Final Degree Project
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ENZYMES ACTING ON CARBOHYDRATES IN FOOD INDUSTRY: CELLULASES AND PECTINASES

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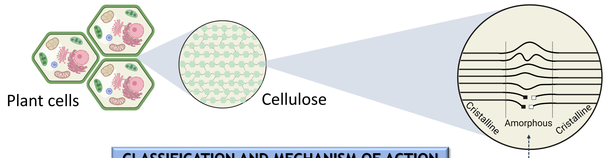
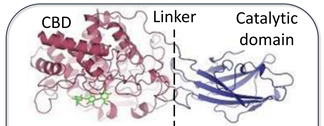
CELLULASES

- Mainly produced by fungi, bacteria and protozoa and found in plants and animals.
- Cellulases catalyze the hydrolysis of plant wall cellulose into glucose.



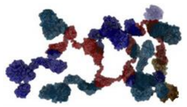
STRUCTURE AND ENZYMATIC COMPLEXES



Non-catalytic domain with cellulose binding function

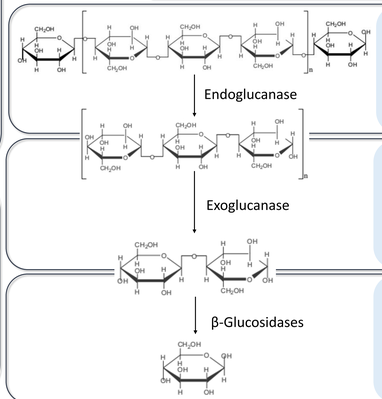
Domain where the catalytic site is located

Cellulases can be organized into cellulosomes, which have higher catalytic efficiency.



Structure model of a cellulosome (Sarai et al. 2019)

CLASSIFICATION AND MECHANISM OF ACTION



Endoglucanases act mainly on amorphous cellulose, randomly hydrolyzing glycosidic bonds and generating oligosaccharides and new terminal chains.

Exoglucanases act on reducing and non-reducing endings of the polymer generated by endoglucanases, releasing glucose or cellobiose molecules.

β-Glucosidases degrade cellobiose to glucose monomers, which may have two possible final configurations.

OBJECTIVES

To understand the basic concepts of food biotechnology and the applications in food industry of carbohydrates related enzymes.

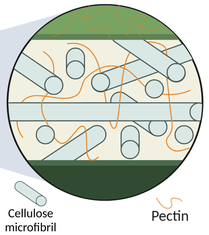
To characterize the biochemical properties and characteristics of pectinases and cellulases.

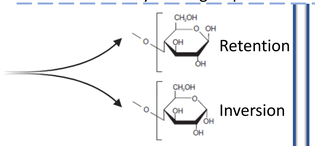
To understand how these families of enzymes work and determine and develop their main applications in food industry.

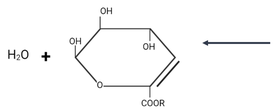
BACKGROUND: CELLULOSE AND PECTIN ON CELL WALLS

Cellulose is the principal component of the primary wall. It is a linear polymer of glucopyranose molecules linked by glycosidic bonds

Pectin is located in the primary cell wall and middle lamella. Consisting mainly of poly-α-galactopyranosyl-uronic acid units with some methyl ester groups





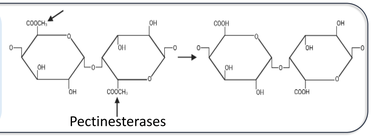


PECTINASES

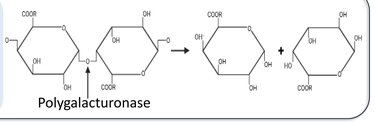
- Pectinases catalyze the degradation of pectic substances.
- The diversity of forms of pectic substances in plant cells probably accounts for the existence of various forms of these enzymes, usually known as pectinolytic enzymes.
- Widely distributed in higher plants and microorganisms.
- The most common source of pectinases for commercial use is filamentous fungi (***Aspergillus***), as well as bacteria, yeasts and actinomycetes.

CLASSIFICATION AND MECHANISM OF ACTION

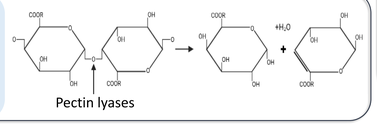
Pectinesterases catalyze metoxyl group deesterification of the esters of galacturonic acids.



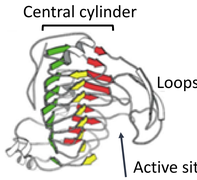
Polygalacturonases hydrolyze the galacturonic acid chain splitting glycosidic linkages next to free carboxyl groups.



Pectin lyases catalyze the random hydrolysis of pectin, breaking glycosidic bonds through β-elimination reactions and generating unsaturations.



PECTINASES STRUCTURE



Structure model of pectinase (Jenkins i Pickersgill 2001).

It consists of a single domain of three parallel β-strands (PB1 in yellow, PB2 in green and PB3 in red) which form a central cylinder.
The substrate binding site is found between PB1 and the protruding loops.

APPLICATIONS IN FOOD INDUSTRY

JUICE AND FRUIT PROCESSING

- To avoid problems caused by colloids present in juice (basically pectin and starch), it is previously treated with pectinases.
- Together, macerating enzymes are also used **to speed up filtration**, **improve stability and texture**, and **achieve higher yields**.

WINE PRODUCTION

- Pectinases are used in different stages during the winemaking process.
- In addition to pectinases, the use of macerating enzyme blends has been shown to have better results in grape processing.
- They can **prevent the use of chemicals**, **improve color extraction**, **skin maceration**, **must clarification**, **filtration** and **wine quality and stability**.

COFFEE AND TEA FERMENTATION

- In coffee fermentation, pectinases are used to remove mucilage and pulp from beans.
- In tea fermentation, the process is accelerated with pectinases and some properties of instant tea are improved.

BREWING PROCESS

- Cellulases are used during maceration stage to hydrolyze β-glucans' excess and **reduce viscosity**, thus **facilitating the separation** of the must from the grains.
- The incorporation of cellulase-forming gene expressions in yeasts such as *Saccharomyces cerevisiae* is being studied to combine processes.

OIL EXTRACTION

- Cellulases and pectinases are also used in the processes of extraction and clarification of vegetable oils.
- The main advantages of using macerating enzymes are **greater extraction**, **better fat fractionation**, production of oils with **higher levels of antioxidants and vitamin E** and **slowing of rancidity**.

CONCLUSIONS

CELLULASES

- Cellulases degrade cellulose into glucose.
- They are divided into endoglucanases, exoglucanases and β-Glucosidases.
- They are formed by a catalytic and a non-catalytic domain, joined by a linker.
- These enzymes can be organized into cellulosomes.

PECTINASES

- Pectinases degrade pectic substances (pectin).
- They are divided into pectinesterases, polygalacturonases and pectin lyases.
- These enzymes are formed by a domain with parallel β-sheets that form a central cylinder.

APPLICATIONS

- Cellulases and pectinases are used collectively with other enzymes.
- Both enzymes optimize processes due to their hydrolytic action of cellulose and pectin.
- They are mainly used in the processing of fruits, vegetables or grains.

REFERENCES

Jenkins, J., & Pickersgill, R. (2001). The architecture of parallel β-helices and related folds. *Progress in Biophysics and Molecular Biology*, *77*(2), 111-175. https://doi.org/10.1016/S0079-6107(01)00013-X

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