide bonds" during the proteolysis with immobilized chymotrypsin. Results demonstrated the obstacles present during sequential hydrolyses of cheese whey [21].

2.3 Using of Thermo-chemically Modified Corn Cobs as Adsorbents

The removing of Phe from the protein hydrolisates are based on adsorption of this amino acid then continued with desorption and crystallization. Most of the adsorbent used such as activated carbons zeolites, ion exchanges and resins are associated with high production and generation cost, that's why recent studies are focused on potential adsorbent obtained from agricultural wastes due to their low costs and renewable properties. In a study done by Clark et al. [22], defective coffee bean pres cakehave been exploded. Since using of such materials lead to some physico-chemical changes in precursor material, the other material such as corn cobs were also evaluated [23]. In the conducted study are explained the adsorption mechanism (hydrophobic reactions and ionized carboxylic group of Phe at the adsorbent surface) of the removal of Phe.These examples emphased the potential of agricultural wastes to be used as an absorbent material.

2.4 Using of Enzymatic Membrane Reactor for the Production of Whey Hydrolysates

As was stated before, one of the most important problems with commercial protein hydrolysates which are obtained by chemical hydrolysis of different protein sources are the unpleasant tastes of products. In case of batch-type hydrolysis, hydrolyzed peptides may have competitive inhibition effects over enzymes and reduced the productivity. Considering these disadvantages, CPA was used as immobilized enzyme (on glyoxyl –agarose gel beds) and membrane was used for separation of the product. The results demonstrated higher productivity (conversion of 85%, product $275x10^{-7}$g/mg Phe $U_{H\text{-}PHE}$) than conventional procedure (83%, product $186x10^{-7}$g/mg Phe $U_{H\text{-}PHE}$). It was confirmed that the removal of products promoted by the enzymatic membrane reactor enhances reaction rates, due to the reduction of inhibition effects. A mathematical model of the enzymatic membrane reactor was also presented and validated [24].

2.5 Using of Protein Hydrolysates Prepared with Rice

Some researches demonstrated that foods rich in oligopeptides, tripeptides could be utilized more effectively during preparation of foods for patients with PKU. In the first stage, oligopeptides are formed. After this stage, peptides are broken down to di- and tripeptides.

Finally, proteins are absorbed in the form of di- and tripeptides or free amino acids. In some studies were reported advantages of di- and tripeptides over free amino acids in terms of absorption speed. The highest Phe removal were determined for di- and tripeptide content, 79% and 77%, respectively [25]. Additionally, such proteins have higher nutritional values comparing with intact proteins. Considering these advantages, Lopez et al. [26] proposed rice protein, after hydrolyzing it with two pancreatines and removing Phe with active carbon as adsorbent. Results demonstrated that both pancreatines could be used effectively for obtaining the rice protein hydrolysates with low Phe content. With one of the pancreatine was obtained much more smaller large peptides and higher oligopeptide contents.

3. CONCLUSION

Proteins used to support the amino acid mixtures have unpleasant taste, smell and cause difficulties in diet applications. The dietary compliances could be overcome by the advancements in food biotechnology and bring new strategies in the treatment of PKU. The removal of phenylalanine by active carbon, by tailored enzymatic hydrolyses of cheese whey, by modified corn cobs as adsorbents, by using enzymatic membrane reactor and by using of protein hydrolysates prepared with rice are promising studies to be continued.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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