# REVIEW ON DRYING PROCESSES AND DAMAGE PROTECTION MECHANISM OF LIQUOR YEAST
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# 白酒酵母干燥工艺及损伤保护机制综述

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*DOI: https://doi.org/10.35633/inmateh-68-73*

***Keywords:*** *Liquor yeast drying process; drying damage mechanism; drying protection strategy; cell membrane perforation.*

***ABSTRACT***

*From the perspective of liquor brewing technology, the quality of liquor yeast undoubtedly determines the quality of liquor products, but the problems such as inconvenient storage, difficult transportation and easy deterioration of liquid liquor yeast greatly restrict the development of liquor industry. Aiming at this problem, the author firstly summarized the research status of drying technology, damage mechanism and protection strategy of white spirit yeast. Then, on the basis of studying the damage mechanism of yeast in the drying process of yeast, the optimization of drying process and the formulation of protective strategies of yeast were discussed. Finally, new research methods are proposed from three perspectives: optimal design of drying process, damage mechanism and protection strategy.*

***摘要***

*从白酒酿造工艺出发，白酒酵母的品质无疑决定了白酒产品的质量，但液态白酒酵母储存不便、运输困难、易变质等问题，极大地制约了白酒行业的发展。 针对这一问题，作者首先总结了白酒酵母的干燥工艺、损伤机理和保护策略的研究现状。然后，在研究白酒酵母干燥过程中酵母损伤机理的基础上，对干燥工艺的优化和酒酵母保护策略的制定进行了研究展望。最终分别从干燥工艺优化设计、损伤机制和保护策略三个角度，提出了新兴的研究方法。*

## INTRODUCTION

There were countless beautiful poems about Liquor in ancient times of China, so Liquor culture is undoubtedly one of the treasures of traditional Chinese culture. At the same time, the liquor industry is also one of the main sources of the national economy (*Wang D. et al., 2019*). However, the inconvenience of transportation and storage of liquid liquor yeast greatly restricts the development of my country's liquor industry (*Jorgensen H., 2009*). Therefore, it is the key to promote the development of the liquor industry to first study the damage protection mechanism of liquor yeast drying, then optimize the drying process, and finally improve the survival rate and efficiency of liquor yeast drying.

The drying of microorganisms represented by liquor yeast is essentially different from ordinary drying of fruits and vegetables. Drying of fruits and vegetables, only needs to consider conventional factors such as drying efficiency and the taste, colour, and shelf life of the dried product, while drying of microorganisms such as liquor yeast must also be considering the biological activity, rehydration survival rate, biological function and other unique factors of the dry product (*Huan-Qin Li et al., 2021*). Therefore, it is particularly important to study the damage mechanism of its drying from the molecular scale, and then formulate an effective protection strategy and optimize the drying process. In the field of drying containing microorganisms, the commonly used drying processes are vacuum drying, freeze drying, fluidized bed drying and spray drying.

In view of the contradiction between the survival rate and efficiency of microbial drying, this paper summarizes the previous researches in-depth and concisely from the drying process, damage mechanism and protection strategy of liquor yeast. Finally, on this basis and in combination with the existing technology, three research directions of microbial drying such as white wine yeast in the future were put forward.

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## 1. Review on the mechanism of drying damage of liquor yeast

Understanding the damage mechanism of liquor yeast drying is the basis for formulating drying protection strategies and optimizing drying processes. It is generally believed that thermal damage, dehydration damage, oxidative damage, osmotic pressure damage and shear damage are the typical damage mechanisms of yeast drying (*Spreutels, L. et al., 2014*).

### *1.1. Dehydration damage*

The water content in microbial cells is about 80-90%, which often exists in the form of bound water and free water, which is not only an indispensable part of biological reaction, but also a necessary component to maintain biological macromolecular structure and fluidity (*Ball P.J.C.R., 2008*). Among them, bound water refers to the water combined with cell components, accounting for about 4.5% of the total water, and its loss is the core cause of cell dehydration damage (*Santivarangkna C. et al., 2007*). Previous studies have shown that dehydration damage is inevitable for microorganisms in drying. On the one hand, because the moisture content of microorganisms dried products is generally required to be less than 7% for long-term preservation (*Peighambardoust S.H. et al., 2011*), but the minimum water content to maintain microorganisms physiological state is 33.33%. When the dry water content is less than 33.33%, it is bound to be quickly inactivated due to dehydration damage. On the other hand, because the cell water loss is not strictly in the order from free water to bound water, but when the water content is lower than 22.3%, the bound water in the cell begins to lose.

A large number of studies have shown that the loss of bound water reduces the stability of the microbial cell substructure, especially the decrease in the fluidity and selective permeability of the cell membrane, which is the main reason for the inactivation of dehydration damage (*Golowczyc M.A. et al., 2011*). Because the cell membrane is the main place for the exchange of substances between microorganisms and the outside world, its unique fluidity and selective permeability have become necessary conditions to maintain the survival of microorganisms. The cell membrane is composed of a phospholipid bilayer, and it is the interaction between phospholipid molecules, water molecules and adjacent phospholipid molecules through hydrophobic interaction, chemical bonds and hydrogen bonds, which ensures the fluidity and selective permeability of the cell membrane. However, the loss of bound water greatly increases the force between phospholipid molecules, resulting in an increase in the lateral stress between the phospholipid bilayers, resulting in a gel phase of the cell membrane and a decrease in cell fluidity, eventually leading to cell death. Many studies have shown that the water content of gel phase transition in microbial cell membrane is about 16.67% (*Santivarangkna C. et al., 2008*). With the continuous loss of water and the increase of transverse stress, the polar head of phospholipid molecules in the bilayer aggregates and nucleates, while the non-polar hydrophobic groups are exposed to the periphery to form Inverse Hexagonal Phase (as shown in Fig. 1) (*Gong P. et al., 2014*). The formation of the anti-hexagonal phase will completely destroy the selective permeability of the cell membrane, so that the substances inside and outside the cell membrane can freely enter and exit the cell, and even the cell membrane will rupture and collapse, resulting in the death of a large number of microorganisms (*Moayyedi M. et al., 2018*). Current studies have shown that the moisture content of Inverse Hexagonal Phase transition in microbial cell membrane is about 10.8%. The relationship between Survival rate of yeast and water content of liquor yeast in the process of traditional drying has shown in Fig. 2.

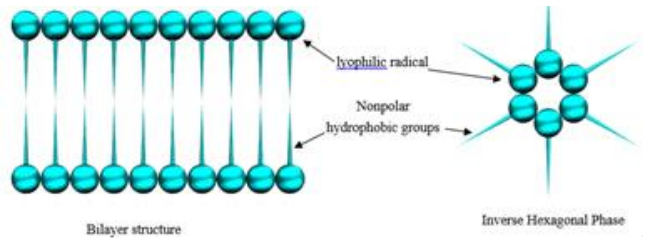



**Fig. 1 - The phospholipid bilayer and its inner hexagonal shape**

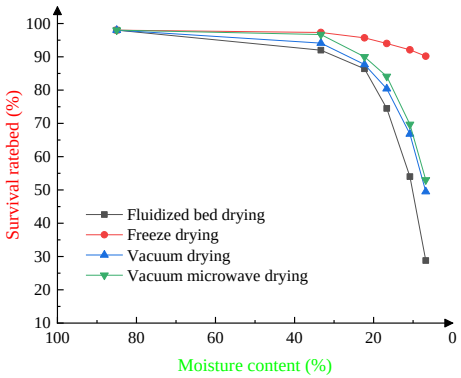



**Fig. 2 - Inactivation by desiccation and dehydration damage in sake yeasts**

Comparing the change curves of yeast moisture content and fermented rehydration survival rate under each traditional drying process in Fig. 2, then it is not difficult to find that the yeast survival rate has dropped sharply near the points where the moisture content is 22.3%, 16.67% and 10.8%. With the loss of water, the cytoplasm will continue to thicken, and at the same time, the aggregation density of macromolecules in the cytoplasm will continue to increase. When the cytoplasmic viscosity more than the1014 Pa·s, it will degenerate into a metastable with free molecular arrangement and solid physical properties (*Fonseca F. et al., 2016*). This phenomenon is often referred to as the glass transition, and the temperature at which the transition occurs is often referred to as the glass transition temperature. Glass transition could maintain the stability of microbial intracellular structure and inhibit the further occurrence of microbial dehydration damage. However, the water content in the microbial cells is inversely proportional to the glass transition temperature, that is, as the drying progresses, the water content in the bacteria decreases, and the glass transition temperature increases instead. When the moisture content of liquor yeast is 22.3%, the glass transition temperature-Tg=52-58°C, so the glass state is extremely difficult to form during the Liquor yeast drying process.

Studies by Sachie Fujii, a scholar from Yamamoto University in Japan, show that dehydration damage will decrease with the decrease of drying rate (*Sachie Fujii et al., 2014*). Therefore, the author believes that in reducing dehydration damage, the key moisture content point (22.3%) could be used as the conversion point, and the combination drying process and parameters of high and low drying rates are adopted before and after.

### *1.2. Thermal damage inactivation*

Thermal damage inactivation is also one of the main reasons for microbial inactivation in the process of drying. Many studies have shown that heating can damage the functional structures and components of microorganisms, such as cell wall, cell membrane, ribosome, RNA, protein, enzyme and so on. The critical injury temperature of each cell structure is shown in Fig. 3 (*Cebrián G et al., 2019*).

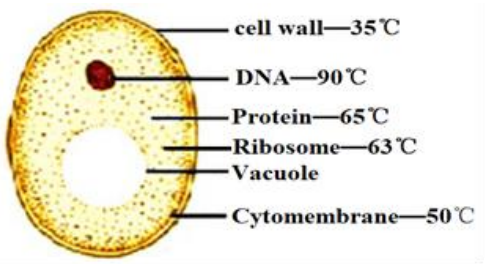



**Fig. 3 - Schematic diagram of the critical temperatures for each structure of liquor yeast**

The higher the drying temperature, the higher the drying efficiency of microorganisms, which is more conducive to enterprise mass production (*Russell A.D., 2003*). However, the drying temperature is too high, which will increase the thermal damage of the cells, resulting in a large number of microbial deaths. It is generally believed that the drying temperature of microorganisms represented by liquor yeast should be controlled within the range of 60-90°C (*Huang S. et al., 2017*). Based on the above analysis, the author believes that in order to ensure the efficiency and survival rate of microbial drying at the same time, the moisture content should be equal to 33.33% as the transposition point. The drying temperature higher than 70°C should be used in the early stage to improve the drying efficiency, and then the drying temperature below 50°C should be used to ensure the drying survival rate of microorganisms.

### *1.3. Osmolality damage*

With the loss of water, the concentration of cytoplasm will increase, which first leads to the increase of osmotic pressure on the outside of the cell before the glass transition, then causes the cell to contract and separates the cell membrane from the cell wall, and finally leads to the osmotic damage and inactivation of microorganisms. At the same time, in the process of rehydration of liquor yeast dry products, if the rehydration speed is too fast or the osmotic pressure on both sides of the cell membrane is too high, the microbial cells will expand rapidly and excessively, thereby causing osmotic damage and inactivation of the microbial cells (*Meneghel J. et al., 2017*). The authors believe that the reconstruction of osmotic pressure balance on both sides of cell membrane is the essence of solving the problem of cell osmotic damage and inactivation during microbial drying. Loading edible compatible media into bacteria instead of water molecules to combine with cell membranes and other cell structures is the most direct and effective protection method.

### *1.4. Shear damage*

Now studies have shown that although atomization might cause shear damage to microorganisms in the process of spray drying, the direct fatality rate is very low, which is mainly due to the inactivation of microorganisms in the later stage of drying (*Broeckx G. et al., 2016*). Shear damage occurs in the atomization process of spray drying and different atomization methods, nozzle structure size, spray parameters and so on would have a great impact on the degree of shear damage inactivation.

### *1.5. Oxidative damage*

The reactive oxygen species produced during drying can combine with the ribosome, protein and cell membrane of microbial cells, and then resulting in microbial cell damage. The latest study proposed that the oxidative damage of cell membrane lipid is an important cause of cell membrane damage (*Dijkstra A.R. et al., 2014*). Under normal conditions, microorganisms could produce antioxidant enzymes to eliminate free oxygen, but in the drying process, the normal metabolic activities of microorganisms are greatly restricted, so they could not produce enough enzymes to eliminate the active free oxygen accumulated in the environment, finally resulting in oxidative damage inactivation (*Ghandi A. et al., 2012*).

## 2. Protection mechanisms were reviewed

The current research usually takes the time as the clue and divides the protection strategy into three parts: pre-treatment before drying, adding protective agent and optimizing drying process parameters.

### *2.1. Optimization of culture conditions and stress treatment*

The optimization of culture conditions and stress treatment are the core components of microbial pre-treatment before drying, in which the optimization of culture conditions mainly includes two parts: the optimization of culture parameters and the optimization of culture medium. According to the treatment conditions, stress treatment is mainly divided into heat shock, acid stress and salt stress.

The optimization of culture medium usually includes five steps: primary selection of basic medium, optimization of basic medium, single factor drying experiment of additives, single factor experiment of optimization of culture parameters and response surface analysis. The evaluation indicators are generally set as the growth of yeast and the rehydration survival rate of dry particles.

In the process of culturing microorganisms, the optimization of culture parameters is usually based on the pH of the medium, culture time, culture temperature, environmental humidity, and rotational speed as variables. Then, using the growth amount and survival rate of microorganisms as evaluation indicators, the culture orthogonal test is carried out, and finally the response surface analysis is completed.

#### *2.1.1. Optimization of liquor yeast culture conditions*

In the selection of basic medium for drying liquor yeast, current research is mainly focused on YEPD medium, YPD medium, yeast complete medium, yeast basic medium, soybean sprout medium and potato medium (*Senz M. et al., 2015*). Most of them have carried out a large number of experimental studies, and all think that YEPD medium and yeast complete medium are the best basic culture medium for liquor yeast. The author also added the rehydration survival rate, glass transition temperature and particle size distribution of the dried particles as evaluation indicators on the basis of the original experiment, and carried out a new experimental optimization. The results showed that YEPD medium was more suitable as the basic culture medium for liquor yeast than the complete yeast medium.

In the aspect of medium additive optimization, at present, it is mainly to optimize the types and dosage of four kinds of additives: carbon source, nitrogen source, inorganic salt and auxin (*Lin J. et al., 2013*). The main carbon sources are glucose, fructose, lactose, sucrose and soluble starch. The nitrogen sources are beef extract, yeast extract, peptone, $NH_4N0_3$, $(NH_4)_2SO_4$ and soybean sprouts. The main inorganic salts are $KH_2PO_4$, $CaCl_2$, $MgSO_4$, NaCl and $ZnSO_4$. The auxins are mainly $VB_2$, inositol, $VB_1$, nicotinamide and calcium pantothenate. The commonly optimized method is single factor test to determine the OD600 value of the culture as evaluation indexes, and then take response surface analysis to complete the optimal selection.

On the optimization of liquor yeast culture parameters, the current research mainly focuses on single-factor experiment optimization research on culture temperature, rotation speed and pH. Among them, the culture temperature is the main reason that affects the growth of microorganisms (*Pichler H. et al., 2001*). Too high or too low temperature will have a great impact on the growth of liquor yeast.

Most studies showed that the growth of liquor yeast increased at first and then decreased in the temperature range of 28-32℃ and reached the maximum at 30℃ (*Shiroma S et al., 2014*). The culture rotation speed indirectly affects the growth of liquor yeast by affecting the oxygen content of the culture. If the rotation speed is too low, the yeast will grow slowly, and if the rotation speed is too high, the bacteria will burst. A large number of studies have shown that the optimal speed of liquor yeast culture is 180 r/min.

At present, almost all researches on the optimization of culture conditions in microbial drying only take the culture growth as the evaluation index, and few studies combine the rehydration survival rate, particle size distribution, and glass transition temperature of the dried particles as the evaluation index. This is undoubtedly a defect of the current research, so it could be optimized in the follow-up research.

### *2.1.2. Stress processing*

In the face of adversity, microorganisms could use innate defence mechanisms to produce protective cellular components to enhance their own tolerance to adverse environments (*Lv Y.J. et al., 2014*). Stress treatment is a method to use the characteristics of microorganisms to expose them to adversity to activate the stress system to produce protective components and reduce the drying damage rate of microorganisms (*Su J. et al., 2011*). Common stress treatment methods include heat shock, acid stress and salt stress. Among them, in addition to activating the stress system, heat shock can also induce the production of specific heat shock proteins in microbial cells to repair damaged proteins and DNA in the bacteria, and the heat shock parameters are usually 58 ℃, 15-30 min. Acid stress is a process in which microorganisms can regulate the amount of proton pump of F0F1-ATP enzyme and amino acid decarboxylation metabolism to promote the production of stress protein (GSP) and chaperone protein, repair and degrade damaged DNA or protein, maintain intracellular PH balance and proton transfer characteristics, and treat liquor yeast in the environment with pH ≤ 4 (*Silva J. et al., 2005*). Salt stress is a process that induces the accumulation of intracellular compatible substances (such as trehalose, glycine, etc.), balances the osmotic pressure inside and outside of microorganisms, and resists osmotic damage in the drying process (*Peighambardoust S.H. et al., 2011*).

### *2.2. Extracellular protection of proteins and enzymatic treatment*

Protein is one of the two most important microbial drying protectants, which mainly acts outside the microbial cell membrane (*Khem S. et al., 2016*). By filling the gap between the cell membrane and the cell wall, at the same time reacting with both at the molecular scale to form chemical bonds or hydrogen bonds, the molecular movement rate could be reduced, which ultimately slows down the rate of damage to the cell membrane and cell wall and prevents the cell wall from collapsing (*Morgan C. et al., 2006*).

Enzyme treatment can promote protein cross-linking or semi-cross-linking reaction, and further overcome the damage of electrostatic interaction between microorganisms and proteins. However, excessive cross-linking reaction will lead to the reduction of the space between microorganisms and the decrease of the water migration rate, which will lead to the increase of the viscosity of the microbial feed (*Gaspar A.L.C. et al., 2015*).

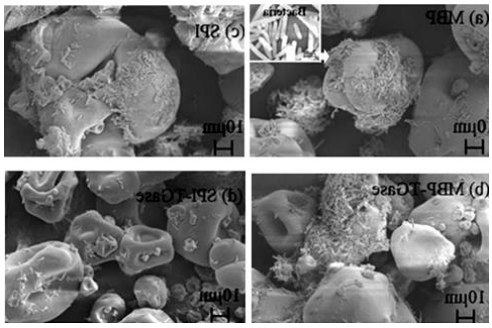


**Fig. 4 - The effect of protein and bacterial action on this**
(*Gong P. et al., 2018*)

Protective proteins used in microbial drying are often divided into two categories: acidic and alkaline. According to the liquor brewing process and the principle of acid stress treatment, the pH of liquor yeast culture solution is 3-5 and the cells are negatively charged. According to the characteristics of yeast and the principle of pH-PI (*Malhotra A et al., 2004*), it could be inferred that in the acidic culture medium, the liquor yeast has a negative charge, and the acid (alkaline) protein has a negative (positive) charge.

According to the charge principle, it could be inferred that the alkaline protein with positive charge could protect better on liquor yeast cerevisiae with negative charge than the acidic protein with negative charge (*Gottenbos B. et al., 2001*). In view of the verification of this theory, *Gong Pimin et al.* of Harbin Institute of Technology carried out a drying experiment comparative of Liquor yeast cell with bovine milk basic protein (MBP) and acid soybean protein isolate (SPI). The complex morphology of cell protein was observed by scanning electron microscope, as shown in a and c of Fig. 4 (*Gong P. et al., 2018*). Comparing the picture *a* and *c*, it is obvious that MBP can recombine with bacteria better. Finally, it had proved that the combination of the above charge and PH-PI theory is suitable for the drying of microorganisms.

Modern studies have shown that the enzymatically treated protected protein produces a cross-linking reaction to provide more sites for protein and bacterial binding, and at the same time, the secondary structure would change, to result in an increase in hydrophobicity. Ultimately, the protein and the bacterial cells are better combined, which plays a role in enhancing the drying protection effect of the protein on the microorganisms. Gong Pimin (*Gong P. et al., 2019*) et al also carried out TGase treatment experiments on the above two drying experiments, and the complex morphology of bacterial protein was observed by scanning electron microscope as shown in *b* and *d* of Fig. 4. Comparing *b, d* with *a, c* is not difficult to find TGase enzyme treatment could enhance the recombination of protein bacteria and enhance the protective effect of protein.

### *2.3. Intracellular protection studies with loading compatibility media*

As an extremely important intracellular protective agent for microbial drying, disaccharide accumulation in microorganisms can better reduce microbial drying damage and improve drying survival rate. At present, a large number of studies have shown that trehalose is the best protective disaccharide, and has been used as a protective agent in the field of microbial drying on a large scale (*Câmara et al., 2019*). In terms of the protective mechanism of trehalose to microorganisms, there are two hypotheses: "water substitution" and "glassy state" (*Santivarangkna C. et al., 2008*). Trehalose can only play its protective function in microbial cells, while in the drying process microbial cells are dormant and couldn't synthesize trehalose. At the same time, due to the selective permeability of the cell membrane, macromolecular substances such as trehalose and antioxidant enzymes cannot directly enter the microbial cells. Therefore, how to load macromolecular protective agents into cells without damaging the cell membrane has become a hot research topic.

The methods of loading macromolecular substances into microbial cells mainly include co-cultivation method, electroporation method and particle gun method (*Ali Shamsaie et al., 2007*). Among them, the co-cultivation method is the most commonly used method in actual production. The principle is to co-cultivate macromolecular substances with microorganisms for a long time, and enter the microorganism cells through the phagocytosis of the cell membrane. Although this method has low cost and little damage to microorganisms, the co-cultivation time is generally 1-3 weeks, the efficiency is too low, and the macromolecular substances phagocytosed into cells are often uneven (*Feng S. et al., 2015*). Electroporation is a method in which macromolecules such as trehalose and antioxidant enzymes are loaded into microbial cells by using an externally enhanced electric field to change the permeability of the cell membrane. This method greatly improves the loading efficiency, but there are three inherent problems: difficult to control the electric field parameters, excessive irreversible damage to the cell membrane, and polarized and uneven distribution of the loaded substance in the cell (*Saswati Mishra et al., 2020*). The particle gun method is a method in which the particles attached to the trehalose bio-macromolecule are shot by the particles and injected into the bacteria at the high speed. Although this method is highly efficient and simple to control, the damage to the microbial cell membrane is too great, which will cause a large number of inactivation, so it is hardly used in practice.

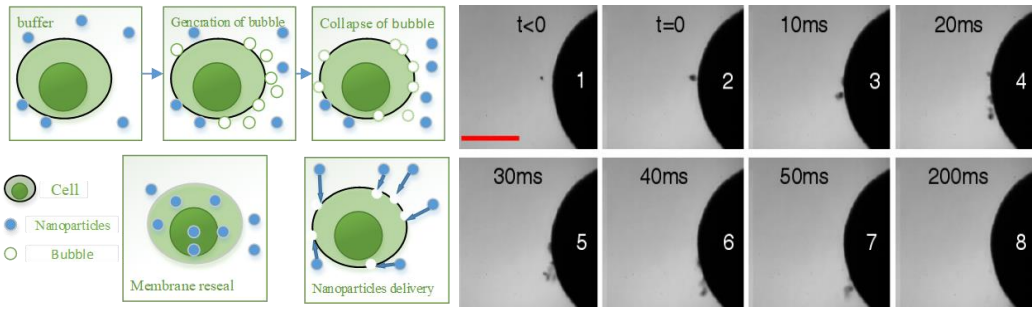



**Fig. 4- Ultrasonic perforation process**

Ultrasound perforation is an emerging technology that is widely used in high-end medical fields. First, it uses the sound pore effect produced by the ultrasonic wave to stimulate the continuous violent contraction and explosion of the tiny bubbles around the cell membrane, resulting in a reversible pore structure on the cell membrane surface. At the same time, it uses ultrasonic energy to stimulate macromolecular substances such as trehalose and antioxidant enzymes to move at a high speed in a monodisperse state. Finally, the high-speed moving biological macromolecules quickly enter into the cell and distribute evenly through the reversible pore structure formed by the cell membrane (the process is shown in Fig. 4) (*Caixia Jia et al., 2021*). Compared with the above three loading methods, ultrasonic piercing load technology has the advantages of high efficiency, low damage failure rate and uniform load distribution. Therefore, this technology has great potential for development.

## 3. Review on drying Technology of liquor yeast

It is undoubtedly the ultimate goal of microbial market-oriented large-scale drying to improve the rehydration survival rate and rate of microbial drying at the same time. Therefore, it is particularly important to compare the commonly used liquor yeast drying processes and design the optimal drying process by combination.

The commonly used liquor drying process and its cost and drying survival rate is shown in Table 1.

**Table 1**

**Comparison of cost and typical survival rate of traditional drying technology**

| Drying process | Cost of equipment (%) | Production cost (%) | Survival of dry products (%) |
|---|---|---|---|
| Freeze drying | 100 | 100 | ≥90 |
| Vacuum drying | 52.2 | 51.6 | 49.5 |
| Vacuum microwave | 65.4 | 53.8 | 53 |
| Spray drying | 12 | 20 | 44.3 |
| Fluidized bed drying | 8.8 | 17.9 | 28.8 |

### *3.1. Freeze drying*

The principle of freeze-drying is as follows: firstly, the yeast liquid with protective agent is rapidly frozen in a freeze dryer at-80 °C under atmospheric pressure, and then vacuum to make the air pressure ≤ 6 mbar to quickly reduce the vapour pressure of water in the cell, and finally make the frozen water sublimate and transfer quickly (as shown in Fig. 6) (*Dimitrellou D. et al., 2016*). From the point of view of the damage mechanism, because it is carried out in the environment of vacuum, low temperature and no shear, the main ways of cell damage and inactivation are dehydration damage and osmotic damage. The damage mainly occurs in the damage to the cell structure during the formation of intracellular ice crystals and the damage to the cell membrane caused by the hyperosmotic environment during water sublimation. Due to the single mechanism of freeze-drying damage, the rehydration survival rate of dried microorganisms is generally more than 90%. However, its equipment structure is complex (the structure diagram is shown in Fig. 7) and precise and the energy consumption of vacuum pumping and freezing is extremely high, so its equipment and use costs are the highest in traditional drying. At the same time, freeze-drying also has the defects of long drying time and intermittent drying, so it is not suitable for the optimization of combined drying process (*Romano N. et al., 2016*).

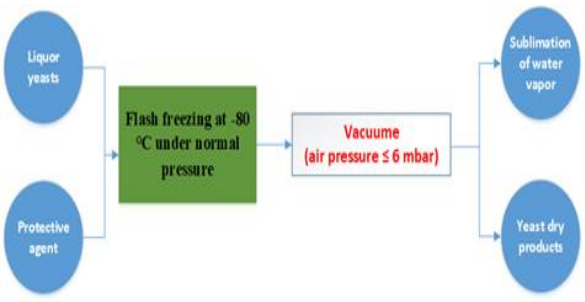
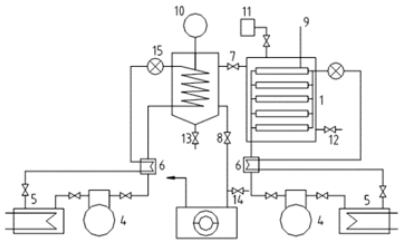



Fig. 6- freeze drying flow chart



1. Freeze drying box, 2. Condenser, 3. Vacuum pump, 4. Refrigeration compressor, 5. Water cooler, 6. Heat exchanger, 7 8 12 15. Valve, 9 10. Temperature monitor, 11. Vacuum gauge, 13 14. Express

Fig. 7 schematic diagram of integrated freeze-dryer structure

### *3.2. Vacuum drying versus vacuum microwave drying*

Vacuum drying is a kind of drying process, which reduces the vapour pressure of water in bacteria and reduces the drying temperature by vacuuming. Different from non-vacuum high temperature drying, medium temperature vacuum drying could reduce the drying temperature of microorganisms below 50 °C by controlling the vacuum, to reduce the occurrence of thermal damage to death (*Foerst P. et al., 2012*). Therefore, dehydration damage is the main cause of inactivation of microorganisms in it. Although the higher vacuum reduces the drying temperature and makes the rehydration survival rate of liquor yeast generally higher than 45%, it also reduces the heat and mass transfer efficiency, which increases the drying time and high drying cost. Therefore, it is not suitable for enterprise mass drying of yeast alone.

Vacuum microwave drying is essentially an optimization and improvement of vacuum drying process, which uses microwave generator to replace the traditional radiation heat source of vacuum drying. It takes advantage of the high heat transfer efficiency of microwave in vacuum to improve the drying efficiency of ordinary vacuum drying; at the same time, the characteristics of microwave heating from intracellular to extracellular have used to improve the drying survival rate of microorganisms. Finally, the efficiency and quality of vacuum drying have improved at the same time. However, it still does not overcome the problem that it is difficult to produce continuously in large quantities (*Ambros S. et al., 2018*). Therefore, it could be used as one of the alternative processes for combined drying process design.

### *3.3. Spray drying*

Spray drying is an extremely efficient drying process. The drying process is as follows: first, the feed liquid composed of microorganisms, protective agents, carriers, etc. is transported to the spray head by a pump to be dispersed into countless fine droplets. Then, the droplets are allowed to fall freely under the combined action of spray pressure, fluid force and gravity. Finally, in the process of falling, heat and mass transfer occurs in contact with hot air to complete the drying of microorganisms. According to the different flow directions of hot air and materials, spray drying is usually divided into convection drying, co-current drying and mixed-flow drying (as shown in Fig. 8) (*Vega-Mercado H. et al., 2001*).

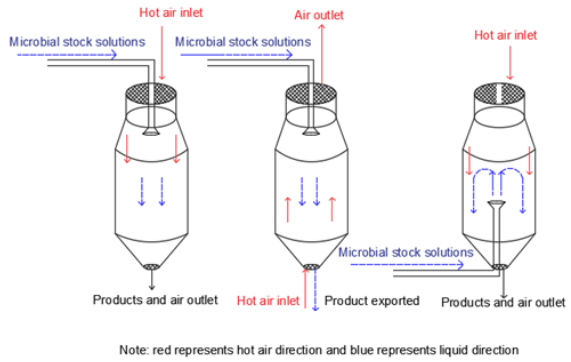
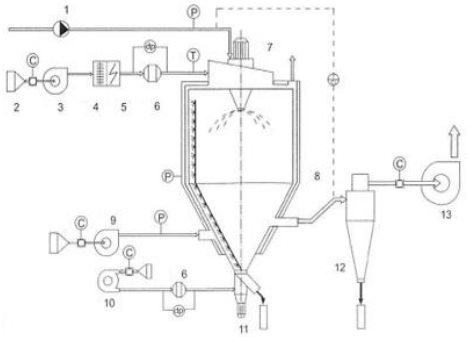



**Fig. 8 - Spray drying classification**



**1. Feed pump, 2 6. Filter, 3 9. Fan feeders, 4. Steam heaters, 5. Electric heaters, 7. Nebulizers, 8. Drying column, 10. Air pump, 11. Gas sweep device, 12. Cyclone separator.**

**Fig. 9 - Schematic of the parallel flow drying column structure**

In convection drying, the material and the airflow go in the opposite direction, which can prolong the residence time of the droplets in the drying tower and reduce the height of the drying tower. However, the outlet temperature is the highest and it is in direct contact with the drying particles, so it is only suitable for the drying of non-biologically active materials. In the co-current drying, the droplets first contact with the high temperature air and the material is always in a low wet bulb temperature environment. Only when the drying reaches the stage of decreasing the speed of drying, the material is in direct contact with the air close to the outlet temperature. This process greatly reduces the thermal damage and deactivation of materials, so it is suitable for the drying of active substances such as microorganisms. Although mixed flow drying combines the characteristics of convection and co-current drying, the sprinkler is easy to block upward, so it is rarely used.

The drying of droplets in the co-current drying has divided into four stages: atomization, constant speed drying, slow speed drying, and separation of particles and gases (*Broeckx G. et al., 2016*). Among them, in the atomization stage, the microorganisms are mainly subjected to shear damage. In the constant-speed drying stage, the droplets are in contact with hot air, and the moisture on the particle surface can maintain the gas-liquid two-phase balance.

The direct contact of microorganisms is the lower wet bulb temperature, and the microorganisms are mainly subjected by dehydration and osmotic damage. In the slow drying stage, the moisture on the particle surface cannot maintain the gas-liquid two-phase balance, and the microorganisms directly contact with the high-temperature air and finally reach the outlet temperature. The microorganisms are mainly subjected by dehydration and heat damage. In the dry product particle and gas separation stage, the larger particles remain at the bottom of the tower, and the tiny particles are separated by the cyclone. Thus, it could be seen that the inactivation of microorganisms mainly occurs in the third and fourth stages.

Compared with other traditional drying, spray drying has the advantages of low cost, high drying efficiency and continuous production. Although the survival rate of microbial rehydration after drying is slightly lower, it is one of the potential drying technology to replace freeze-drying, and it is one of the current research hotspots of microbial drying. Therefore, it could be used as the core process of combined drying process design.

### *3.4. Fluid bed drying*

Fluidized bed drying is a drying process of atomizing and spraying the microbial suspension on the dry carrier particles, and then placing it on the fluidized bed to adjust the heat and mass transfer by adjusting the flow of the hot air to change its movement in the hot air. This process has high drying efficiency and low cost, but the rehydration survival rate of the dried product is extremely low, so it is generally not used for microbial drying independently, but is often combined with other processes as an auxiliary drying process.

### *3.5 Emerging drying technologies*

Spray-freeze-drying is a new drying process improved by adding spray module on the basis of freeze drying. The basic principle is the same as freeze-drying, which mainly includes three steps: microbial liquid atomization, refrigerant contact solidification and vacuum low-temperature sublimation (*Ishwarya S.P. et al., 2015*). The material size was reduced by adding spray module, thus the efficiency of freeze-drying is greatly improved. However, the equipment and operating cost is 3 times higher than that of freeze-drying, so it is only suitable for small-scale drying of high-value bioactive substances.

Electrospray drying is a drying process, which uses electrostatic action to optimize the spray head of spray drying, and its characteristics are consistent with the traditional spray drying. The electrostatic spray can not only avoid shear damage, but also refine the droplets, and finally improve the rehydration survival rate of spray-dried particles. However, the electrostatic effect is greatly affected by the properties of the microbial feed liquid, so it could only be applied to the drying of microorganisms with specific electrical properties.

The microwave freeze-drying process is a drying process based on freeze-drying, replacing the original heat source with a microwave generator to improve heat and mass transfer. However, microwaves in high vacuum will stimulate plasma generation, causing uneven heating of microorganisms and additional damage to microorganisms. Therefore, the process is not yet mature and could only be used for small batch production.

## 4. Summary of current research status and future perspectives

### *4.1. Summary of current research status*

Current research on the mechanism of microbial drying damage inactivation. At the cellular scale, firstly, a set of relatively complete damage inactivation principles has derived from the five macroscopic dimensions of dehydration, heat, oxidation, shear, and osmotic pressure. Then, the characteristics of each damage mechanism is reviewed in detail. Finally, based on the damage mechanism, the commonly used drying processes are analysed in a targeted manner. At the scale of cell substructure, firstly, the damage analysis is carried out, and the specific principle and sequence of the effect of each damage inactivation mechanism on each cell substructure is obtained. Then, the main damage sites for drying inactivation of microorganisms is sorted as follows: cell membrane > intracellular protein > ribosome > cell wall. Overall, the current research has established a relatively complete theory of cell injury inactivation mechanism at the macroscopic and cellular substructure scales.

In terms of microbial drying protection, the current research was carried out from three aspects: pre-treatment before drying, addition of protective agents and optimization of drying process parameters. Firstly, from the two dimensions of culture conditions and stress treatment, the pre-treatment before drying was studied by means of basal medium optimization, additive optimization, culture parameter optimization, and stress treatment.

Then, the protective mechanism of adding protective agent was studied from the two dimensions of extracellular protection of enzyme-treated protein and intracellular protection of loaded compatible mediators.

The current research regards the drying process of microorganisms. Firstly, based on the microbial drying damage mechanism and protection strategy, the advantages and disadvantages of each common drying process was analysed. Then, combined with the microbial drying protection strategy, a number of emerging drying processes were combined and optimized. Finally, the survival rate and efficiency of drying of microorganisms such as liquor yeast were improved.

### *4.2. Outlook*

On the damage mechanism and protection strategy of microbial desiccation, there is no report on molecular scale research. For example, the intracellular protection mechanism of trehalose, there are still two hypotheses of "water replacement" and "Glass transition". Therefore, advanced instruments and optimized detection method would be used to conduct molecular-scale research on it in the later stage.

The preliminary idea is that, firstly, the advanced ultrasonic perforation loading technology would be transferred to the drying protection research of liquor yeast, and silver nanoparticles, trehalose and catalase would be loaded into the cells and evenly distributed. Then, the liquid chip, fluorescent probe and field emission transmission electron microscope would be combined to build a microbe observation platform at the molecular scale. Finally, the molecular-scale observation and research on the drying damage and protection mechanism of liquor yeast would be realized.

In terms of drying process optimization, the combined design of drying process can be carried out on the basis of comparative analysis of traditional processes, combined with the characteristics of damage mechanism of microbial drying under each drying process.

The preliminary idea is that, firstly, an innovative combination of spray drying, vacuum microwave drying and fluidized bed drying is carried out in principle, and the combined drying process of spray + vacuum + vibration + microwave is optimized. Then, the damage mechanism of microorganisms in the new combined drying process is further studied at the molecular scale. Finally, the emerging combined drying protection strategies and equipment are optimized in a targeted manner.

**ACKNOWLEDGEMENT**

This work was supported by the National Natural Science Foundation of China [No. 51876109] and the Key Project of International Science and Technology Cooperation Program for Shaanxi Province [No.2020KWZ-015].

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