

Application Of Genetic Technology And Fruit Extracts To Improve Yogurt

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Abstract. Yogurt is a ubiquitous daily drink in our life, which is popular among the public because of its unique flavor and taste. Lactobacillus in yogurt is a symbiotic bacterium in human body, it promotes human health, and as probiotics is widely used in food processing industry and plays an important part in the processing and production of yogurt. Now, people's living standards are gradually improving and the pursuit of a healthy and happy life, it is particularly important to develop lactobacillus, which is more in line with the needs of human health and has more excellent traits, to improve the quality such as flavor improvement, taste enhancement and growth shelf life of yogurt. Yogurt quality is generally improved by adding some fruit / plant extract to the yogurt and mixing fermentation by lactic acid bacteria, resulting in a large improvement in flavor, taste, production time, and shelf life. Such as chitosan, bacteriocins, CMC (carboxymethylcellulose) can extend shelf life and Lotus / lily bulb powder thereby further increasing free amino acids, citric acid and free fatty acids concentrations in yogurt. And mouth feel by inducing protein-protein interactions to further increase the viscosity of yogurt; The inclusion of both grape seed extract and banana peel extract in yogurt substantially increases the post shelf life of yogurt so that nutrients are not easily lost. Moreover, mutagenesis breeding and CRISPR-Cas9 technology can obtain mutant strains with acid tolerance and weak H⁺-ATPase activity to avoid post-acidification.

Keywords: Yogurt, Plant Extracts, CRISPR-Cas9, Post-acidification, Fermented milk.

1. Introduction

Being one of the oldest fermentation food, yogurt is welcome. In global, milk and dairy fermentation is day-to-day food for a great number of families. Yogurt is defined as the milk component fermented by the lactic acid-developing bacteria. Therefore, the flavor and taste of yogurt are enriched via lactic acid fermentation. Likewise, it increases the nutrition inside the products. The unique flavor of yogurt and its various nutritious value attract more consumers. Nowadays, it appears that multiple methods can improve the flavor, taste, efficacy and shelf life of yogurt products remarkably, such as the addition of some beneficial compounds, the addition of extra substances, blended fermentation and the application of CRISPR-Cas9 gene engineering. Some fruit or plant, such as grape seed and banana peel, plays a role in prolonging the term of nutrient loss. Furthermore, Lotus /lily bulb powdered material, chitosan, bacteriocins, CMC can result in the improvement of yogurt. Apart from the addition of beneficial substances, mutagenesis breeding and CRISPR-Cas9 technology can be applied to the intensified capability of defending against post-acidification. This article summarizes various ways to improve the quality of yogurt to provide a new perspective on the industry value-added of yogurt.

2. Yogurt Manufacture

2.1. Procedure of Yogurt Production

Generally, there are several steps in yogurt manufacturing (Figure 1).

Blending: As the first step of the procedure, raw materials including milk and other solid components are dissolved in the tank, being blended homogeneously.

Sterilization: In the following compulsory stage, the mixed compounds would be sterilized by heating, which is aimed to destroy all pathogenic microorganisms, reducing the activity of enzymes in the milk. Pasteurization is applied to dairy products for more efficient sterilization effects and lower nutrition loss [1].

Homogenization: In order to inhibit the creaming phenomenon in milk, it is general to homogenize dairy products, especially for yogurt production [2]. To some extent, high-pressure homogenization may improve the yogurt's nutrition value and sensory flavor [3].

Fermentation: After a series of procedures, milk and its mixture would be converted to primary yogurt products via the fermentation effect of the Lactic acid bacterium (LAB). LAB utilizes nutritious elements, such as carbohydrates, amino acids, and lipids, altering them into lactic acid and complicated flavor compounds respectively [4].

Cooling: Cooling the fermented mass is considered to play a role in restricting the activity of LAB and enzymes; therefore, maintaining the optimum rank of PH, body, and texture of yogurt products [5].

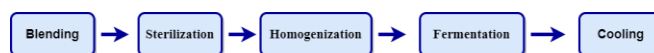


Figure 1 Yogurt processing

2.2. Problems Exist in Yogurt

2.2.1 Microbial Spoilage

Due to different polluting sources in the process of yogurt manufacture, microbial spoilage (some species of fungi, gram-negative and gram-positive bacteria), can be observed in polluted dairy products [6]. Furthermore, fungal contamination is the most common phenomenon since the majority of spoilage fungi can develop in the lactic acid circumstance, even adapting to the low temperature [7,8]. The growth of contaminating bacterial organisms would generate the spoilage and loss of yogurt.

2.2.2 Post-acidification

Because of the proceeding fermentation effect of lactic acid bacteria, the fermented yogurt may occur post-acidification phenomenon, which leads to being over the optimum product term. Besides, it results in undesirable problems, such as reducing shelf life, disrupting the flavor and texture, destabilizing the stability of probiotics, and decreasing the nutritious value [9].

2.2.3 Singular Quality

Apart from its simple quality, the flavor and nutrients of plain yogurt can be enriched through the insert of other beneficial ingredients.

3. Adding Plant/Fruit Extracts To Improve Yogurt

Yogurt is a kind of drink in People's Daily life. With the development of biology, more and more probiotics are added to yogurt, aiming to bring health benefits to the human intestine and other areas. Starting from providing a variety of beneficial probiotics to improve the quality and flavor of yogurt, studies have shown that some fruit and plant extracts can be added in the fermentation process of yogurt, which are rich in a large number of bioactive compounds. These bioactive compounds can have a variety of effects on the flavor, taste, viscosity and shelf life of yogurt.

3.1. Add Plant/Fruit Extracts to Improve Flavor

The main compounds which play important role in flavor of yogurt in yogurt were ketone (3-hydroxybutane-2-ketone, 2, 3-butanedione, 2-butanone). These compounds contribute to yogurt flavor as well as free amino acids, citric acids and free fatty acids [10]. The pyruvate is the metabolism of lactose and citric acid, and turn into 2,3-butanedione [11], which play an important role in flavor of yogurt. Previous studies have shown that 2, 3-butanedione has the ability to combine with 3-hydroxy-2-butanedione (ethyl ketone) to create a distinctive buttery flavor. This flavor gives yogurt a different flavor from other flavored beverages [12]. Adding lotus seed/lily bulb powder (LBP) and dairy active peptide(DAP) improve the production of 3-hydroxy-2-butanedione and 2, 3-butanedione.

3.2. Adding Plant/Fruit Extracts to Improve Viscosity

Adding bioactive double emulsions (DE) to high-protein yogurts is an appropriate strategy for designing nutrient-rich foods for the elderly [13]. Proanthocyanidins bind to casein and whey proteins to induce protein-protein interactions [14]. These interactions have a great influence in yogurt to form a more viscous structure. In Ozturk's research, it was found that adding LBP to yogurt increased its viscosity. LBP fibers contain hydroxyl groups that provide water interaction through hydrogen bonding [15]. Adding fibers with high water absorption capacity to protein matrix can improve its cohesion. When DAP and LBP were added, protein-protein and protein-carbohydrate interactions were more extensive, strengthening the network in the yogurt [16]. Adding bioactive peptides to dairy products can also improve the viscosity index of yogurt [17]. The hydrolysate significantly increased the viscosity of yogurt and thus improved the quality of yogurt. In the study of Srisuvor, it was found that adding milk protein into yogurt can greatly improve the viscosity of yogurt. Therefore, the addition of moringa extract (ME) can reduce the dehydration value of yogurt, thus improving the viscosity of yogurt. This may be due to the interaction between ME components and proteins in yogurt (Figure 2) [18]. In Kabir's study, adding banana peel extract to yogurt also increased its viscosity. Banana peel is as rich in dietary fiber as LBP, which explains why banana peel extract can give yogurt a thicker structure.

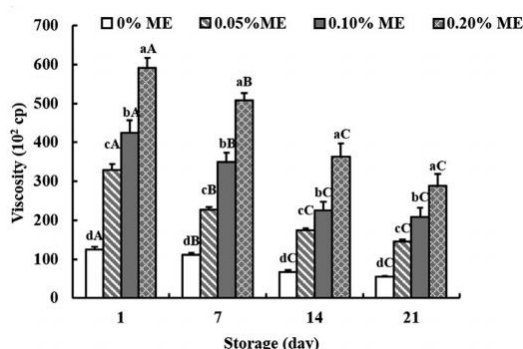


Figure 2 Changes in the viscosity of the yogurt supplemented with 0–0.2% ME during fermentation.

3.3. Adding Plant/Fruit Extracts to Improve Fermentation Time

Ozturk's research found that LBP has high levels of starch, protein and dietary fiber, which, if added to yogurt, can be a large carbon source for fermentation [19]. For lactic acid bacteria can greatly shorten the fermentation time.

3.4. Adding Plant/Fruit Extracts to Improve Shelf Life of Yogurt

In the Vaya experiment, it was found that the physical and chemical properties, total phenolic content and antioxidant capacity of yogurt were not affected after the addition of capsule grape seed extract (GSE) in yogurt for 3 weeks. Microencapsulated GSE can be used as a functional additive

in yogurt to improve its antioxidant capacity [20]. In Kabir's study, adding banana peel extract to yogurt increased its antioxidant capacity. They found that banana peels contain high levels of polyphenols, such as phenolic acids, flavonoids, catecholamines, dopamine and L-dopamine, and anthocyanins, so banana peels may have a high free radical scavenging capacity. In their study, it was found that after adding banana peel extract, the reduction rate of total phenolic content in yogurt during storage was significantly lower than the degradation rate of fortified yogurt with grape residue extract, so banana peel extract had a better retention effect on total phenolic content in yogurt during storage. However, in terms of antioxidant capacity, under the same concentration of extracts, the antioxidant capacity of banana extract fortified yogurt was not as high as grape residue extract fortified yogurt. Some studies have discovered that the presence of milk protein in yogurt reduces the free radical scavenging ability and antioxidant ability of polyphenol compounds because of the formation of polyphenol-milk protein complex. In addition, thermal induces the proteins in yogurt to degenerate, which are more trends to synthesis polyphenol - milk protein complexes due to increased exposure to polyphenol binding sites. Therefore, the hydroxyl cinnamic acid in casein and banana peel extract of yogurt may have covering effect that explain the decreased free radical scavenging ability of fortified yogurt in the period of storage. During the shelf life at the same time it is important to ensure that the nutrients in the food product is not lost in the loading of double emulsion products add vitamin, the storage period of stability is significantly higher than directly add vitamins, the results show that the load DE structure makes it during the whole storage can well resist yogurt products of heat treatment and acidic environment (Figure 3, Figure 4).

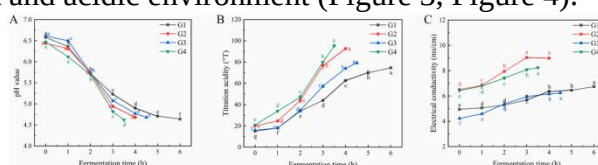


Figure 3 Effect of additives on pH (A), conductivity (B) and TA (C) of goat milk yogurt during fermentation.

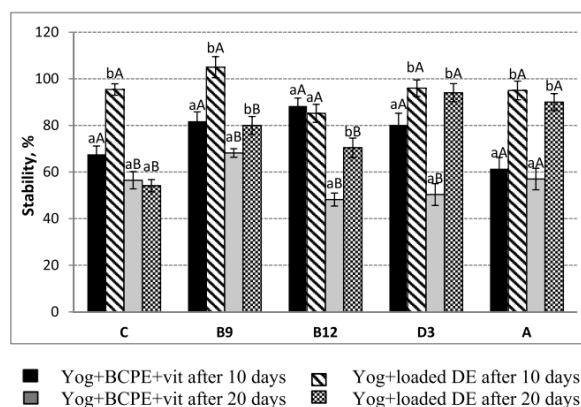


Figure 4. Stability of vitamins in different high-protein yogurt type product formulations during storage at 4°C in the dark.

4. Post-Acidification After Fermented Milk As Well As Control Methods

4.1. Post Fermented Milk Acidification

In the process of storage and transportation, the acidification caused by the metabolism of some active substances will reduce the quality of products and the consumption rate will decline. Therefore, people expect to ensure the flavor of yogurt and control the formation of post acidification to the greatest extent without destroying the beneficial bacteria and components in yogurt. Such as emerging technologies such as HHP, PEF, ultrasound or the addition of preservatives should be considered, provided that sensory properties are not altered. Bacteriocin purification is costly and has poor

stability in food matrices, hindering direct incorporation of bacteriocins into yogurt. Bioprotective culture and strain modification by random mutagenesis efficiently avoid post-acidification.

Post acidification during refrigeration of fermented milk mainly occurs in three stages:

(1) Acid production before the temperature drops to 20°C, i.e., by the cooling process. Lactic acid producing bacteria are more acid producing at this stage and more so when cooled slowly (30 – 60 min or more). This stage is mainly caused by lactic acid bacteria proliferation and the action of bacteriophage enzymes.

(2) The temperature decreased from 20°C to 10°C stage, i.e., acid production after cooling. Mainly caused by enzyme action within the bacteroids.

(3) Temperature of 7°C, acid production during refrigerated stage. A higher pH at the end of fermentation results in greater acid production during post ripening and, conversely, less acid production. Excessive acidification after fermented milk can not only affect its flavor, but also affect the quality of fermented milk, which may cause whey separation and affect the activity of lactic acid bacteria, and studies have shown that the number of viable bacteria is greatly reduced when the environmental pH decreases below 4.2 [21]. High quality fermented milk with pH 4.2 - 4.3, while in real production fermented milk pH may decrease to 3.5 and although factory stores will weaken its acidification by methods such as cryopreservation, often fermented milk shelf life is only about 7 days and better flavor characteristics can only be maintained for about 3 days. Therefore, a simple and less costly means of controlling postacidification of fermented milk products during sale and storage has become the research focus of fermented dairy products.

4.2. Acidification Control Measures After Fermented Milk

The key to control post acidification of fermented milk is not only to maintain lactic acid bacteria activity but also to attenuate the acid producing capacity of lactic acid bacteria under low temperature conditions. There are many relevant studies, mainly as the following.

4.2.1 Addition of Exogenous Substances

Researchers have added CMC (carboxymethylcellulose) and other stabilizers to fermented milk, which can prolong the fermented milk storage time by about 10 d [22]; To change the water utilization of lactic acid bacteria, the growth characteristics of strains generally in the milk added sugar substances such as sucrose, reinoculated lactic acid bacteria fermentation, exogenous substances so that the water activity available to lactic acid bacteria changes [23]. Changes in water activity can also further affect the live to Bacteroides ratio, which ultimately leads to reduced acidification after fermented milk. Preservatives can inhibit lactic acid bacteria growth and can also achieve to attenuate post fermentation milk acidification [24]. Nowadays, a wide range of exogenous preservatives are used, including chitosan, bacteriocins, and so on.

4.2.2 Mutagenesis Breeding

Treatment of microbial cell populations with mutagens greatly increases the mutation rate so that the mutant strains of interest can be screened. Temperature sensitive strains have been developed by others, and a temperature of 42°C has no effect on acid production of the strain, but the fermentation capacity for acid production under low temperature conditions is significantly weakened [25]. Acid sensitive bulgaricus strains have been obtained by mutagens, and the acid phenomenon can be controlled after yogurt. Some studies have shown that the activity of lactic acid bacteria with weakened H⁺-ATPase is decreased compared to the activity of the strain before the mutation, and the growth Acid producing properties were significantly reduced [26].

4.2.3 Application of Genetic Engineering Techniques

Mutagenesis breeding, although also yielding targeted strains, is labor intensive, less accessible, and difficult to control. Genetic engineering techniques to engineer lactic acid bacteria genes are relatively simple, and shape enables stable inheritance. The β -GAL(β -galactosidase) gene is altered by genetic mutations and can be screened for lactic acid bacteria with weak postacidification [27].

Using gene knockout technology to obtain mutant strains with acid tolerance and weak H⁺-ATPase activity, druesne et al changed the lactose permease codon, attenuated the enzyme activity, and the degree of post acidification was also significantly improved.

4.2.4 Screening for Lactic Acid Bacteria with Weak Acid Production at Low Temperature

The best way to control postacidification of fermented milk is to screen lactic acid bacteria with excellent characteristics, and researchers have isolated and identified p-gal (p-galactosidase) - deficient lactic acid bacteria, because the modified strains cannot metabolize lactose normally in bovine milk to produce acid, which attenuates postacidification of fermented milk [28]. Ongol et al study considered that H⁺-ATPase deficient *Lactobacillus bulgaricus* had weak post acidification and the selected natural mutant strains were stable in character, which can fundamentally solve the post acidification of fermented milk and be simple to operate, thus can greatly reduce the production cost.

4.3. Mixed Fermentation by Lactic Acid Bacteria

Control of acidification after fermented milk in many ways, artificial mutagenesis is one of the most direct ways, but its safety issues are highly debated, and the way of mixed culture uses the characteristics of lactic acid bacteria themselves to attenuate acidification after fermented milk, is simple and easy, and has a high safety profile, which is conducive to industrial applications [29]. Combined *S. thermophilus* with different lactobacilli, and in the study, the growth and metabolic properties of different strain combinations were different, and the post acidification of milk fermented with the mixture of *S. thermophilus* and *L. helveticus* was the most severe, and the fermentation was the fastest in combination with *L. acidophilus*. The researchers have used *Lactobacillus helveticus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum* as well as *Lactobacillus bulgaricus* with *Streptococcus thermophilus* respectively in soymilk fermentation to develop a milk beverage with better outlet flavor and health care function. It was finally concluded that *S. thermophilus* had a synergistic effect with *L. helveticus* to improve the product flavor and shorten the fermentation time. Kefir has a special texture and aroma, which is formed because lactic acid bacteria co cultured with yeasts, and vedamuthu research found that lactic acid bacteria in Kefir grains have a symbiotic relationship.

4.4. Adjust for Cocci and Lactobacilli Ratio for Post Acidification Effects

Increasing the proportion of coccobacter in a certain range can extend the shelf-life of fermented milk. *S. thermophilus* grew faster in the initial stages of fermentation, *L. bulgaricus* accelerated the growth when the pH of fermented milk decreased to about 5.5, while the growth of cocci slowed, and when the pH decreased to 5.0, the main strain of fermented acid producing bacteria was bacilli, when the pH was set to about 4.6, curd was formed, and put into low-temperature storage, mainly bacilli metabolized acid producing, the pH of fermented milk continued to decrease, and when it was set to about 3.5, cell membrane permeability increased, The intra - phage pH stabilization was compromised and the intra - phage enzyme activity was inhibited. Lactic acid bacteria intracellular pH maintained stable near neutral with no effect on intracellular enzyme activity when the environmental pH was in the range of 4.0 to 4.5, while lactic acid bacteria cell metabolism was inhibited when the environmental pH continued to decrease to 3.5. Research by Russell J B et al [30] showed that fermentation was slower when the proportion of coccus was higher in mixed fermentation, and although there were relatively few flavor substances, the posterior acid was significantly weakened, which prolonged the shelf life of the fermented milk. Study appropriately adjusted the coccobacterial ratio when fermentation was optimal at a coccobacterial ratio of 1:1.5, resulting in a long shelf life. At present, more methods for controlling acidification after fermented milk have their own benefits, and the addition of exogenous stabilizers and preservatives, although they can ameliorate the state of yogurt and prevent heterobacterial contamination, the problem of excessive flavor of fermented lactic acid cannot be solved, and the production cost is high, so there is a certain danger in the stability of genetic engineering breeding and mutagenesis breeding legacy as well as expression. In this study,

based on the already optimized fermentation milk preparation process, the post acidification was further attenuated by screening of weak post acidification strain combinations, and the process conditions of weak post acidification composite starter preparation were optimized, which enriched the variety of fermenters.

5. Crispr-Cas9 In Improving The Quality Of Yogurt

5.1. Improve the Quality of Yogurt Through CRISPR-Cas9 Technology (Taking Lactobacillus as an Example)

Lactobacillus is widely used in the food processing industry as probiotics. It is of great practical significance to create better Lactobacillus through CRISPR-Cas9 gene editing technology. The purpose is to improve the traits of Lactobacillus and improve the quality of products, to reduce the risk of lactic acid bacteria releasing pollutants and harmful substances to pollute the environment, in order to improve the quality of yogurt.

5.1.1 Improves the Stress Resistance of Lactobacillus

In the food industry production process of yogurt, lactobacillus needs to face the stress of various physical and chemical factors. Meanwhile, when lactobacillus enters the human body, it needs to survive under harsh conditions in the gut to exert its activity. Therefore, the CRISPR-Cas9 technology was used to adapt the gene to the living environment under different conditions. Lactobacillus can produce organic acids itself during industrial fermentation and often faces acid stress, which results in reduced cellular activity and production of Lactobacillus. The glutamate decarboxylase system is one of the most important acid resistance systems of Lactobacillus. Because of its acid resistance ability, lactobacillus can grow normally under acid stress environment [31]. Therefore, it is very important to improve the stress resistance of Lactobacillus in food processing. The stronger the stress resistance of Lactobacillus is, the more it can maintain a high degree of cellular activity in food processing, thus showing its unique physiological function in yogurt processing and production.

5.1.2 Improving the Antimicrobial Activity of Lactobacillus

With the continuous progress of technology, the codons on some beneficial Lactobacillus chromosomes can be accurately mutated by CRISPR-Cas9 technology to have strong antibacterial activity. The bacteriocin produced by Lactobacillus can be further improved and enhanced in terms of antimicrobial profile, yield and stability. Jee-Hwan et al used CRISPR-Cas9 for site-directed saturation mutagenesis of the NisR and NisK genes of *L. reuteri* to transform an oligonucleotide containing the NNK motif, in which one codon was modified to encode all 20 amino acids, and found that this recombinant enhanced the antibacterial activity of *L. reuteri* [32]. With the continuous improvement of the technology, the researchers use CRISPR-Cas9 technology to accurately mutagenize the codons in some beneficial Lactobacillus chromosomes to create a strong antibacterial Lactobacillus, which is conducive to the improvement of yogurt quality.

5.1.3 Altering the Immunomodulatory Properties of Lactobacillus

Researchers can alter the immunomodulatory properties of Lactobacillus by editing and modifying related genes contributing to immune regulation in Lactobacillus. Steidler et al. constructed recombinant *L. lactis* secreted mouse interleukin 10 (IL-10), when mice oral recombinant strains can significantly reduce intestinal inflammation [33], providing new ideas for the treatment of inflammatory bowel disease. Therefore, we can also enhance the beneficial function of such strains stably and long time, so as to improve the immunomodulatory characteristics of Lactobacillus and improve the quality of yogurt.

5.1.4 Reduce the Release of Harmful Substances by Lactobacillus

Lactobacillus can also produce some substances harmful to the human body, such as: D-lactic acid. Miroslav Pohanka demonstrated that D-lactate is an important marker and a toxic metabolite that can

cause health problems and complicate other pathologies, and that intoxication is not easy to diagnose. Because D-lactate dehydrogenase does not exist in human and animals, D-lactate acid cannot be catabolized. If the human body consumes too much D-lactic acid, it will lead to metabolic disorders, or even the symptoms of poisoning [34]. Therefore, reducing the release of harmful substances by *Lactobacillus* plays a vital role in human health.

5.2. Mechanism of Action of the CRISPR-Cas9 Technology

The CRISPR-Cas9 system of today, as the most mainstream gene's gene editing system, CRISPR-Cas9 system has the advantages of high efficiency, easy to use and low cost. So, it is widely used in gene modification of bacteria, especially *Lactobacillus*.

The CRISPR-Cas9 system consists of two parts, one CRISPR and the other is Cas9. The CRISPR-Cas system functions to prevent foreign DNA from invading cells. This is achieved by incorporating foreign DNA fragments (for example, phage DNA) into the genome [35]. CRISPR has a database for storing viral DNA fragments. It is primarily used to identify and locate viral DNA. Cas9 is responsible for cleaving and deleting viral DNA and bringing a small number of fragments back to the database for storage. If the same virus attacks again, they can quickly match the virus, cut off its DNA, eliminate infection, and thus immune bacteria against the virus. Most importantly, this database can also be passed on to offspring, so that bacteria can identify and eliminate viruses encountered by their ancestors.

6. Conclusion

In current studies, adding bioactive substances to yogurt and using genetic engineering technology to transform yogurt fermentation strains are relatively popular aspects. This article focuses on the addition of plant/fruit extracts, the addition of exogenous substances, mixed fermentation and the use of CRISPR-Cas9 gene technology. In the study, it was found that the methods of adding extracts and exogenous substances into yogurt were widely studied and the results were significant. The added substances were rich in some compounds that could interact with yogurt or strain, which could improve the efficiency of yogurt, chitosan, bacteriocins, CMC (carboxymethylcellulose) can extend shelf life and Lotus / lily bulb powder thereby further increasing free amino acids, citric acid and free fatty acids concentrations in yogurt. At the same time, genetic technology is used to modify the strain to make certain directional changes in its metabolic mode, which complement each other. Therefore, when the pH is guaranteed to be 4.5-5.0, adding extracts or exogenous substances to yogurt modified by genetic technology may further find ways to improve yogurt, mutagenesis breeding and CRISPR-Cas9 technology can obtain mutant strains with acid tolerance and weak H⁺-ATPase activity to avoid post-acidification, give yogurt higher value, and make it have higher economic benefits in the market.

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