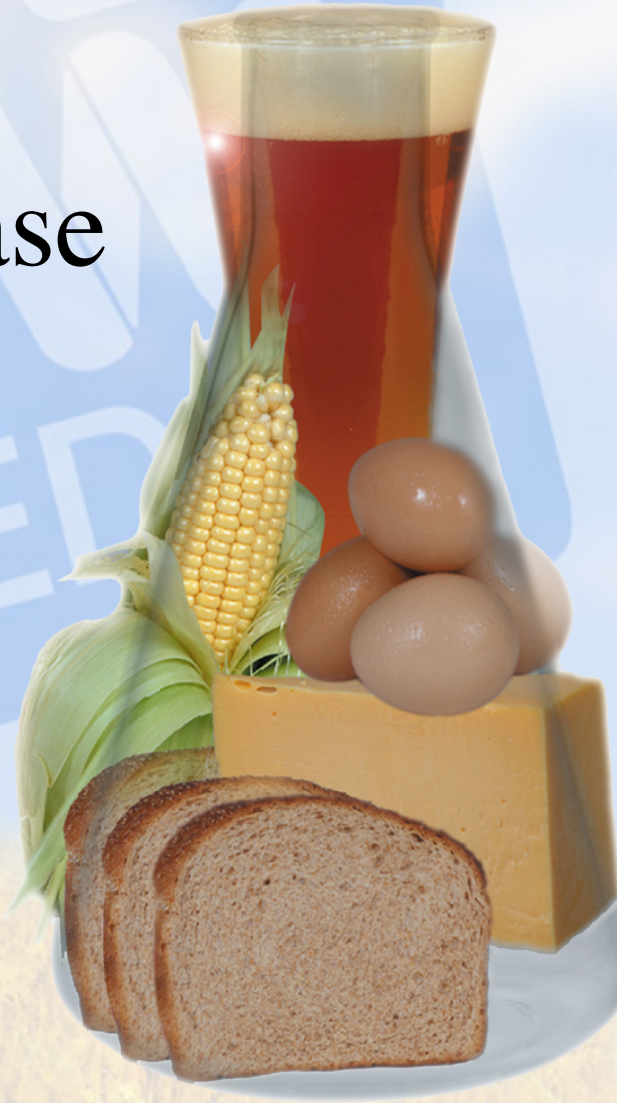
A New Aspartic Protease for use in foods, Cynzyme®

Presented by
Enzyme Development



A natural extract from the flower of the Thistle, *Cynara cardunuculus*

Cynara cardunculus

Also know as Cardoon and is a relative of the artichoke.

History of Use

- Documented use as early as the time of the Roman Empire
  - ~50 BC Lucius Junius Columella in his treatise *De Re Rustica*
  - A method to clot milk to produce cheese

History of Use

- Mostly used in Portugal and Spain for the production of cheese from sheep or goat

Estimated 5,000,000 kilos of cheese / year

Typically soft bodies cheeses

Typical Cheeses Produced

Country	Cheese	Type of Milk
Portugal	Serra da Estrella	ewe
	Serpa	ewe
	Azeitão	ewe
	Nisa	ewe
	Castelo Branco	ewe
	Évora	ewe
Spain	Casar de Cáceres	ewe
	Torta del Casar	ewe
	La Serna	ewe
	Los Pedroches	ewe
	Los Ibores	Goat
	Flor de Guía	Ewe and goat

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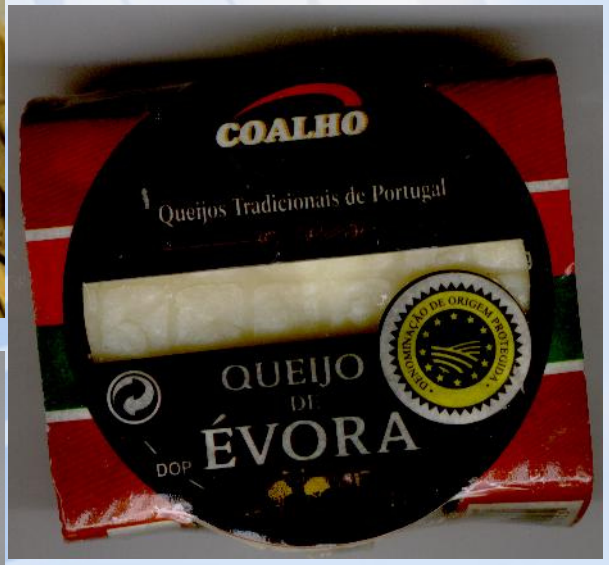

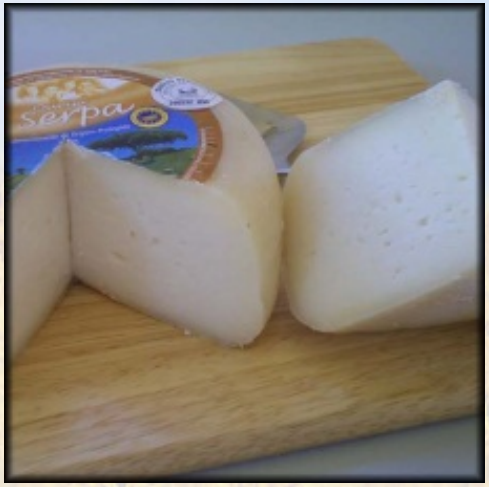

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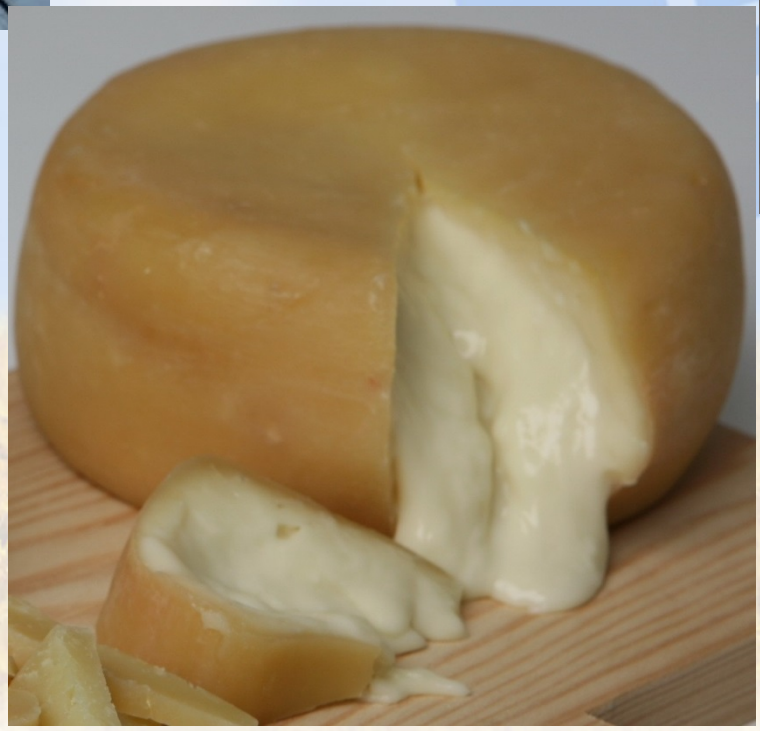

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1.Queso de Flor
2. Azeitão
3. Évora
4. Serra da Estrela
5. Serpa

Properties of the Aspartic Protease

- Optimum temperature 40 – 45 ° C
- Optimum pH 4.5 ± 0.5
- Easily inactivated above 55° C

Properties of the Aspartic Protease

- Clots milk similar to Chymosin

- Specificity on κ-casein
  - Only cleave the $Phe_{105} - Met_{106}$

  - Unlike Chymosin, also hydrolyses α_s-casein

Problems with use of the flowers

- Protease activity is unstandardized
  - Current practice is to grind up flowers and make a liquid extraction (tea). Unknown activity can cause a batch of milk to be lost.
  - **Solution:** Liquid is standardized @ Scranton PA so each batch will have exactly the same clotting strength.

Problems with use of the Flower

- No control over long term storage of the flowers
  - Current practice is to hold the flowers at ambient conditions at the cheese factory. This can lead to excessive loss of activity and potential mold
  - **Solution:** Large scale processing allows frozen storage of the flowers for year round use.

Problems with use of the Flowers

- No testing for other quality issues
  - Molds (aflatoxins), Heavy Metals, Pesticides
  - The problem is that limited amounts of flowers prevent testing by individual factories
  - **Solution:** Larger scale batches allow for complete testing on an economical basis.

Regulatory Concerns

- While common use in Europe and well documented, it has not been used in the USA.
  - **USA – FDA self affirmed GRAS is done as of the end of 2014.**
  - Canada – It is approved as cyprosin in standardized cheese as of December 2014.

Potential new applications

- All assumptions are based on the fact that this Aspartic Protease is different from rennet.
  - It has a completely different action pattern.
  - Like other botanical proteases, it clots milk but, like other botanical proteases may work to hydrolyze other proteins, e.g. Papain & Bromelain.

Potential New Applications

- Enzyme Modified Cheese
  - Produces unique cheese flavors over traditional proteases.
  - Protease has a lower temperature of inactivation that other botanical or bacterial proteases.
  - Initial “bitter notes” mellow over time.
    - “Bitter notes” can be eliminated by adjusting the enzyme dosage

Fresh Cheeses and Whey Cheese

- Has shown good texture and flavor in Queso Fresco products
- Possible modification of the ricotta process to reduce energy requirement by pre-clotting
- Possible texture improvement in low fat cheese (softer, less grainy)

Protein Hydrolysis

- Work to begin shortly on soy and wheat hydrolyses.
  - Possible different peptide profiles
  - Expected mild hydrolysis so likely will be combined with other proteases
  - Easy to inactivate protease without other side activities

For the Cheese industry

- Cynzime® liquid produced in USA
- Kosher, Halal, Vegan
- Sold in the USA and Canada by
  Enzyme Development Corporation

Check with EDC for Samples

- Thank you for your time.