

THE USE OF ENZYMES IN YEAST EXTRACTION

The flavour industry is constantly seeking new flavours and new flavour combinations to wow customers, but sometimes it is the old ones that are the best. One such flavour is that of yeast extract, the meaty savoury flavour so beloved by the food industry.

Biocatalysts Ltd manufacture enzymes which play an important role in the extraction of yeast. This includes a microbial alternative to the traditionally used papain. The microbial origin of this comparative enzyme not only allows for use in kosher, halal and vegetarian products, but also removes variability of quality and supply associated with plant derived material.

This technical bulletin will take you through the yeast extraction process giving recommendations to achieve the best possible extraction. This includes:

- Accelerating the process
- Increasing yields
- Reducing viscosity
- Improving solubilisation of debris .
- Aiding the clarification of the extract
- Compensation for damaged/inactive yeast cells .
- Flavour enhancement .



Figure 1.0 Saccharomyces cerevisiae

The enzymes covered in this technical bulletin are from our current off-the-shelf range. If you find that these are not suitable, Biocatalysts has the enzyme development & manufacturing capabilities to create a completely unique enzyme for you, which can open up endless possibilities.

BACKGROUND

Yeast extract is well known for its use as a food flavouring agent in many food products, e.g. soups, sauces, gravies, snack foods, and meat products. It is the main component of savoury spreads and is produced by hydrolysing baker's yeast or spent brewer's yeast to provide a paste or spray dried product with a very strong savoury flavour. It is essentially a concentrate of the soluble cellular components of the yeast (such as amino acids, nucleotides, peptides, proteins, sugars, vitamins and flavour compounds) and is an excellent source of protein, B vitamins and nucleotides. The constituents from different Saccharomyces yeasts are usually similar. Baker's yeast tends to have a higher carbohydrate and lower protein level than brewer's yeast.

An essential part of yeast processing is the breakdown of the cell wall. The yeast cells can be disrupted using mechanical methods or 'burst' when suspended in water. Once some of the cells have been disrupted, endogenous enzymes released from within the yeast start to hydrolyse the cell content by autolysis. Exogenous enzymes are added to accelerate this process resulting in decreased processing times and higher yields.

Enzymes can also be used effectively to break down the yeast cell wall. The characteristic components of the yeast cell wall include beta-1,3-glucan, beta-1,6-glucan, mannan, protein, chitin and lipids. In addition to aiding cell wall digestion, the presence of enzymes will also improve the flavour of the end product.



Figure 2.0 yeast cell 'bursting' in water releasing endogenous enzymes.

A MICROBIAL ALTERNATIVE TO PAPAIN

Promod[®] 950L is a microbial endopeptidase preparation with a broad substrate specificity. It was developed to hydrolyse baker's and brewer's yeast proteins to increase solubility and yields during the manufacture of yeast extracts. The enzyme is a sulphite-free microbial protease and can be used to manufacture yeast extracts with lower sulphite content. It is also a suitable alternative to Papain (100TU/mg) and the microbial origin of the product removes variability of quality and supply associated with plant derived material.

FLAVOUR ENHANCEMENT OF YEAST EXTRACTS

Flavorpro® UMAMI 852MDP can be used on its own or in combination with Promod® 950L to produce a yeast extract with a savoury flavour. Flavorpro® UMAMI 852MDP is an exopeptidase with endopeptidase and glutaminase side activities. The enzyme releases high levels of glutamic acid, an amino acid giving strong umami flavour. An umami flavour note will increase the apparent saltiness of the yeast extract and can be used for salt reduction in yeast extract applications. Yeast extracts with high glutamic acid content can also be used to replace monosodium glutamate (MSG).

Flavorpro® 937MDP is an expopeptidase preparation from a fungal source with low levels of endopeptidase activity. In yeast extract production, Flavorpro® 937MDP can be used to avoid bitterness and to create high levels of free amino acids to enhance the flavour intensity of the yeast extract. It can also be used in combination with Promod® 439L, an endopeptidase used to help lyse the yeast cell wall.

Promod® 950L Efficient lysis of yeast. Good hydrolysis of yeast proteins. Acceleration of yeast extract production process.	Promod [®] 144GL Accelerated process (shorter time). Higher yields of peptides. Efficient release of other valuable yeast well	 Promod[®] 439L Efficient lysis of yeast and yeast proteins. Accelerated process (shorter time). When used in combination with F937MDP it will increase levels of free amino acids, including glutamic acid.
Increased yields of yeast extract. Suitable for production of yeast extracts with lower sulphite content.	Flavour enhancement.	
Flavorpro [®] Umami 852MDP It creates an umami / savoury type flavour. It can be used to reduce salt (health benefit). It can be used to avoid addition of monosodium glutamate.	Flavorpro® 937MDP It can be used in debittering of yeast extracts. It increases the concentration of free amino acids. It can be used to enhance the flavour intensity of the yeast extract.	

PROCESS FOR ENZYME ASSISTED YEAST EXTRACT PRODUCTION

Yeast Cream 28% DM, 600 g/L

Adjust pH to 5

(Plasmolysis)

Baise temperature

Although the details of the production process might vary from manufacturer to manufacturer, with regard to temperature, pH or the duration of each step, most follow the general pattern of plasmolysis, autolysis, pasteurisation, clarification and extract concentration. As an example, typical conditions for preparing an extract of a Baker's type yeast are outlined below:

1. Dilute yeast cream to 600g/l (28% dry weight) and adjust pH of veast cream to about 5. (plasmolysis)

$\widehat{\mathbf{\omega}}$	Q	to 55°C over 5-8h	yeast cream to about of (plasmolysis)
(+ Autolysis		Hold at 55°C for 24h	 2.8kg of Promod[®] 950L and 3.5kg of sodium chloride are added per 1,000 litres of diluted yeast cream. Raise temperatures over 5-8hours to 55°C. (plasmolysis and autolysis)
			0.50% w/w of Flavorpro [®] 937MDP yeast dry weight.
ation)	4	Raise temperature to 70°C and hold for 15h	-O Sodium Chloride 3.5 kg per 1000L of diluted yeast cream.
teuris			O Promod [®] 950L 1% w/w of yeast dry weight.
(Pas	¢	70 - 75°C for 2-5h	
			3. Hold at 55°C for 24 hours (plasmolysis and autolysis).
	0	Centrifuge (remove debris) (clarification)	4. Raise temperature to about 70°C and hold for 15 hours (completes autolysis and starts pasteurisation).
		First evaporation under	5. Centrifuge to remove cell debris. Counter current washing for maximum yield of extract (clarification).
		partial vacuum to >30% solids (extract concentration stage 1)	6. Heat the extract to 70-75°C for 2-5 hours (completes pasteurisation).
	þ	Filtration (remove precipitate)	7. First evaporation under partial vacuum to above 30 per cent solids (extract concentration stage 1).
		Second evaporation under Vacuum to 70 – 75%	8. Polish filtration to remove any precipitate produced during the first concentration step.
	9	solids (or spray dry) (extract concentration stage 2)	9. Second evaporation to 70-75% solids using vacuum. Ensure temperature does not exceed 55°C. (extract concentration stage 2)
		final product	Note: Variations in temperature, holding time, pH, proteases and the level of salt used will affect yield and flavour.

Note: The above flow diagram outlines the process that is used in the United Kingdom, but the process is subject to variability in other countries, especially the plasmolysis and autolysis stages.

STEP 1: PLASMOLYSIS

A simple method for the initiation of cell disruption. Favourable conditions include raised temperature - sufficient to kill the yeast but not to inactivate its enzymes and the addition of chemicals, in particular salt, or organic solvents such as isopropanol.

The plasmolysing agents have some bacteriostatic or bactericidal effect and therefore perform the additional role of reducing the growth of any contaminating bacteria which can cause a high viscosity in the extract, hamper clarification and impair the flavour of the final product.

Salt, as a condiment, contributes to the flavour. However, if the extract is made by heat alone (or with an organic plasmolysing agent), it will be more bland but will be low in salt, making it suitable for use in special applications e.g. food for convalescents, infant and baby foods.

Suitable conditions for a temperature-alone plasmolysis are pH 5.5 and 40 - 48 hours at 58°C, to achieve yields of around 65%. Similar results are obtained if isopropanol is used at 0.5% v/v, but with a lower temperature for the first 5 hours.

STEP 2: AUTOLYSIS

Self-digestion of the yeast cell contents. During this stage enzyme-catalysed hydrolysis is achieved commercially by relying on the yeast's own enzymes, possibly augmenting them with an added protease such as Promod[®] 950L at about 1% w/w of yeast dry weight. The added Promod[®] 950L is useful for increasing solubilisation in long autolysis processes only.

STEP 3: PASTEURISATION

This serves to kill vegetative cells of bacteria, and the temperature used is sufficiently high to inactivate Promod[®] 950L and yeast proteases.

In the process typified by figure 1, no further solubilisation occurs during the pasteurisation, but

interaction of small molecules, in particular amino acids and sugars, leads to flavour development.

STEP 4: CLARIFICATION

Clarification removes insoluble cell wall debris, some protein, glucans and mannans, which, despite the presence of mannanases and glucanases in the yeast, are only slightly degraded during autolysis.

Some solubilisation of this debris can be achieved with a glucanase used during the autolysis stage. Insoluble material is discarded as waste or can be incorporated into animal feed, or may find use as a flavour carrier.

A second clarification/filtration step may follow the first concentration stage to produce a clear extract by removing the haze formed during concentration. This haze is partly composed of less soluble oligopeptides precipitated at the concentration stage.

STEP 5: CONCENTRATION

This is usually in two stages, with a final filtration interposed. During the extraction concentration, further development of flavour occurs, influenced by the increasing concentration of the components and by the temperature.

To avoid the development of burnt flavours during the final evaporation, a temperature not exceeding 55°C is achieved by the application of vacuum techniques.

Note: Heat Denatured Yeast

Many breweries now operate an alcohol recovery programme from the waste yeast. This process involves heat which kills a lot of the yeast and inactivates many of the yeast's own enzymes required for autolysis. In this case additional protease/exopeptidase is required over and above Promod® 950L. Our exopeptidase product Flavorpro® 937MDP can be used to compensate for the partial destruction of the endogenous enzymes when used at a dose rate of 0.50% w/w of yeast dry weight.

COMMON PROBLEMS ENCOUNTERED IN YEAST EXTRACTION

Problem	Enzyme Solution
Enzyme not working.	Ensure no direct heat is denaturing enzyme solution and rendering it ineffective.
Low yield following autolysis.	 Check pH and temperature of slurry at each stage. Extend autolysis time. Increase dose of Promod[®] 950L.
Heat (e.g. During alcohol recovery stage) inactivates yeast proteases, preventing efficient autolysis.	Add Flavorpro [®] 937MDP 0.50% w/w of yeast dry weight.
Evaporator capacity limiting.	Extend pasteurisation time.
Burnt flavours.	Keep temperature below 55°C.



Developing #BiobasedValue

Contact Biocatalysts' scientists to learn more about enzymes used in Yeast Extract production or to create something bespoke to your application.

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