

## Yeast Extracts : Production, Properties and Components

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### Production of Yeast Extracts

Yeast autolysates are concentrates of the soluble components of yeast cells and in Europe are predominantly produced by autolysis. That is to say, that the cell hydrolysis is performed without addition of other enzymes. Yeast autolysates are known under the name of "yeast extracts" and are mainly used in the fermentation industry as substrates and in the food industry as flavour improvers.

In Europe, the major raw material for yeast extract is primary grown high protein yeast (strains of *Saccharomyces cerevisiae*), which is grown on molasses based media. In the United Kingdom and in the United States, yeast extracts are also manufactured from debittered brewers yeasts (strains of *Saccharomyces cerevisiae* or *Saccharomyces uvarum*). Other raw materials in use are yeasts such as *Kluyveromyces fragilis* (fermented on whey) or *Candida utilis* (grown on high carbohydrate waste-products of the timber industry or on ethanol).

Autolysis is the most frequently used disruption method in yeast extract production. During this process, yeasts are degraded by their own endogenous enzymes. The autolysis process can be initiated by a controlled temperature or osmotic shock, causing the yeast cell to die off without inactivating its own endogenous enzymes (particularly the proteases). Controlled pH, temperature, and duration of the autolysis are decisive factors for an optimal and standardized autolysis process. By addition of salt or enzymes (for example proteases or mixtures of proteases and peptidases) the protein degradation of the yeast cell can be controlled. For this, both the ratio of the peptide fractions and the ratio of the peptides to the free amino acids have to be modified.

Modifications of that kind result, on the one hand, in new product properties of interest to the food and fermentation industries and, on the other hand, in more neutral flavour profiles compared to the "classic" yeast autolysates. For the production of yeast extracts containing five prime nucleotides special strains of primary grown bakers yeast are used as raw materials.

The yeasts ribonucleic acid (RNA) is extracted under controlled conditions and then enzymatically hydrolysed. Fresh bakers yeast contains 6 to 8 % RNA, special strains may contain 13 % or more (all figures referring to dry solids contents). Under normal autolysis conditions, the RNA is mainly degraded to three prime nucleotides. These do not have any flavour enhancing properties. Under controlled enzymatic hydrolysis, 5 prime nucleotides of guanine (GMP), adenine (AMP), cytosine (CMP) and uracil (UMP) are formed. However, flavour enhancing properties have only been found in 5 prime GMP. By use of adenylic

deaminase adenine-5 prime-monophosphate (AMP) is converted to inosine-5 prime - monophosphate (IMP). Yeast extracts containing 5 prime IMP have significantly increased flavour enhancing properties.

Other disruption methods used for yeast extract production are:

- thermolysis (for example boiling of yeast in water at 100o C)
- plasmolysis (for example treatment with high salt solutions at
- temperatures well below 100o C)
- mechanical disruption (for example by high-pressure homogenisation or a ball mill)

Dependent on the process, duration of the autolyses can vary from 15 to more than 60 hours. After completion of the autolysis the soluble cell components are separated from the insoluble cell walls and then concentrated by agitating evaporators or falling film evaporators. Particularly for applications for the fermentation sector the concentrate may then have to undergo filtration or "polishing" steps. A further concentration step, carried out in a partial vacuum, and a short-time sterilisation provide the typical product types:

- liquid yeast extracts (with dry solids contents of 50 to 65 %)
- highly viscous paste types (with dry solids contents of 70 to 80 %)
- Dried powder types are mainly obtained by spray drying. Some manufacturers also use vacuum belt driers or drum driers.

During all those process steps (with the exception of short-time sterilisation) temperatures are manipulated to maintain active vitamins and other heat sensitive components. Only during short-time sterilisation, have temperatures to exceed these levels, to inactivate residual enzyme activity and provide product stability.

( Process of Yeast Extract Production)

Yeast cell walls are marketed either as liquid or as spray-dried products. By additional alkaline extraction steps a product with glucan contents up to 70 % can be obtained.

(Production of Yeast Cell Walls)

## **Yeast extract properties in foods**

Yeast autolysates and yeast extracts are mainly used as flavour improvers, but they can also serve as flavour enhancers or even as pure flavours. Other yeast autolysates can mask bitterness or sour taste, increase aroma and serve as colouring agents or antioxidants.

In 5 prime IMP and 5 prime GMP enriched foods (ratio of the ribonucleotides: 1 : 1), sweet and salty tastes are only slightly enhanced, whereas sour and bitter tastes are suppressed. The flavour improving properties come from the interaction of various amino acids (the most important being glutamic acid) in combination with 5 prime nucleotides, peptides and reaction products.

To put it simply, the flavour enhancing effect of 5 prime GMP, 5 prime IMP and glutamic acid is a continuous stimulation of the receptors in the taste buds which creates a greater sensory potential for flavours. I would now like to give a few comments on glutamate, the most important flavour enhancing component of yeast autolysates. With regard to taste, the threshold value of glutamate is 100 - 300 ppm, the threshold values of 5 prime inosinate and 5 prime guanylate are 120 ppm or 35 ppm respectively (tested in aqueous solutions). The flavour enhancing properties of 5 prime guanylate are three times higher than those of 5 prime inosinate. In combination with the 5 prime nucleotides glutamate is an effective synergist. Its impact on taste is intensified by factor 10 to 15 when used in combination with 5 prime GMP and 5 prime IMP. However, these effects are dependent on the concentration of the 5 prime nucleotides and on the other food components.

### Flavour Enhancing Effects of MSG

- Flavour enhancing effects with **dissociated MSG** only
- Dissociation degree dependent on pH value

<i>pH</i>	<i>Dissociation Degree</i>
4	36.0 %
5	84.9 %
6	98.2 %
7	99.8 %
8	96.9 %

L glutamic acid

H

HOOC - CH<sub>2</sub> - CH<sub>2</sub> - C - COOH

NH<sub>2</sub>

L glutamic acid (dissociated)

H

- OOC - CH<sub>2</sub> - CH<sub>2</sub> - C - COO -

NH<sub>2</sub><sup>+</sup>

With glutamate this effect can only be achieved within a pH range of 5 - 8 (slightly acid to neutral), as only dissociated glutamate has any flavour enhancing properties.

Just a comment on the negative image of MSG:

For example, glutamate is suspected of being the cause of the Chinese restaurant syndrome. This opinion is misleading and pejorative. The American Medical Association as well as the European Community have declared that MSG does not represent any health hazards (Statement of the IFF in Food Technology, October 1995).

A mixture of 95 parts of MSG and 5 parts of 5 prime IMP and 5 prime GMP is recommended as a food additive. The flavour enhancing potential of these components is revealed when

taking into consideration their *naturally occurring amounts* in a salt-free special yeast extract. It contains:

- total glutamic acid 11 % approx.
- free glutamic acid 4 - 6 %
- 5' IMP 1.5 % approx.
- 5' GMP 1.5 % approx.

Glutamic acid and the 5 prime nucleotides are chemically stable substances. They are scarcely altered or degraded by standard food processing. However, attention should be paid to enzymatically active foods (such as raw meats, raw fish and vegetable tissues) which contain transaminases, deaminases and decarboxylases, as those enzymes can degrade glutamic acid.

### **Degradation of Glutamic Acid**

H H

HOOC - C - C - COOH

H NH<sub>2</sub>

- Decarboxylation by Decarboxylase
- Deamination by Deaminase

Phosphatases degrade the phosphoric acid residues of the 5 prime nucleotides. Through this, 5 prime IMP and 5 prime GMP lose their flavour enhancing properties. It is said that the 5 prime nucleotides in combination with glutamic acid and cysteine contribute to a foods typical meat flavour. However, the primary amino groups of the free amino acids also play an important role.

They react with the carbonyl groups of the reducing sugars and form Schiff's bases. By Maillard reactions melanoidines with distinct brown colouring properties are formed. They cover a wide spectrum of flavour intensive compounds. Important process control parameters are temperature, reaction time, concentration and structure of the solutions.

The following list shows which odours and tastes are obtained when dry heating a pure amino acid and glucose to 180°C.

### **Amino Acids : (Odour / Taste)**

#### **Odour / taste obtained when dry heating a pure amino acid and glucose to 180°C :**

- Glycine (Caramel)
- Alanine (Caramel)
- Valine (Chocolate)
- Leucine (Baked cheese)
- Isoleucine (Baked cheese)

- Proline (Cakes and pastries)
- Hydroxyproline (Crackers)
- Methionine (Cooked potatoes)
- Phenylalanine (Violets)
- Tyrosine (Caramel)
- Asparaginic acid (Caramel)
- Glutamic acid (Toffee)
- Histidine (Corn bread)
- Lysine (Fresh bread)
- Arginine (Burnt sugar)

*Reference: Ajinomoto Comp., Japan*

Control of downstream processing allows further changes in flavour profile. Thus yeast extracts with meat like or roasted notes or with brothy and grainy flavour profiles can be manufactured.

Further applications of salt-free yeast extracts containing 5 prime nucleotides are:

- childrens foods
- oncological preparations
- cancer therapy

The antioxidative properties of yeast extracts come from their contents of glutathione, Maillard reaction products and sulfur-containing amino acids.

### **Amino Acids (Effects / Applications)**

- **Alanine and Asparaginic Acid** Masking of sour taste [ fruit juices etc. ]
- **Tryptophan and Methionine** Anti-oxidative properties [ fat ]
- **Tryptophan and Histidine** Anti-oxidative properties [ milk powder etc. ]
- **Histidine** Anti-oxidant [ frozen sausages ] Inhibition of formation of rancid notes [ biscuits] *in combination with glucose)*
- **Cysteine** Anti-oxidant [ Improvement of bread quality ]
- **Methionine** Increase in nutritional value [ Animal food sector ]
- **Lysine** Supplement to cereal proteins (*in combination with threonine and tryptophan*)
- **Glycine** Masking of unpleasant sharpness caused by artificial sweeteners Provision of a fuller taste (*in combination with artificial sweeteners*) Bacteriostatic [ food sector ] Increase of keeping properties [ fish products, ham, sausages ]

The antioxidative effect of Maillard reaction products is dependent on the amino acid sequence of the dipeptides. And dipeptides can be components of yeast autolysates. It was found that the inhibitory activity of the melanoidines on autoxidation is much stronger than of butylhydroxyanisole (BHA) or propyl gallate.

In butter we tested the antioxidative properties of glutathione. It is known that at room temperature and exposed to light and oxygen butter is quickly turned rancid. The tests showed that glutathione dosages of 0.01 % and 0.02 % slow down this process significantly.

Glutathione is the tripeptide of glutamate, cysteine, and glycine. Fresh baker's yeast contains approx. 0.65 % (dry basis) glutathione. By fermentations of selected yeast strains glutathione contents up to 5 % can be achieved. This is the basis for high glutathione yeast extracts (with up to 15 % glutathione). High glutathione yeasts or yeast extracts can act as dough conditioners and thus as substitutes for cysteine. In dough, glutathione acts in several ways. It can be involved in the enzymatic reduction of dehydroascorbic acid, or act directly on gluten. By degradation and/or inter-exchange of the gluten disulfide bonds, glutathione can be used to adjust the dough rheology. Glutathione is highly reduced and can be used as food and pharmaceutical grade antioxidant. It has important properties for therapeutical preparations:

- Glutathione is the highest non-protein thiol in mammalian cells.
- Glutathione promotes the detoxification of xenobiotic compounds.
- It plays an important role in the antioxidation of reactive oxygen species and free radicals.
- By acting as an antioxidant or by binding with cellular mutagens, glutathione can serve as an anticarcinogen.
- The human overall plasma level of glutathione is a result of both liver synthesis and dietary intake.
- Experimental carcinogenesis can be prevented by dietary intake of Phase II enzyme inducers. These substances even occur naturally in vegetables.
- Glutathione promotes the proliferation of lymphocytes.
- It slows down HIV replication.
- It helps in liver or angina therapies. *etc.*
- Naturally occurring glutathione in raw fruit and vegetables (e.g. broccoli) decreases the risks of oral cancer.

## **Glucans / Yeast cell walls**

Another yeast component with growing importance is glucan, derived from the crude yeast cell walls. Approx. 20 % of the total dry weight of yeast is cell wall material. It has a two-layered structure. An outer mannoprotein layer encloses an inner skeletal layer of -glucan. Shape and strength of the cell are dependent on the -glucan layer. It also consists of two layers, an outer alkali soluble non-chitin linked layer and an inner alkali insoluble chitin linked layer.

The alkali insoluble fraction is of major commercial interest. Pure glucan is white in colour with a slightly sweet taste and a fat-like mouthfeel.

### **Glucan Production :**

- Cell walls
- Alkaline extraction
- Neutralisation
- Acid extraction
- Neutralisation
- Spray drying

### **Glucan Applications**

Standard product for feed and food applications:

- Dry matter **96 %**
- NaCl **< 0.1 %**
- Protein in dry matter **< 5 %**
- Ash **< 4 %**
- Total polysaccharides **78 %**
- Glucan **> 70 %**

Glucan applications range from the pharmaceutical and veterinary sectors to cosmetics and foods.

- Glucans can be used as immunostimulants in medical and veterinary applications.
- Glucans promote the production of monocytes, neutrophils, fibroblasts, collagen and elastin.
- Glucans can stimulate the macrophage to increase the metabolic activity which leads to an increased IL-1 (interleukin) production. This is essential for the IL-2 formation in the lymphocytes.
- Particularly for ruminants, glucans have important properties as immunostimulants. They were found to increase phagocytosis and have been shown to protect ewes against staphylococcal mastitis.

There already is a considerable amount of patent literature on the production of different glucan types.

### **Recent patents stress the following applications :**

- thickeners for health foods, soft texture foods, cream cheeses, juices and sour milk
- fat replacers

- cell sacs for encapsulation
- cosmetics for skin repair
- cholesterol reduction
- adjuvants for wound healing
- immunostimulation in plant, animal and human health
- vaccine adjuvants
- feed supplements for aquaculture and pig industries.

However, the range of yeast extract applications is not limited to the food, feed or pharmaceutical sectors.

## **Yeast Extracts for Fermentations**

### **Composition of a standard salt-free powder yeast extract for the fermentation industry :**

Average protein contents are 73 - 75 %. Sodium and polysaccharide contents do not exceed 0.5 % or 5 % respectively. (The predominant polysaccharides in salt-free yeast extracts being mannans and glucans.) Oligosaccharide contents are less than 1 %. They consist of mannan and glucan fractions. In combination with polysaccharides oligosaccharides frequently cause problems during yeast extract processing. Fat contents less than 0.5 % do not have significant effects on the fermentation of microorganisms.

The yeast extracts protein content is broken down as follows :

Free amino acids amount to 35 - 40 %. Di-, tri-, and tetrapeptides with molecular weights less than 600 MW (Dalton) contribute 10 - 15 % to the total protein. With a total of 45 - 55 %, those two fractions are the largest portion of the yeast extract protein.

The portion second in size are oligopeptides with 2000 - 3000 MW (Dalton). These oligopeptides amount to 40 - 45 % of the total protein. With contents of 2 - 5 % only, oligopeptides with molecular weights of 3000 - 100000 MW (Dalton) are the smallest fraction. In yeast autolysates produced by autolysis the ratios of the free amino acids to the di-, tri-, tetra-, and oligopeptides are relatively stable. By enzymatic disruption processes those ratios can be altered significantly, thus providing new applications for the fermentation industry. Very often, yeast autolysates serve as essential nutrients in fermentations. However, details of the exact mechanisms have still to be identified.

## **Salt-Free Yeast Autolysate**

### **Amino acid and free amino acid contents :**

<i>Amino Acid</i>	<i>% in product</i>	<i>% free AS in product</i>
<i>Asparaginic acid</i>	6.66	2.49
<i>Threonine</i>	3.20	2.02
<i>Serine</i>	3.28	2.35
<i>Glutamic acid</i>	9.18	6.01
<i>Glycine</i>	3.17	1.11
<i>Alanine</i>	5.53	4.78
<i>Cystein</i>	0.45	--

<i>Valine</i>	4.09	3.30
<i>Methionine</i>	1.12	1.08
<i>Isoleucine</i>	3.38	2.64
<i>Leucine</i>	4.83	4.34
<i>Tyrosine</i>	1.92	1.46
<i>Phenylalanine</i>	2.80	2.72
<i>Histidine</i>	1.63	1.80
<i>Lysine</i>	5.51	3.08
<i>Arginine</i>	1.71	0.24

This list clearly shows that there is no constant ratio of amino acids to free amino acids. On the one hand, at least 85 % of the methionine, leucine, alanine, and phenylalanine contents are in the form of free amino acids. On the other hand, free amino acid portions of asparaginic acid, glycine, and arginine are 14 - 37 % only.

The reason is the typical degradation process of the yeasts own proteases and peptidases. During the autolysis process proteases endogenously break the amino acids at defined bond points. Carboxypeptidases and aminopeptidases, however, break the amino acids exogenously beginning either at the acid ends or at the amino ends.

Just a remark on glutamic acid: It is essential for the assimilation of amino acids.

### **Vitamin contents**

- Thiamine - B1            3 mg / 100 g
- Riboflavin - B2        11.9 mg / 100 g
- Niacin                    68 mg / 100 g
- B6                         2.3 mg / 100 g
- Folic acid                3.1 mg / 100 g
- Ca-Pantothenate      30 mg / 100 g
- Biotin                    0.25 mg / 100 g

Vitamin contents and ratios may differ significantly, dependent on production process and processing of the yeast autolysates. pH adjustment and sterilisation conditions are important factors for keeping vitamins active.

### **Mineral contents**

- Calcium    120 mg / 100 g
- Magnesium 200 mg / 100 g
- Potassium    3.3 g / 100 g
- Sodium      < 0.5 g / 100 g
- Iron            5 mg / 100 g
- Phosphorus   1.8 g / 100 g

Processing and filtration steps may decisively change the mineral contents and ratios. Through this, the subsequent growth of microorganisms can be affected considerably. However, it has been found that standard yeast autolysates are not sufficient to meet the specific requirements of the fermentation industry. Bacteria, yeasts, and other micro-organisms can firmly hold on to the slightest cracks and openings in surfaces of industrial equipment. For its delicate fermentations the pharmaceutical industry uses electropolished special steel fermenters. Surface roughnesses have to be less than 0.8  $\mu\text{m}$  to minimize the formation of a biological layer consisting of cells and extracellular metabolites (such as polysaccharides, proteins, lipids, and colloids).

However, it has to be taken into consideration that chloride-containing substrates may cause corruptions. To avoid any corruptions, yeast extracts with chloride ion contents higher than 0.05 % cannot be used as substrates for those fermentations. Molasses is the main raw material for yeast and yeast autolysate fermentations. However, to obtain yeast autolysates with chloride ion contents less than 0.05 %, molasses has to be substituted by highly refined carbon sources, such as glucose syrup or ethanol. Similar effects are to be observed in fermentations for the isolation of special metabolites. The delicate extraction can be made even more difficult by polysaccharides of molecular weights higher than 50000 MW (Dalton). Typical examples are mannans, glucans, cell fractions, crystalline components of insoluble amino acids, and minerals such as calcium, magnesium, copper, and iron. As fermentation substrates ultrafiltered yeast autolysates have been used successfully. Even after sterilisation they show neither cloudiness nor sediments.

In future the demand for yeast autolysates with special components to support defined functions/reactions will increase rapidly.

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