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Application of melon serine protease in food processes

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ABSTRACT

Proteases are one of the industrially most important enzymes, the catalytic function of which is to hydrolyze the peptide bonds of proteins. Serine proteases are a subfamily of endopeptidases that are useful in a variety of applications, especially in the food processing industry. Cucumisin is an extracellular thermostable alkaline serine protease that is expressed at high levels in melon fruits (*Cucumismelo*L.). This focused review encompasses an overview on plant serine proteases (especially cucumisin) and their classification, mechanism and food applications mainly of melon source in a handy module.

KEYWORDS

Cucumisin;
Milk clotting;
Meat tenderness;
Bakery,
Plant coagulant;
Proteolytic activity.

INTRODUCTION

Melon (*Cucumismelo*L. 2n=24) is an economically important vegetable which is subdivided into six cultivar groups Cantaloupensis, Inodorus, Flexuosus, Conomon, Chito-Dudaim and Momordica[1]. Melon (Cucumismelo L) is an important and a popular horticultural crop in the world with an overall annual production of 26.8 million tons and planted area of about 1.3 million ha[2] where China, Turkey, Iran, United States and Spain are the major producers. In 2010, China had the largest melon-cultivating area[3]. Melon can grow anywhere in the world due to its good adaptation to various soil types and climatic conditions. The melon fruit contains 93% water and small amount of carbohydrates, sugars, vitamins, minerals and enzymes. Nutritionists claim that daily consumption of melon can prevent stroke, decrease cholesterol and add energy.

Proteases are one of the most important classes of proteolytic enzymes widely distributed in the animal, plant and as well as microbes (bacteria, fungi and yeasts). Proteases catalyse the cleavage of polypeptides to oligopeptides or amino acids. The importance of proteases lies in their versatility as agents of many critical biological processes that include the vital functioning of the regulating metabolism, gene expression, pathogenicity, modification of enzymes, and in the hydrolysis of larger proteins to short peptides for transportation and metabolism[4]. These enzymes carry out proteolysis i. e. break down proteins by hydrolysis of the peptide bond that exists between two amino acids of a polypeptide chain. Proteolytic enzymes are very important in digestion as they breakdown the peptide bonds in the protein foods to liberate the amino acids needed by the body. Additionally, proteolytic enzymes have been used for a long time in various forms of therapy. Their use in medicine is notable based on several clinical studies indicating their benefits in oncology, inflammatory conditions, blood rheology control, and immune regulation. Protease also has an ability to digest unwanted debris in the blood including certain bacteria and viruses. Therefore, protease deficient people are immune compromised, making them susceptible to bacterial, viral and yeast infections and a general decrease in immunity. Proteases were initially classified into endopeptidases, which target internal peptide bonds, and exopeptidases (aminopeptidases and carboxypeptidases), the action of which is directed by the NH2 and COOH termini of their corresponding substrates. However, the availability of structural and mechanistic information on these enzymes facilitated new classification schemes. Proteolytic enzymes belong to the hydrolase class of enzymes (EC 3) and are grouped into the subclass of the peptide hydrolases or peptidases (EC 3.4). Based on the catalytic mechanism and the presence of amino acid residue(s) at the active site the proteases can be grouped as aspartic proteases, cysteine proteases (EC 3.4.22), glutamic proteases (EC 3.4.19), metalloproteases (EC 3.4.24), asparagine proteases (EC 3.4.23), serine proteases (EC 3.24.21), threonine proteases (EC 3.4.25), and proteases with mixed or unknown catalytic mechanism[5,6].

Proteases are used in a wide range of foods and food processing applications. Dairy (milk coagulation, flavor development),baking (gluten development), fish and seafood processing (fishmeals, enhanced oil recovery, aquaculture), animal protein processing (improved digestibility, reduced allergenicity, improved flavor, meat tenderization), plant protein processing (improved functionality and processing, generation of bio-active peptides), yeast hydrolysis (flavor compounds) and production of Value-Added Food Ingredients. Proteases are a powerful tool for modifying the properties of food proteins(soy Protein Modification for example). Improved digestibility, improved solubility, modified functional properties (emulsification, fat-binding, water-binding, foaming properties, gel strength, whipping properties, etc.), improved flavor and palatability, Improved processing (viscosity reduction, improved drying, etc.).

Plant proteases have been well-known for ages for their industrial applications due to their broad substrate specificity and their activity over a wide range of pH and temperature values. Quantitatively, more than half of the total commercially used industrial enzymes are proteases[7]. They offer a better opportunity for people whose eating habits, health, religious beliefs, biotechnological level, law and financial situation constrain the use of animal and microbial enzymes.

The scope of this review encompasses biochemical and structural approaches toward characterization of plant serine proteases, especially those most recently reported in the literature, as well as hypotheses on their possible applications in food processes.

SERINE PROTEASESES(EC 3.4.21)

Serine proteases are one of the largest groups of proteolytic enzymes involved in numerous regulatory processes. In plants, they are widely spread among different taxonomic groups and are found to be involved in a number of physiological processes such as protein degradation and processing, microsporogenesis, symbiosis, hypersensitive response, signal transduction and differentiation, and senescence[8]. More than 500 entries of proteolytic enzymes are documented in which serine proteases are grouped together into six classes; the second largest is the subtilisin class. The first characterized plant subtilase was cucumisin from sarcocarp of melon fruit (*cucumismello*). With more than 200 members, serine proteases are the largest class of proteolytic enzymes in plants. Plant serine proteases are divided into 14 families. These families belong to 9 clans that are evolutionarily unrelated to each other[9].

Despite being the largest class of proteases in plant, the functions and regulatory roles of plant serine proteases are poorly understood, probably due to a lack of identification of their physiological substrates[8]. Once thought to be rare in plants, in recent years, several serine proteases have been isolated and purified from different plant parts of various plant species including seeds, latex and fruits. They

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have indeed been found and extracted from the seeds of barley (*HordeumvulgareL.* cv. Morex)[8],*Holarrhenaantidysenterica*[10], *Canavaliaensiformis* DC (L.)[11], soybean (*Glycine max* [L.] Merr.), rice (*Oryza sativa* L.)[8],Caesalpiniaechinata Lam. (Brazilwood)[12], *Cucumismelovaragrestis*[13] and *Solanumdubium*[14, 15]; from the latex of *Euphorbia supine*[8], Figure (Ficuscarica var. Brown Turkey)[16], dandelion (*Taraxacumoffýcinale* Webb. Sl.), African milkbush (*Synadeniumgrantii*Hook)[8], Ficusreligiosa[17], jackfruit (*ArtocarpusheterophyllusL.*)[8], *Cryptolepisbuchanani*[18], *Euphorbia neriifolia* Linn[19] and Streblusasper[20]; from the flowers, stems, leaves and roots of *Arabidopsis thaliana*; from the storage roots of sweet potato (*Ipomoea batatas*L.) and maize (*Zea mays* L.)[8]; from the sprouts of bamboo (*Pleioblastushindsii*Nakai)[8]; from the leaves of tobacco (*Nicotianatabacum*L.), common bean(*Phaseolus vulgaris* L. cv. Cesnjevec),lettuce (Lactuca sativa)[21], tomato (*Lycopersiconesculentum*L.) and Kesinai Plant (*Streblusasper*) [22]; from the peel of mango (*Mangiferaindica* cv. Chokanan)[23] and from the fruits of *Cucurbitaficifolia*L., osage orange (*Maclurapomifera*), suzumeuri (*Melothria japonica* Thunb. Maxim.), "Ryukyu white gourd" (*Benincasahispida*var. Ryukyu), Japanese large snake gourd (*Tricosanthesbracteata*Lam. Voigt) and yellowsnake gourd (*Tricosantheskirilowii*Maxim. var. japonica Miq. Kitam)[8], tomato (*Lycopersiconesculentum*Mill.)[24]; Kachri fruit (*Cucumistrigonus*Roxburghi)[25]; honeydew melon (*CucumisvarInodorus*. Naud)[26] and melon (*Cucumismelo*L.)[27].

CLASIFICATION AND MECHANISM

Serine endopeptidases and exopeptidases are of extremely widespread occurrence and diverse function. Many distinct families of serine proteases exist and they have been grouped into six families[8,28], of which the two largest and most well-known are the (chymo)trypsin-like and subtilisin-likefamilies. Both superfamilies use the same catalytic triad (Asp: a nucleophile - Ser: an electrophile- His: a base), which is thought to have evolved through convergent evolution[8]. According to Dunn[29], the basic mechanism of action of serine proteases involves transfer of the acyl portion of a substrate to a functional group of the enzyme. The two basic steps of catalysis by this group of enzymes thus include:first, the formation of an ester between the oxygen atom of serine and the acyl portion of the substrate-which produces a tetrahedral intermediate and releases the amino part of the substrate and second, the attack of water on the acyl-enzyme intermediate, which breaks it down and releases the acidic product-while regenerating the original enzyme form[8, 30].

Cucumisin

Cucumisin (EC 3.4.21.25; CAS Number:82062-89-3) is an extracellular thermostable alkaline serine protease that is expressed at high levels in melon fruits (*Cucumismelo*L.). It comprises more than 10% of the total juice protein and is synthesized in the central parts of the fruits. The molecular weight of native form of cucumisin from the juice of melons has been reported 67 kDa[31]. It was found that the amino acid sequence Gly-Thr-Ser-Met, around the reactive serine of cucumisin is identical with that of subtilisin, which is a microbial serine protease[32]. Cucumisin was the first characterized plant subtilase[33]. Subsequently, more cucumisin-like proteases were isolated from plants. Little direct evidence is available about the proteolytic activities of plant subtilases, except for those with broad specificity such as cucumisisn and cucumisin-like protease. Several cucumisin-like enzymes were isolated from other Curcubitaceae and from rice, maize, wheat, and barley[8]. Most of these enzymes show Mr-values in the range 60–70 kDa and exhibit broad specificity, preferring hydrophobic amino acid residues in the P1 position[34]. Mature cucumisin (54 kDa) shows optimum pH in the range 8–10 and is stable at 60 °C and over a broad pH range (4–11). Cucumisin is composed of multiple domain modules, including catalytic, protease-associated, and fibronectin III-like domains[35]. Cucumisin is not affected by trypsin inhibitor from soybean (SBTI), ovomucoid, cysteine proteinase inhibitors, or ethylene ediaminetetraacetic acid (EDTA), but is strongly inhibited by chloromethyl ketone derivatives of peptide substrates, phenylmethylsulphonyl fluoride (PMSF), and diisopropylfluorophosphate[33, 35,36].

APPLICATIONS

Plant coagulants are of growing interest, as the use of animal rennet may be limited for religious reasons (e.g., Judaism and Islam), diet (vegetarianism), or consumer concern regarding genetically engineered foods (e.g., Germany, Netherlands and France forbid the use of recombinant calf rennet).More recently, the incidence of bovine spongiform encephalopathy has reduced both supply and demand for bovine rennet[37].

Apparently, most of the isolated and characterized plant proteases have been classified as cysteine proteases, which are widely used in several processes in the food industry[38]. The major drawback in the use of cysteine proteases is that their activity is readily reduced by air oxidation and metal ions. Thus, the application of these proteases requires reducing agents and chelating agents, which restricts their commercial application, as they are not so economic and handy[39]. By contrast, plant serine proteases are both stable and active under harsh conditions of raised temperatures. So, the search for new potential plant serine proteases to make industrial processes cost-effective is still in demand.

Proteases have a large variety of applications in food industry. They have been routinely used for various purposes such as, cheese making, baking and meat tenderization[39,40].

Dairy industry

Milk-clotting by proteolytic enzymes is very important in dairy technology. The enzymatic coagulation of milk involves a specific hydrolysis of the Phe_{105}-Met_{106} bond *kappa*-casein covering the protein micelles,depilating the casein micelle and provoking the milk clotting[41].

However, the increasing cheese production and consumption, the high price and reduced supply of rennets, as well as the associated ethical issues have led to the search for alternative or additional rennet substitutes produced either from plants or from genetically modified microorganisms[14,15]. The use of plant coagulants in cheese making is associated with many advantages: they are natural, cheap, easy to prepare, allow straightforward process and used to produce cheese for ecological markets[37]. Plant proteases employed for cheese production in various areas of the world include papain, bromelin, ficin, oryzasin, cucumisin, Sodom apple and Jacaratiacorumbensis[42]. The milk clotting activity of cucumisin suggests that it might be suitable for milk-clotting production. Cucumisin exhibited the same milk-clotting activity of cysteine proteases such as papain, but inaddition, it produced much less bitter-tasting peptides than those formed by more typical plant cysteine proteases. Uchikoba et al shown the milk-clotting activity of cucumisin was the same to that of papain and was half value of that of ficain[43]. Dairy farmers in some parts of the Sudan use the berries of *Solanumdubium*to make white soft cheese using goat and sheep milk[44]. Reaserchesperformeda chymotrypsin-like serine protease (dubiumin) from the seeds of *Solanumdubium*Fresen has been recognized[15,45,46,47]. Lo Piero et al. shown that Lettucine from *L. sativa* leaves is able to provoke a significant disorganization of the micellar structure of casein andanalyzed its proteolytic activity under various technological parameters, such as temperature and pH, and the results were highly consistent with the milk-clotting process[21].

Yoghurt is an increasingly popular cultured dairy product in most countries. Fruit additions have an increasing effect on yoghurt consumption. Also, using different fruit additives give more yoghurt choices to the consumer in the market[48]. Fruits and vegetables are good sources of vitamins, minerals, antioxidants and fibers. So, certain fruits can be used in yoghurt production for improving their nutritional values and sensory properties (e.g. strawberry, apple, cornelian, rosehip, morello cherry, grape, date and other fruit homogenates)[48,49, 50]. Papaya, kiwi, pineapple and khaki[51] and melon[52] fruits are known for their high nutrients and fibers content and they have highproteolytic enzymes.

Meat processing

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Tenderness of meat is the major factor affecting consumer satisfaction and eating quality. In order to improve tenderness, different physical and enzymatic methods are adopted. Tenderness is a complex trait. Generally, the two primary structural features of muscle that influence tenderness are integrity of the myofibrils (termed the actomyosin effect) and the connective tissue contribution (termed a background effect)[53]. Naveena et al.[54] evaluated many proteolytic substances of plant origin and concluded that cucumis and ginger gave better results to tenderize buffalo meat. Kim et al.,[55] shown that ginger rhizome improves the palatability and acceptability of lean beef from carcasses of marginal quality. Traditionally, the Kachri fruit, C. trigonusRoxburghi, has been used as meat tenderizer in the Indian subcontinent. Asif-Ullah demonstrated that the proteolytic activity of the fruit was in part due to a serine protease (cucumisin-like proteases). This protease was stable at basic pH values and at high temperature, suggesting its potential application in the food industry[25]. Zochowska-Kujawskashown that enzymes of raw plant juices (mango, kiwi and papaya) could be used as tenderizers in dry sausage production[56].

Bakery industry

Proteases are used on a large commercial scale in the production of bread, baked goods, crackers, pastries, biscuits and cookies[57]. They act on the proteins of wheat flour, reducing gluten elasticity and therefore reducing shrinkage of dough or paste after moulding and sheeting[58,59], for instance, hydrolysis of gluten proteins, which are responsible for the elasticity of dough, has considerable improving effects on the spread ratio of cookies[58]. Also these enzymes can be added to reduce mixing time, to decrease dough consistency, to assure dough uniformity, to regulate gluten strength in bread, to control bread texture and to improve flavour[60,61]. In addition, proteases have largely replaced bisulfite, which was previously used to control consistency through reduction of gluten protein disulfide bonds, while proteolysis breaks down peptide bonds. In both cases, the final effect is a similar weakening of the gluten network[62]. These enzymes have great impact on dough rheology and the quality of bread possibly due to effects on the gluten network or on gliadin[63].

OTHER APPLICATIONS

Serine proteases are most widely used in industries. The largest application of proteases is in detergent industry where removal of proteinaceous substances occurs at alkaline pH. Another important application of serine proteases is in the leather industry for dehairing of hides and skins. Commercially successful uses of serine proteases are also mentioned inleather processing, tanning, silk processing,waste treatment,photographic, textile,chemical and medical, biotechnology and pharmacology industries[64].

CONCLUTION

Considering that mentioned about potential properties cucumisinof melon juice concentrate can be use it in different food processes, such as tenderizing meat, coagulating milk for production of special foods, production of jelly and fruit dessert as digestion aid and baking processes. Also using this concentrate causes enrichmentand improvement of flavor and aroma products. Since the melon grows in a wide range of climate, melon production for extraction of cucumisin can be a source of earning a high income to farmers and associated industries. This fruit contains a valuable natural protease that can be used as a rich, natural, and abundant media source for commercial production of the enzyme.

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