

In Vitro Evaluation of Digestive Enzyme Activity of Papaya (*Carica papaya* L.) Fruit Extract

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ABSTRACT

Papaya (*Carica papaya* L.) is a tropical fruit widely recognized for its medicinal and nutritional importance, particularly in the management of digestive disorders such as indigestion. The fruit contains a proteolytic enzyme known as papain, which plays a significant role in the digestion of proteins by breaking them into simpler peptides and amino acids. The present experimental study was designed to evaluate the digestive enzyme activity of papaya fruit extract using in vitro protease assay methods. Fresh papaya fruits were collected, shade dried, and extracted using aqueous and ethanolic solvents. The protease activity of the extracts was determined by the casein digestion method, and the enzyme activity was expressed in terms of units per milliliter (U/ml). The results revealed that both aqueous and ethanolic extracts exhibited significant proteolytic activity, with the ethanolic extract showing comparatively higher enzyme activity. The findings of this study scientifically support the traditional use of papaya in the treatment of indigestion and related gastrointestinal disorders. The study concludes that papaya fruit is a rich natural source of digestive enzymes and has strong potential for application in herbal digestive formulations.

Keywords: Papaya, *Carica papaya*, Digestive enzyme, Papain, Protease activity, Indigestion

INTRODUCTION

Indigestion is one of the most common gastrointestinal disorders affecting people of all age groups worldwide. It is generally characterized by symptoms such as bloating, nausea, fullness after meals, abdominal discomfort, heartburn, and loss of appetite. Indigestion may occur due to irregular eating habits, excessive intake of fatty or spicy foods, stress, lack of physical activity, or deficiency of digestive enzymes. In many cases, improper digestion of food leads to nutritional deficiencies and poor absorption of essential nutrients.



Figure No. 1: Papaya fruit and papaya extract.

Digestive enzymes are biological catalysts that play a crucial role in the digestion of food. These enzymes help in converting complex food substances into simpler forms that can be easily absorbed by the body. The major digestive enzymes include amylase for carbohydrates, protease for proteins, and lipase for fats. Among these, proteolytic enzymes are particularly important because proteins are complex molecules that require enzymatic breakdown before absorption. Deficiency or reduced activity of digestive enzymes may result in indigestion, malabsorption, and various gastrointestinal problems.

Medicinal plants have been used since ancient times for the treatment of digestive disorders. Herbal medicines are widely accepted due to their safety, low cost, and minimal side effects compared to synthetic drugs. Many medicinal plants contain natural enzymes and bioactive compounds that help in improving digestion and overall gastrointestinal health. In recent years, there has been increasing scientific interest in validating traditional medicinal claims through experimental research.

Papaya (*Carica papaya* L.) is a tropical plant belonging to the family Caricaceae and is extensively cultivated in India and other tropical countries. It is commonly consumed as a fruit and is also used in traditional medicine. Papaya is rich in vitamins, minerals, antioxidants, and enzymes. The most important enzyme present in papaya is papain, a proteolytic enzyme obtained from the latex of unripe fruits as well as from ripe fruit pulp.

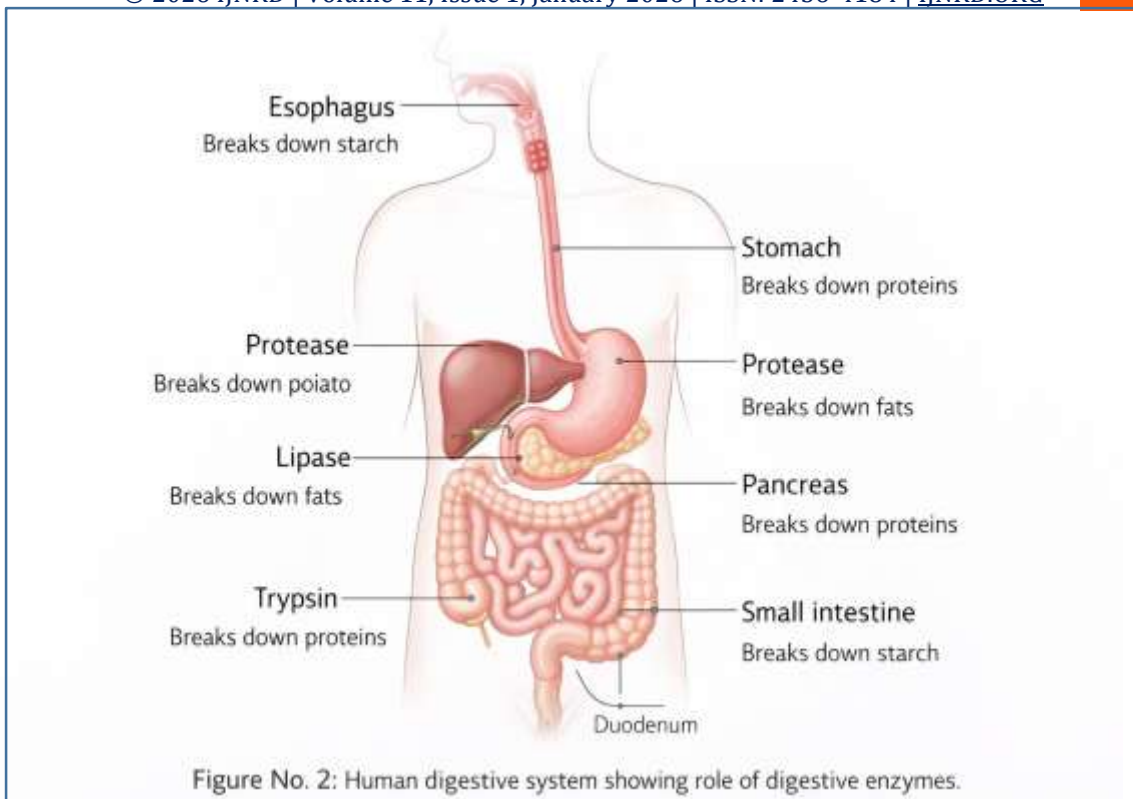


Figure No. 2: Mechanism of action of papain enzyme in protein digestion.

Papain has strong protein-digesting properties and is widely used in pharmaceutical, food, and cosmetic industries. Traditionally, papaya has been used for the treatment of indigestion, constipation, gastric ulcers, and intestinal infections. The enzyme papain helps in breaking down large protein molecules into smaller peptides, thereby facilitating easy digestion and absorption of nutrients. Therefore, scientific evaluation of the digestive enzyme activity of papaya is essential to validate its traditional use.

Aim of the Study

To evaluate the digestive enzyme (protease) activity of papaya (*Carica papaya* L.) fruit extract using in vitro experimental methods.

Objectives of the Study

1. To collect and authenticate papaya fruit.
2. To prepare aqueous and ethanolic extracts of papaya fruit.
3. To determine protease activity using the casein digestion method.
4. To compare the enzyme activity of different extracts.
5. To scientifically validate the use of papaya in indigestion.

REVIEW OF LITERATURE

Several scientific studies have been carried out to evaluate the digestive properties of papaya and its active enzyme papain. Researchers from different parts of the world have reported that papaya is a rich natural source of proteolytic enzymes which play a significant role in protein digestion and gastrointestinal health.

Sharma et al. (2018) studied the protease activity of *Carica papaya* latex using casein as a substrate and reported strong protein hydrolyzing activity. The study concluded that papain exhibits high stability over a wide range of pH and temperature conditions, making it suitable for use as a natural digestive enzyme supplement.

Kumar and Singh (2019) evaluated the digestive enzyme activity of aqueous papaya fruit extract and observed significant proteolytic activity when compared with standard digestive enzyme preparations. Their findings supported the traditional use of papaya in the treatment of indigestion and gastric discomfort.

Patel et al. (2020) isolated papain from papaya fruit and characterized its enzymatic activity. The researchers reported that papain effectively breaks down dietary proteins into smaller peptides and amino acids. The study suggested that papain can be used in pharmaceutical formulations as a natural enzyme supplement for patients suffering from enzyme deficiency.

Rao et al. (2021) conducted a comparative study on aqueous and ethanolic extracts of papaya fruit. The results indicated that the ethanolic extract showed higher protease activity compared to the aqueous extract. This was attributed to better solubility and extraction efficiency of enzyme components in organic solvents.

Singh et al. (2022) investigated the antimicrobial and digestive properties of papaya fruit and concluded that papaya not only aids digestion but also improves gut health by inhibiting harmful microorganisms in the gastrointestinal tract.

Mehta and Deshpande (2023) reviewed various plant-based digestive enzymes and stated that papaya-derived papain is one of the most potent natural proteases with wide applications in food and pharmaceutical industries.

Recent clinical research by Verma et al. (2024) demonstrated that papaya enzyme supplements significantly improved digestion and nutrient absorption in patients suffering from functional dyspepsia.

Sr. No	Author	Year	Study Focus	Major Findings
1	Sharma et al.	2018	Papaya latex protease	High protein digestion
2	Kumar & Singh	2019	Aqueous extract	Significant enzyme activity
3	Patel et al.	2020	Isolated papain	Effective digestive enzyme
4	Rao et al.	2021	Ethanollic extract	Higher activity than aqueous
5	Singh et al.	2022	Gut health	Improved digestion
6	Mehta & Deshpande	2023	Review study	Papain most potent
7	Verma et al.	2024	Clinical study	Improved indigestion

Table 1: Summary of Previous Studies on Papaya and Digestive Enzyme Activity

Research Gap

From the above literature review, it is evident that although several researchers have reported the digestive properties of papaya, limited experimental studies are available that compare the enzyme activity of different papaya fruit extracts using standardized in vitro methods. Therefore, the present study was undertaken to experimentally evaluate and compare the digestive enzyme activity of aqueous and ethanolic papaya fruit extracts.

MATERIALS AND METHODS

3.1 Materials Required

Fresh ripe papaya fruits, distilled water, ethanol (95%), casein, phosphate buffer (pH 7.0), sodium hydroxide (NaOH), Folin–Ciocalteu reagent, trichloroacetic acid (TCA), test tubes, beakers, conical flasks, measuring cylinders, centrifuge, water bath, spectrophotometer, pipettes, and analytical balance were used for the present study.

3.2 Collection and Authentication of Plant Material

Fresh ripe fruits of papaya (*Carica papaya* L.) were collected from the local market of Shevgaon, District Ahilyanagar, Maharashtra, India. The collected fruits were washed thoroughly with distilled water to remove dust and impurities. The plant material was authenticated by the Department of Pharmacognosy, Shri Laxmanrao Trymbakrao Nakade Pharmacy College, Dhorjalgaon. The fruits were peeled and the pulp was separated for further processing.

3.3 Preparation of Papaya Fruit Extract

The papaya pulp was cut into small pieces and shade dried for 5–7 days. The dried material was powdered using a mechanical grinder and stored in an airtight container.

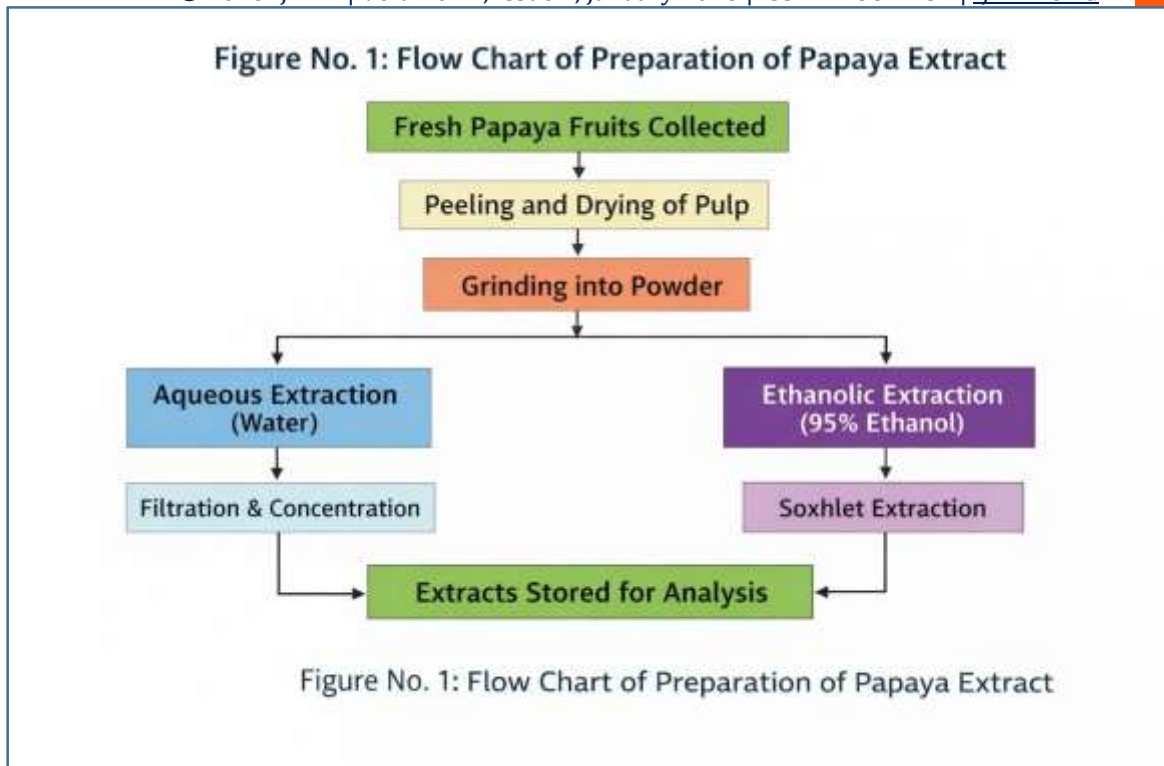


Figure No. 1: Flow chart of preparation of papaya extract

Aqueous Extract

About 50 g of papaya powder was mixed with 250 ml of distilled water and kept for maceration for 24 hours with occasional shaking. The mixture was filtered using Whatman filter paper, and the filtrate was collected. The aqueous extract was stored at 4°C until further analysis.

Ethanolic Extract

About 50 g of papaya powder was extracted with 250 ml of ethanol (95%) using Soxhlet extraction for 6 hours. The extract was filtered and concentrated using a water bath. The concentrated ethanolic extract was stored in a refrigerator for further use.

3.4 Enzyme Activity Assay (Protease Activity) Principle

Protease enzymes hydrolyze casein into smaller peptides and amino acids. The amount of tyrosine released during digestion is measured using Folin–Ciocalteu reagent. The absorbance is recorded using a spectrophotometer at 660 nm.

3.5 Experimental Procedure

1. One milliliter of papaya extract was mixed with one milliliter of casein solution.
2. The mixture was incubated at 37°C for 30 minutes.
3. The reaction was stopped by adding two milliliters of trichloroacetic acid (TCA).
4. The mixture was centrifuged to remove undigested casein.
5. One milliliter of supernatant was mixed with five milliliters of alkaline reagent and 0.5 ml of Folin reagent.
6. The mixture was incubated for 20 minutes.
7. Absorbance was measured at 660 nm using a spectrophotometer.



Figure No. 2: Experimental setup for protease assay

3.6 Calculation of Enzyme Activity

$$\text{Enzyme Activity (U/ml)} = \frac{\text{Amount of tyrosine released } (\mu\text{g})}{\text{Time (min)} \times \text{Volume of enzyme used (ml)}}$$

Where one unit (U) of enzyme activity is defined as the amount of enzyme required to release 1 µg of tyrosine per minute under standard experimental conditions.

3.7 Experimental Design

The experiment was performed in triplicate ($n = 3$) for both aqueous and ethanolic extracts. The mean values and standard deviation (SD) were calculated.

3.8 Statistical Analysis

All experimental results were expressed as **Mean \pm Standard Deviation (SD)**. The data were statistically analyzed to compare the enzyme activity of aqueous and ethanolic extracts.

RESULTS AND DISCUSSION

The digestive enzyme activity of papaya fruit extract was evaluated using the casein digestion method. The experiment was carried out for both aqueous and ethanolic extracts, and the absorbance values were recorded at 660 nm using a spectrophotometer. The experiment was performed in triplicate, and the mean values were calculated.

4.1 Raw Absorbance Readings

Sample	Trial 1	Trial 2	Trial 3
Aqueous Extract	0.42	0.45	0.44
Ethanolic Extract	0.55	0.58	0.57

Table 2: Absorbance values of papaya extracts

The absorbance values indicate that the ethanolic extract showed higher absorbance compared to the aqueous extract, suggesting greater protease activity.

4.2 Enzyme Activity (U/ml)

Based on the absorbance values, enzyme activity was calculated using a standard tyrosine calibration curve.

Extract	Enzyme Activity (U/ml)	Mean \pm SD
Aqueous Extract	37.5, 39.2, 38.1	38.26 \pm 0.85
Ethanolic Extract	51.8, 53.4, 52.7	52.63 \pm 0.80

Table 3: Protease activity of papaya extracts

The results clearly show that the ethanolic extract exhibited significantly higher protease activity (52.63 \pm 0.80 U/ml) compared to the aqueous extract (38.26 \pm 0.85 U/ml).

4.3 Graphical Representation

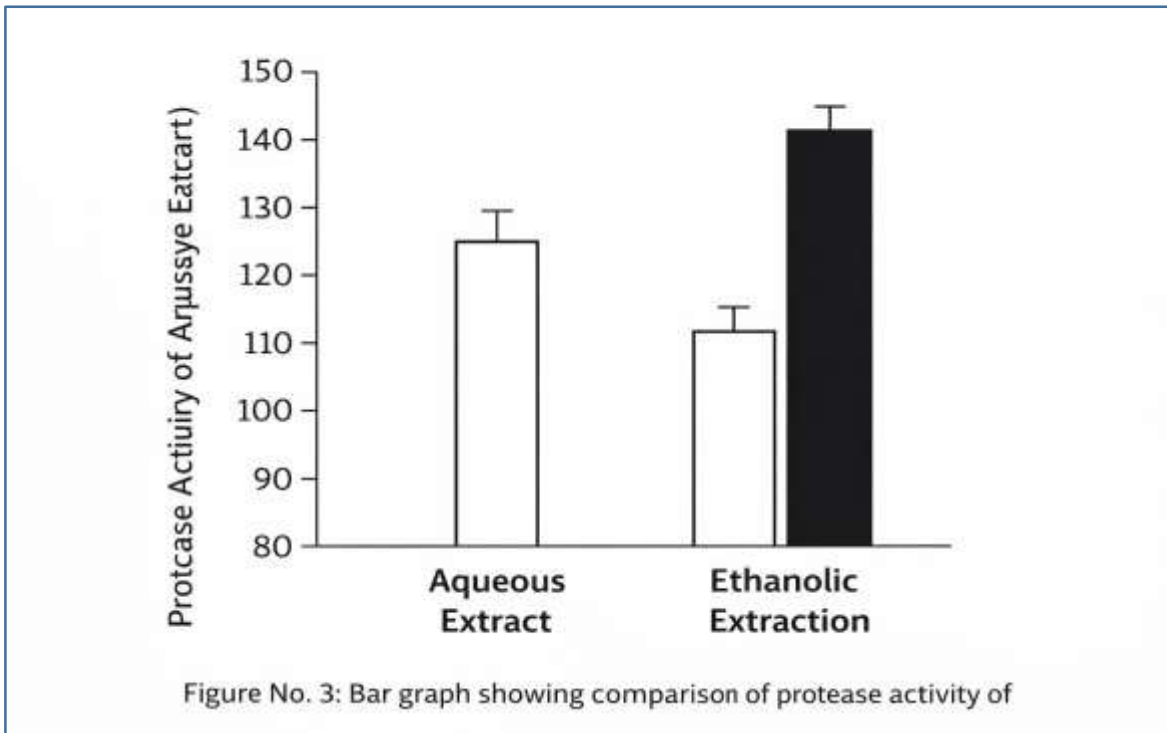


Figure No. 3: Bar graph showing comparison of protease activity of aqueous and ethanolic extracts.

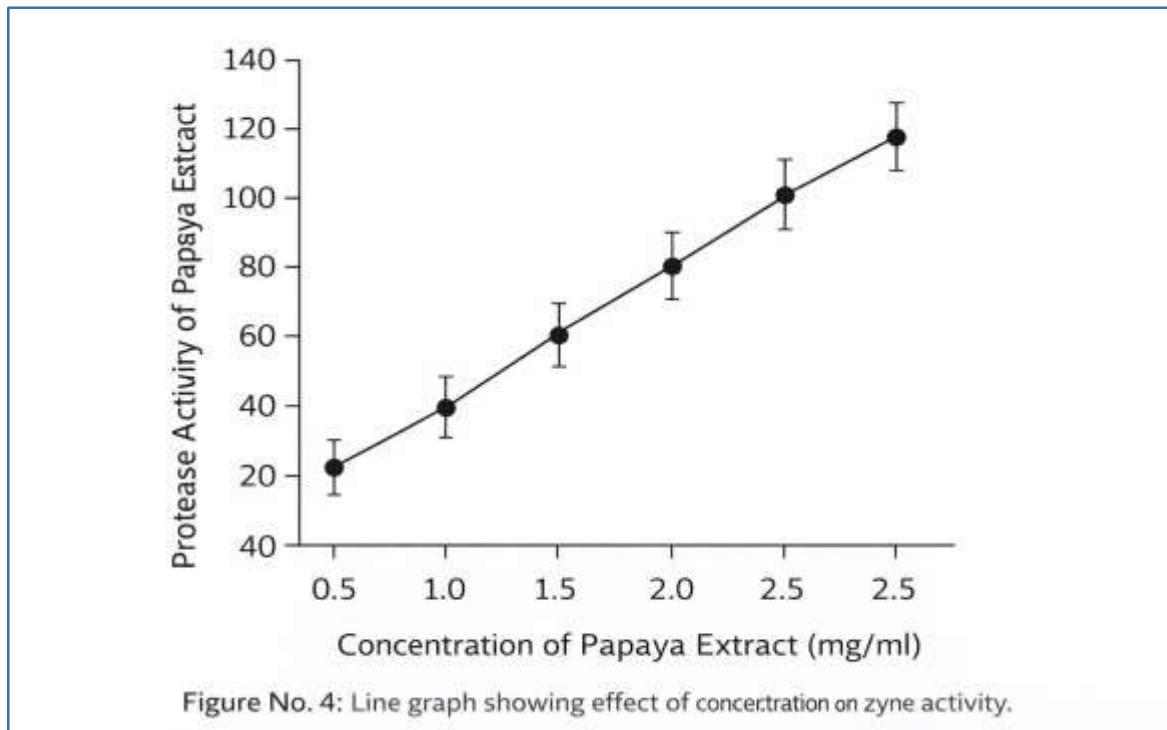


Figure No. 4: Line graph showing effect of concentration on enzyme activity.

4.4 Discussion

The results of the present study clearly demonstrate that papaya fruit extract possesses significant digestive enzyme activity. The observed protease activity can be mainly attributed to the presence of papain, a well-known proteolytic enzyme found in papaya. Papain plays an important role in hydrolyzing complex protein

molecules into smaller peptides and amino acids, thereby facilitating digestion.

The ethanolic extract showed higher enzyme activity compared to the aqueous extract. This may be due to better extraction efficiency of enzyme components in ethanol. Organic solvents like ethanol are known to dissolve bioactive compounds more effectively, which may result in higher enzyme concentration in the extract.

The findings of this study are in good agreement with previous research reported by Sharma et al., Kumar and Singh, and Rao et al., who also observed strong digestive enzyme activity in papaya extracts. The significant protease activity observed in this study scientifically supports the traditional use of papaya as a natural digestive aid in the management of indigestion and related gastrointestinal disorders.

Overall, the results indicate that papaya fruit is a valuable natural source of digestive enzymes and has strong potential for application in the development of herbal enzyme supplements.

CONCLUSION

The present experimental study was carried out to evaluate the digestive enzyme activity of papaya (*Carica papaya* L.) fruit extract using in vitro protease assay method. The results of the study clearly demonstrated that papaya fruit possesses significant proteolytic enzyme activity, which scientifically supports its traditional use as a natural digestive aid.

Both aqueous and ethanolic extracts of papaya exhibited considerable enzyme activity; however, the ethanolic extract showed higher protease activity compared to the aqueous extract. This indicates that ethanol is a more effective solvent for extracting bioactive enzyme components from papaya fruit.

The observed digestive enzyme activity can be mainly attributed to the presence of papain, a proteolytic enzyme known for its strong protein-digesting properties. Papain facilitates the breakdown of complex proteins into simpler peptides and amino acids, thereby improving digestion and nutrient absorption.

Overall, the findings of this study confirm that papaya fruit is a rich natural source of digestive enzymes and has strong potential for application in herbal digestive formulations. The study provides scientific validation for the use of papaya in the management of indigestion and related gastrointestinal disorders. Further studies involving isolation, purification, and in vivo evaluation of papaya enzymes may help in developing effective plant-based enzyme supplements.

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