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RESEARCH ARTICLE

Effect of Butylated Hydroxytoluene on Papain obtained from Fresh Latex of *Carica papaya*

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Abstract

This study was based on the effect of butylated hydroxytoluene (BHT) on the stability of papain extracted and purified from fresh latex of *Carica papaya*. Latex was processed to produce papain by following the classical protocols. The final product of the trials namely the enzyme in the powder state was obtained which was crystallized. The present study was based on tests with the resultant enzyme combined with BHT to estimate the stability for storage and transport purposes. Shelf-life studies were performed for the plain enzyme and the enzyme with the preservative BHT. There was 14% and 26% increase over control in BHT treated papain after 7 and 14 d respectively. About 50% of papain activity was retained after 12 months in both control and BHT treated papain at 4°C. Thus, there was not a very significant difference in the activity between treated and control in both the experiments.

Keywords: Butylated hydroxytoluene, *Carica papaya*, papain, preservative, shelf-life.

Introduction

Papain (EC 3:4:22:2) also known as papaya peptidase-I is a cysteine protease enzyme found in the latex of the fruits of papaya. It has a molecular weight of 23,000 daltons. Though this enzyme is present in a lesser amount (less than 10%) in the latex than other prominent enzymes like chymopapain A and B (26-30%), glycyl endopeptidase III and IV (23-28%), and caricain (14-26%), it has gained great importance globally. It is widely used in various industries replacing harmful chemicals and saving energy and money. Some predominant industries that make use of this enzyme are the meat industry, tanneries, textile, detergent, pharmaceutical and cosmetic industries. Many food processing industries including beverages and feed for livestock also depend on the hydrolytic activity of this enzyme and make best use of it to serve the world its day to day needs.

BHT (butylated hydroxytoluene) are widely used by the food industry as preservatives, mainly to prevent oils in foods from oxidizing and becoming rancid. Butylated hydroxytoluene, also known as dibutylhydroxytoluene, is a lipophilic organic compound, chemically a derivative of phenol useful for its antioxidant properties. European and US regulations allow it in small amounts to be used as a food additive. In addition to this use, BHT is widely used to prevent oxidation in fluids (e.g. fuel, oil) and other materials where free radicals must be controlled. BHT is listed under three categories in catalogues and databases, such as food additive, household product ingredient, personal care product/cosmetic ingredient, pesticide ingredient, plastic, rubber, etc.

BHT is also used to preserve food odor, color, and flavor. Many packaging materials incorporate BHT. It is also added directly to shortening, cereals and other foods containing fats and oils. As papain is sensitive to temperature and air, its active compounds get oxidized and the potency of the enzyme is lost. BHT was used for the processed papain to study the effects of antioxidant property. If the enzyme responded positively, it was planned to incorporate this chemical for enzyme stabilization. Hence, this study was aimed to study the effect of BHT on the stability of papain extracted and purified from fresh latex of *Carica papaya*

Materials and methods

Processing of latex: The papaya latex was procured from Ongole, Prakasam District, Andhra Pradesh. After collection of latex, 0.05% potassium metabisulfite (KMS powder) was added in the fields and it was transported on the same day to the place of processing. Once the latex was brought to the place of processing, it was left in cold storage at 4°C on the same day. The latex was passed through nylon sieves to clear debris and lumps to obtain a uniform slurry or colloid. The extraction of papain was performed according to Kimmel and Smith (1958). An aqueous solution containing a mix of 0.04 M cysteine and 0.054 N of NaOH was added to the latex to obtain a pH of 5 to 5.5. This mix was filtered through Whatman No.1 filter paper under vacuum using Hyflo-Supercel as a support medium to obtain a clear, opalescent bottle green color extract. About 1N NaOH solution was gradually added to the green color extract.

Table 1. Effect of Butylated Hydroxytoulene (BHT) on stability of Papain at 50°C.

14 days		7 days	
BHT 0.01%	Control	BHT 0.01%	Control
94± 2.6	70± 3.6	258± 4.6	226 2.6

Table 2. Effect of BHT and Temperature on stability of Papain at 50°C.

Cold room		Room temperature		Date
0.02% BHT	Control	0.02% BHT	Control	
497±3.6	462±3.6	349±3.6	342±2.6	13 Aug 2015
469±2.6	454±2.6	-	-	14 Sep 2015
449±4.6	442±5.3	-	-	14 Oct 2015
424±4.0	427±4.4	-	-	13 Jan 2015
350±3.6	405±2.6	-	-	14 July 2015
317±4.6	347±3.6	-	-	27 July 2015

This mixture was once again passed through the Hyflo-set up and filtered to remove the inert material present in the mix. The resultant filtrate was subjected to 45% ammonium sulfate saturation and centrifuged at 2500 rpm for 20 min. The precipitate, which is the papain enzyme, was collected and the supernatant was discarded. The precipitate was washed with ice cold solution of 45% ammonium sulfate and once again centrifuged. The wet precipitates were lyophilized and stored at 4°C and used for crystallization and assays.

Assay of papain enzyme: Kunitz's method (1947) was followed for assaying papain using casein as substrate and tyrosine as standard and the activity was expressed as tyrosine units based on the amount of tyrosine released. The absorbance of the filtrates was read against the blank at 280 nm.

Results

In the first experiment, to study the effect of BHT, the enzyme was stored at 50°C along with 0.01% BHT. Papain stored without BHT served as the control. Papain assay was done on the 7th and 14th d. There was 14% and 26% increase over control in BHT treated papain after 7 and 14 d respectively (Table 1).

In the second experiment, the enzyme was stored at room temperature and cold room with and without 0.01% BHT. The initial activity was 652 TU/mL. After 30 d, the activity of enzyme stored at room temperature was comparable with that of 0.02% BHT treated sample. However, this activity dropped approximately by two fold compared to the initial activity. Further analysis was dropped due to this decrease. Whereas, enzyme stored at cold room in the presence and absence of 0.02% BHT showed a decrease of 30% and 29% respectively after 30 d of incubation. After 12 months, the percent decrease were 47% and 51% respectively. About 50% of papain activity was retained after 12 months in both control and BHT treated papain at 4°C (Table 2).

Discussion

Papain has gained popularity as an ingredient in some skin care products besides its various uses. The use of papain for cosmetics was patented in 1994 by Ozlen. It was quoted that digestive enzymes along with hydroxy acids were formulated to treat various types of skin related problems. Papain can have some good influence on collagen, by which it can assist in removing freckles and enable the skin to appear even and white. It protects the cutin, accelerates metabolism of epidermal cells and makes the skin-tender. Some practitioners believe that papain helps to open up clogged pores and is effective for addressing blemish prone skin. This enzyme from the papaya fruit also has antiinflammatory and antibacterial properties. Because of its ability to remove dead skin cells, papain is found in a variety of exfoliating skin care products including facial scrubs, body cleansers, facial masks and peels. As reported by Sim *et al*. (2000), papain was conjugated to a soluble biopolymer produced by Schizophyllum commune. Stability of the conjugated enzymes were significantly enhanced, such that more than 90% of the initial activity remained after a month storage at 45 °C, even in a cosmetic formulation including various oils and surfactants. Cosmetic lotion containing 1% papain conjugate was more effective in exfoliating stratum corneum of skin than the lotion containing 5% lactic acid, one of the popular exfoliating agents. BHT is used in a wide range of cosmetic formulations as an antioxidant at concentrations from 0.0002% to 0.5%. BHT does penetrate the skin, but the relatively low amount absorbed remains primarily in the skin. Recognizing the low concentration at which this ingredient is currently used in cosmetic formulations, it was concluded that BHT is safe as used in cosmetic formulations (Lanigan and Yamarik, 2002). Papain enzyme is widely used in meat processing in the developed nations (Tappel *et al.,* 1956). Physical incorporation of tenderizers into meat is an important factor influencing their effectiveness.

Papain is the active ingredient in most commercial tenderizers. The proteolytic action of the enzyme cleaves or breaks apart the muscle fiber proteins and connective tissue of meat by hydrolysis-in a manner similar to that of digestion-which makes food more absorbable. According to Istrati (2008), papain tenderization of the adult beef determined improvement of tenderness, flavor and juiciness. It is recommended to use papain doses as low as possible, in order to avoid advanced tenderization and obtaining meats with soft structure, with very low resistance to mastication and paste texture. Biomolecules have a tendency to lose their activity on storage. Many ways to protect their activity have been explored universally. Two main uses of papain enzyme were considered and the best way to deliver the enzyme to the market was planned for this study. Since BHT was recommended as safe by the FDA, its addition to the enzyme was sought after as a protective agent. Therefore it was mixed with the enzyme and its activity was studied for a time period. But from the test results, it was revealed that just the plain addition of the BHT to the enzyme did not give any significant protection for stability of the enzyme on storage. Whereas, the studies by Lanigan and Yamarik (2002) project a view that a protected environment like a glycosphere or a formulated condition safeguards the stability of the enzyme thus retaining its potency. The use of papain in cosmetics and meat industry, and BHT as a preservative, both in the cosmetic and food industry, one can easily justify the usage of BHT as a preservative for papain in this study. With this proven data the present study gains support for a combination of papain enzyme with BHT.

Conclusion

Papain is mainly used in the meat industry in the developed nations. To a certain extent papain is used in cosmetics. Since BHT is a food additive and used in cosmetic products too, it was planned to study its preservative effects on the enzyme. The stability and activity of enzyme shown by certain products are due to sophisticated and advanced technology and the compounded cosmetic formulations act as sealants to protect the enzymes, whereas the simple addition of BHT to the enzyme and storing to study its stability did not produce such impressive results.

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