

ROOM-TEMPERATURE ZERO-SUCROSE YOGURT AND MANUFACTURING METHOD THEREFOR

FIELD OF THE INVENTION

The present disclosure relates to room-temperature yogurt, and in particular to room-temperature zero-sucrose yogurt and a manufacturing method therefor.

BACKGROUND OF THE INVENTION

Obesity is a disease of nutritional metabolic imbalance, which refers to a physiological condition in which a person has relatively high body fat or is overweight. According to a survey, at least 1/5 of the world's population are overweight. Weight loss is a behavior aimed at reducing excessive fat and weight, including a variety of means such as adjusting lifestyle (diet, yoga, exercise, fitness, etc.), taking weight loss medicaments and foods, and surgical treatments. With the deepening of research, many people who want to lose weight have realized that dietary adjustment, appropriate aerobic exercise, and a regular life style are more beneficial to increasing energy consumption and fat metabolic consumption. Clinical studies have shown that scientific and reasonable nutritional therapy combined with exercise intervention is still the most effective and safest basic treatment method.

Honeysuckle is the dried bud or newly opened flower of the plant *Lonicera japonica* of the Caprifoliaceae family. It is a commonly used traditional Chinese medicine as well as food having medicinal properties, and has multiple pharmacological effects such as anti-inflammatory, antiviral, and antioxidant. Studies have shown that among the main active ingredients of honeysuckle, chlorogenic acid is the organic acid component of the highest content. Chlorogenic acid, also known as 3-caffeoylquinic acid, is a secondary metabolite produced by aerobic respiration in plants. It has pharmacological activities such as antiviral, blood pressure lowering, blood lipid lowering, liver protection and bile scavenging, and free radical scavenging, and has become one of the hot fields in food and medicament research. Honeysuckle is often used in food to exert its effects because it is rich in chlorogenic acid.

CN101595918B discloses a yogurt product with added polydextrose, inulin and a special combination of fermental bacteria, wherein the product has low calories and can control energy intake, playing a positive role in weight control. CN 102870882B discloses that it achieves the effect of lowering blood sugar by adding a variety of traditional Chinese medicine ingredients with claimed weight loss effects, and claims that the product has a regulating effect on weight.

CN 112273565A discloses a preparation process of liquid beverage containing honeysuckle and chrysanthemum ingredients, wherein honeysuckle is added to the beverage via a mixing and stirring method, thereby giving the beverage the effect of heat-clearing (reducing inflammation) and detoxifying. CN 113142303A discloses that intestinal flora is regulated by adding special lactic acid bacteria and mulberry leaf extract gel particles, and the gavage experiment of high-fat fed mice verifies that the product plays a role in preventing the development of obesity by regulating blood lipids, cholesterol levels and other ways.

At present, there are not many room-temperature yogurt products having weight loss effects for sale. There are still many problems for room-temperature yogurt products with weight loss effects that need to be optimized and improved. The formula design of most fat-reducing yogurt products at this stage is complex and cumbersome. Some yogurts use too many types of fermented strains, resulting in prolonged fermentation time and difficulty to control the fermentation state; some yogurts use multiple functional ingredients to improve the weight regulation ability of the products, but the actual products' flavors, texture and shelf life stability are affected.

SUMMARY OF THE INVENTION

Yogurt is a type of nutritious fermented dairy product with good market and consumer demands. At present, consumers' demands for weight loss lead the development of various types of yogurts, and sugar-free fat-reducing yogurt products have become one of the hot fields for product development.

One purpose of the present disclosure is to provide room-temperature zero-sucrose fermented dairy product of yogurt with a weight control (weight loss) effect.

In order to achieve the purpose of the present disclosure, in the first aspect, the present disclosure provides a method for preparing room-temperature zero-sucrose yogurt, wherein the relative amounts of honeysuckle extract, prebiotic and *Bifidobacterium longum* BBMN68 are (2-10g): (1-6g): ($1 \times 10^{7-8}$ CFU) per 100g of raw materials of the room-temperature zero-sucrose yogurt; wherein the prebiotic is inulin or xylo-oligosaccharide.

The room-temperature zero-sucrose yogurt obtained by the present disclosure does not contain sucrose, further reducing the calories of the yogurt. The obtained yogurt is suitable for people who need to lose fat. The room-temperature zero-sucrose yogurt obtained by the present disclosure has good flavors and stability, and the zero-sucrose yogurt provided by the present

disclosure does not bring too much weight burden to consumers while meeting the shelf-life stability requirement.

Due to the addition of chlorogenic acid, the yogurt fermentation system is not stable, and the shelf life of the fermented yogurt product is short and easy to exude water. The present disclosure significantly improves the stability of the fermentation system by adding a prebiotic.

Inulin and xylo-oligosaccharide are both prebiotics that are currently on the market and used frequently. They are mixtures of oligosaccharides or polysaccharides with different degrees of polymerization. The beneficial effects of prebiotics on the human body, such as regulating intestinal flora, enhancing immunity, and weight control, have been confirmed by a large number of literatures and clinical trials.

The experiments of the present disclosure also confirmed that after adding inulin and xylo-oligosaccharide, not only the stability of the fermentation system is improved, but also the production of short-chain fatty acids in the fermentation process is effectively improved, and the efficacy of yogurt in fat reduction and weight control is improved.

In the prior art, functional yogurt products that promote intestinal health are the most widely developed, but functional yogurt products related to weight control still have a large potential market. The yogurt made from honeysuckle in the present disclosure has a fresh smell, and its main component chlorogenic acid has an effect on weight control.

In a preferred embodiment, the extraction method of the honeysuckle extract is as follows: the honeysuckle is pre-freezed and freeze-dried to obtain honeysuckle powder; the honeysuckle powder is dissolved and extracted in an insulated tank at 65-75°C for 10-20 min at a material-liquid (sterile water) ratio of 20g : 1L to 30g : 1L, and then filtered to obtain the honeysuckle extract.

In a preferred embodiment, after fermentation of room-temperature zero-sucrose yogurt and before sterilization and filling, the materials are preheated to 50-55°C and maintained for 5-10 minutes, which can increase the chlorogenic acid content in the final product of the room-temperature zero-sucrose yogurt.

Specifically, in the preparation method of the present disclosure, the room-temperature zero-sucrose yogurt is obtained by fermenting a raw milk with a stabilizer, a sweetener, a prebiotic, a whey protein and a honeysuckle extract by using *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Bifidobacterium longum* BBMN68 as fermentation strains; the stabilizer is high-esterified pectin; the sweetener is erythritol or xylitol.

In a preferred embodiment, the raw materials of the room-temperature zero-sucrose yogurt contain 80-100 parts by weight of raw milk, 2-10 parts by weight of honeysuckle extract, 0.01-10 parts by weight of sweetener, 0.5-2 parts by weight of whey protein powder, 0.1-0.5 parts by weight of high-esterified pectin, 1-6 parts by weight of prebiotic, $1 \times 10^{7-10}$ CFU/100g of effective viable bacteria of *Lactobacillus bulgaricus*, $1 \times 10^{7-10}$ CFU/100g of effective viable bacteria of *Streptococcus thermophilus*, and $1 \times 10^{7-10}$ CFU/100g of effective viable bacteria of *Bifidobacterium longum* BBMN68. The deposition number of *Bifidobacterium longum* BBMN68 is CGMCC NO.2265, which has been disclosed in Chinese patent application CN101649303B.

In a specific embodiment, the present disclosure provides a method for preparing room-temperature zero-sucrose yogurt, wherein:

(1) a raw milk is filtered through a reverse osmosis membrane to obtain a retained solution and a permeate;

(2) the retained solution obtained in step (1) is preheated to 45-55°C, and a sweetener, whey protein powder, a prebiotic, and a honeysuckle extract are added, which are then stirred, homogenized, sterilized, and cooled; and

the cooled material is fermented for 4-8 hours by adding *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Bifidobacterium longum* BBMN68, and then after the pH thereof reaches 4.25-4.35, the material is demulsified and cooled to obtain a first material;

(3) the permeate separated in step (1) is heated to 50-55°C, added with a stabilizer, stirred evenly, homogenized, sterilized, and cooled to obtain a second material;

(4) the first material obtained in (2) and the second material obtained in (3) are statically mixed, preheated to 50-60°C for 2-5 minutes, then heated to 65-80°C for 15-60 seconds for sterilization; cooled to 20-25°C, aseptically homogenized at 20-30 bar, and aseptically filled to obtain the room-temperature zero-sucrose yogurt.

In a specific embodiment, in step (2) of the preparation method, the parameters for homogenization before fermentation are 55-65°C, 150-160 bar, the parameters for sterilization before fermentation are 120-130°C, 5-10 seconds, and the cooling temperature before fermentation is 40-42°C; the cooling temperature after demulsification is 20-25°C.

In a specific embodiment, in step (3) of the preparation method, the parameters for homogenization after adding a stabilizer and stirring are 55-65°C, 150-160bar, the parameters for sterilization are 110-120°C, 15s, and the cooling temperature is 20-25°C.

As a specific embodiment of the present disclosure, a method for preparing room-temperature zero-sucrose yogurt is provided, wherein:

(1) honeysuckle is screened and cleaned, and then pre-frozen in a -80°C refrigerator, and freeze-dried for 24 h under the conditions of a cold trap temperature of -50°C, a partition heating temperature of 20°C, and a vacuum degree of 0.12 mbar to obtain honeysuckle powder. The freeze-dried powder is crushed, and sterile water is added at a material-liquid ratio of 30g : 1L, and dissolved and extracted in an insulated tank at 65-75°C for 20 min, and then filtered to obtain a honeysuckle water extract;

(2) the raw milk is filtered with a reverse osmosis membrane to obtain a retained solution and a permeate;

(3) the retained solution obtained in (2) is preheated to 55°C, and a sugar substitute, a whey protein powder, inulin or xylo-oligosaccharide, and the honeysuckle extract are added, which are then stirred evenly, and homogenized at 60°C and 150bar;

(4) after homogenization, the material is sterilized at 121°C for 6s, cooled to 40°C after sterilization, and fermented for 6h by adding yogurt starter (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and probiotics (*Bifidobacterium longum* BBMN68) until a pH of 4.35 and an acidity of $\geq 65^\circ\text{T}$ are reached, and then it is demulsified and cooled to 20°C to obtain a first material, wherein the fermentation temperature is 40~45°C for 4~8h, and the material is demulsified when the pH reaches 4.35 and the acidity is $\geq 65^\circ\text{T}$, which is beneficial for the honeysuckle extract to release chlorogenic acid;

(5) the permeate separated in step (3) is heated to 55°C, and a stabilizer is added, which is then stirred evenly, homogenized at 60°C 150bar, sterilized at 110°C for 15s, and then cooled to 25°C to obtain a second material;

(6) the first material obtained in (4) and the second material obtained in (5) are then statically mixed, preheated to 55°C and maintained for 5min, which is beneficial to the further release of chlorogenic acid, and then heated to 65°C for 60s for sterilization;

(7) the sterilized material obtained in (6) is aseptically homogenized at 25°C 20bar and aseptically filled to obtain the room-temperature zero-sucrose yogurt.

The recommended consumption of the room-temperature zero-sucrose yogurt of the present disclosure is 2 times a day, 100g each time.

In a second aspect, the present disclosure is directed to room-temperature zero-sucrose yogurt made by the above methods.

As understandable to those skilled in the art, the present disclosure is also directed to a use of the above room-temperature zero-sucrose yogurt in the preparation of fat-reducing and body-shaping health products.

Advantages

The present disclosure relates to room-temperature zero-sucrose fermented dairy product with a weight control (weight loss) effect, which contains inactivated *Bifidobacterium longum* BBMN68, inulin and a honeysuckle extract. In the present disclosure, the processing of honeysuckle extract is optimized, the chlorogenic acid content in the final product is increased, and the product's effect on fat reduction is improved. Secondly, a probiotic (*Bifidobacterium longum* BBMN68) is further added to the fermenting agents, which promotes the release of chlorogenic acid from the honeysuckle extract, assisting in the functional improvement of the final product. In addition, the flavors and shelf life stability of the product is ensured by adding inulin, and facilitating the effect of weight loss. The regulatory effects of the product on rat weight, body fat percentage and other factors during the shelf life are verified through animal experiments, compared with other room-temperature yogurt formulas.

More specifically, the beneficial effects of the present disclosure lie in one or more of the following:

(1) The present disclosure solves the problem of low chlorogenic acid release from a honeysuckle extract. Compared with the existing honeysuckle extract processing methods, a vacuum freeze-drying and a leaching process are combined in the present disclosure to increase the chlorogenic acid content of a honeysuckle extract; a preheating process is performed before filling, thereby increasing the chlorogenic acid content in the final product of the room-temperature zero-sucrose yogurt; chlorogenic acid is an organic acid of the highest content in the honeysuckle extract, which can inhibit fat absorption, promote cholesterol excretion, and has the effect of weight loss.

(2) In the product of the present disclosure, a probiotic (*Bifidobacterium longum* BBMN68) is added to the yogurt starter to participate in the fermentation, which promotes the release of chlorogenic acid from the honeysuckle extract, thereby increasing the chlorogenic acid content in the final product, and improving the fat-reducing effect of the product.

(3) The present disclosure solves the defect that the yogurt fermentation system is unstable due to the addition of chlorogenic acid. Inulin is added, which ensures the stability of the room temperature product during the shelf life and simplifies the product formula. At the same time, as a type of prebiotic, inulin is beneficial to promote the metabolism of human intestinal flora to

produce short-chain fatty acids, wherein the short-chain fatty acids in intestines facilitate the effect of weight control.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to more clearly illustrate the technical solutions of the present disclosure or the prior art, the drawings of the Examples or prior art will be briefly introduced below. Obviously, the drawings described below are only some embodiments of the present disclosure. For ordinary technicians in this field, other drawings can also be obtained based on these drawings without creative work.

Figure 1 shows the chlorogenic acid content in the honeysuckle extract of the present disclosure before and after the optimization of the extraction process; wherein, “a” indicates no significant difference from the direct leaching group, “b” indicates a significant difference from the direct leaching group ($p < 0.05$), and “c” indicates a significant difference from both the direct leaching group and the vacuum freeze-drying group ($p < 0.05$).

Figure 2 shows the effect of heat treatment on the chlorogenic acid content in the product, wherein the “b” indicates a significant difference in chlorogenic acid content from the extract before heat treatment ($p < 0.05$).

Figure 3 shows the effect of the addition of probiotics on the chlorogenic acid content in the product and the effects of the process parameters on the chlorogenic acid content in the product. For the lowercase letters in the figure, different letters indicate differences between different groups ($p < 0.05$), and groups marked with the same letters indicate that there is no significant difference between these groups.

Figure 4 shows the detection results of short-chain fatty acids between the Examples and the Comparative Examples; wherein, “a” indicates no significant difference from Comparative Example 4, “b” indicates a significant difference from Comparative Example 4 ($p < 0.05$), and “c” indicates a significant difference from both Comparative Example 4 and Example 4 ($p < 0.05$).

Figure 5 shows the effect on weight gain of rats between the Examples and the Comparative Examples; wherein, different letters (*i.e.*, a, b, c, d and e) indicate differences between different groups ($p < 0.05$), and groups marked with the same letters indicate that there is no significant difference between these groups.

Figure 6 shows the effect on body fat percentage of rats between the Examples and the Comparative Examples; wherein, different letters (*i.e.*, a and b) indicate differences between

different groups ($p < 0.05$), and groups marked with the same letters indicate that there is no significant difference between these groups; wherein “b” indicates no significant difference from the model group, and a significant difference from the blank group ($p < 0.05$).

Figure 7 shows the detection results of acetic acid in rat feces between the Examples and the Comparative Examples; wherein, the * indicates that there is a significant difference from other groups ($p < 0.05$).

Figure 8 shows the detection results of propionic acid in rat feces between the Examples and the Comparative Examples; wherein, the * indicates that there is a significant difference from other groups ($p < 0.05$).

Figure 9 shows the detection results of butyric acid in rat feces between the Examples and the Comparative Examples; wherein, the * indicates that there is a significant difference from other groups ($p < 0.05$).

DETAILED DESCRIPTION OF THE INVENTION

In order to make the purpose, technical solutions and advantages of the present disclosure clearer, the technical solutions of the present disclosure are described clearly and completely below. Obviously, the described Examples are only part but not all of the Examples of the present disclosure. Based on the Examples of the present disclosure, all other Examples obtainable by ordinary technicians in this field without creative work are within the scope of the present disclosure.

In the Examples of the present disclosure, the preparation method of the honeysuckle extract is as follows: honeysuckles with full buds, emerald green color, no mildew, no insect bites and no miscellaneous leaves and branches are selected, and washed with clean water to remove impurities such as surface mud and dirt. After pre-freezed in a -80°C refrigerator, the honeysuckles are put into a vacuum freeze-drying device and freeze-dried for 24 h under the conditions of a cold trap temperature of -50°C , a partition heating temperature of 20°C , and a vacuum degree of 0.12 mbar to obtain honeysuckle powder. The freeze-dried powder is crushed, and added with sterile water at a material-liquid ratio of 20-30 g: 1L, and dissolved and extracted in an insulated tank at $65-75^{\circ}\text{C}$ for 20 min, and then filtered to obtain a honeysuckle water extract

Lactobacillus bulgaricus and *Streptococcus thermophilus* are common strains available on the market.

Example 1 Preparation of room-temperature zero-sucrose yogurt

The fermented dairy product comprises the following raw materials (by mass percentage): honeysuckle extract 2%, erythritol 0.7%, xylitol 0.9%, whey protein powder 0.6%, high-esterified pectin 0.2%, inulin 3%, *Lactobacillus bulgaricus* 0.0001% (the bacterial concentration in the fermentation agent is 1×10^{12} CFU/g), *Streptococcus thermophilus* 0.0001% (the bacterial concentration in the fermentation agent is 1×10^{12} CFU/g), *Bifidobacterium longum* BBMN68 0.02% (the bacterial concentration is 5×10^8 CFU/g, containing 1×10^9 *Bifidobacterium longum*/100g), supplemented with a raw milk.

The preparation method of the room-temperature zero-sucrose yogurt was as follows:

(1) A honeysuckle was screened and cleaned, and then pre-frozen in a -80 °C refrigerator, and freeze-dried for 24 h under the conditions of a cold trap temperature of -50 °C, a partition heating temperature of 20°C, and a vacuum degree of 0.12 mbar to obtain honeysuckle powder. The freeze-dried powder was crushed, and added with sterile water at a material-liquid ratio of 30g : 1L, and dissolved and extracted in an insulated tank at 75°C for 20 min, and then filtered to obtain a honeysuckle water extract, wherein filtration was performed through a filter mesh or filter cloth at room temperature to remove impurities;

(2) A raw milk was filtered with a reverse osmosis membrane to obtain a retained solution and a permeate;

(3) The retained solution obtained in (2) was preheated to 55°C, added with a sugar substitute, whey protein powder, inulin, and the honeysuckle extract, stirred evenly, and homogenized at 60°C and 150bar;

(4) After homogenization, the material was sterilized at 121°C for 6s, cooled to 40°C after sterilization, and fermented for 6h by adding yogurt starter (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and a probiotic (*Bifidobacterium longum* BBMN68) until a pH of 4.35 and an acidity of $\geq 65^\circ\text{T}$ are reached, and then demulsified and cooled to 20°C to obtain a first material, wherein the fermentation time and temperature are beneficial to the release of chlorogenic acid from the honeysuckle extract;

(5) The permeate separated in step (2) was heated to 55°C, added with a stabilizer and stirred evenly, homogenized at 60°C 150bar, sterilized at 110°C for 15s, and then cooled to 25°C to obtain a second material;

(6) The first material obtained in (4) and the second material obtained in (5) were then statically mixed, preheated to 55°C and maintained for 5min, which is beneficial to the further release of chlorogenic acid, and then heated to 65°C for 60s for sterilization;

(7) The sterilized material obtained in (6) was aseptically homogenized at 25°C and 20bar and aseptically filled to obtain the room-temperature zero-sucrose yogurt.

Example 2 Preparation of room-temperature zero-sucrose yogurt

The fermented dairy product comprises the following raw materials (by mass percentage): honeysuckle extract 5%, erythritol 0.7%, xylitol 0.9%, whey protein powder 0.6%, high-esterified pectin 0.2%, inulin 3%, *Lactobacillus bulgaricus* 0.0001% (the bacterial concentration in the fermentation agent is 1×10^{12} CFU/g), *Streptococcus thermophilus* 0.0001% (the bacterial concentration in the fermentation agent is 1×10^{12} CFU/g), *Bifidobacterium longum* BBMN68 0.2% (the bacterial concentration is 5×10^8 CFU/g, containing 1×10^{10} *Bifidobacterium longum*/100g), supplemented with a raw milk.

The preparation method of the room-temperature zero-sucrose yogurt in Example 2 was the same as that in Example 1.

Example 3 Preparation of room-temperature zero-sucrose yogurt

The fermented dairy product comprises the following raw materials (by mass percentage): honeysuckle extract 8%, erythritol 0.7%, xylitol 0.9%, whey protein powder 0.6%, high-esterified pectin 0.2%, inulin 3%, *Lactobacillus bulgaricus* 0.0001% (the bacterial concentration in the fermentation agent is 1×10^{12} CFU/g), *Streptococcus thermophilus* 0.0001% (the bacterial concentration in the fermentation agent is 1×10^{12} CFU/g), *Bifidobacterium longum* BBMN68 0.02% (the bacterial concentration is 5×10^8 CFU/g, containing 1×10^9 *Bifidobacterium longum*/100g), supplemented with a raw milk.

The preparation method of the room-temperature zero-sucrose yogurt in Example 3 was the same as that in Example 1.

Example 4 Preparation of room-temperature zero-sucrose yogurt

The fermented dairy product comprises the following raw materials (by mass percentage): honeysuckle extract 5%, erythritol 0.7%, xylitol 0.9%, whey protein powder 0.6%, high-esterified pectin 0.2%, xylo-oligosaccharide 1.5%, *Lactobacillus bulgaricus* 0.0001% (the bacterial concentration in the fermentation agent is 1×10^{12} CFU/g), *Streptococcus thermophilus* 0.0001% (the bacterial concentration in the fermentation agent is 1×10^{12} CFU/g), *Bifidobacterium longum* BBMN68 0.02% (the bacterial concentration is 5×10^8 CFU/g, containing 1×10^9 *Bifidobacterium longum*/100g), supplemented with a raw milk.

The preparation method of the room-temperature zero-sucrose yogurt in Example 4 was the same as that in Example 1.

Example 5 Preparation of room-temperature zero-sucrose yogurt

The fermented dairy product comprises the following raw materials (by mass percentage): honeysuckle extract 5%, erythritol 0.7%, xylitol 0.9%, whey protein powder 0.6%, high-esterified pectin 0.2%, inulin 3%, *Lactobacillus bulgaricus* 0.0001% (the bacterial concentration in the fermentation agent is 1×10^{12} CFU/g), *Streptococcus thermophilus* 0.0001% (the bacterial concentration in the fermentation agent is 1×10^{12} CFU/g), *Bifidobacterium longum* BBMN68 0.02% (the bacterial concentration is 5×10^8 CFU/g, containing 1×10^9 *Bifidobacterium longum*/100g), supplemented with a raw milk.

The preparation method of the room-temperature zero-sucrose yogurt in Example 5 was the same as that in Example 1.

Comparative Example 1 Preparation of room-temperature zero-sucrose yogurt

In this Comparative Example, no honeysuckle extract was added, and the amounts of other ingredients and the preparation method were the same as those in Example 5.

Comparative Example 2 Preparation of room-temperature zero-sucrose yogurt

In this Comparative Example, 15% honeysuckle extract was added, and the amounts of other ingredients and the preparation method were the same as those in Example 5.

Comparative Example 3 Preparation of room-temperature zero-sucrose yogurt

In this Comparative Example, no *Bifidobacterium longum* was added, and the amounts of other ingredients and the preparation method were the same as those in Example 5.

Comparative Example 4 Different Prebiotic

In this Comparative Example, glucose was selected as the prebiotic, and the amounts of other ingredients and the preparation method were the same as those in Example 5.

Comparative Example 5 Different Probiotics

In this Comparative Example, *Bacillus bifidus* BB-12 (Chr. Hansen, Denmark) was selected as the probiotic, and the amounts of other ingredients and the preparation method were the same as those in Example 5.

Comparative Example 6 Different Fermentation Conditions

Compared with Example 5, this Comparative Example differed in that the fermentation temperature of this Comparative Example is 38°C.

Comparative Example 7 Different Preheating Treatments Before Filling

Compared with Example 5, this Comparative Example differed in that no preheating treatment was performed before filling.

Experimental Example 1 Product Flavors and Stability Analysis

The room-temperature zero-sucrose yogurts prepared by Examples 1 to 5 and Comparative Examples 1 to 4 were tested for sensory suitability. The test method was as follows: 50 persons were selected to conduct a blind test on the above-mentioned room-temperature zero-sucrose yogurts. The blind test indicators included the following parameters: characteristic flavors, mouthfeel (thickness), both of which were represented by numbers: 1 to 2 represented “very weak”; 3 to 4 represented “relatively weak”; 5 to 6 represented “just right”; 7 to 8 represented “relatively strong”; 9 to 10 represented “very strong”. After the test, the above test results were statistically analyzed, and the results were shown in Table 1:

Table 1 Product taste and flavor test results

	Characteristic flavors	Tastes
Example 1	6.1	5.8
Example 2	6.5	6.2
Example 3	7.2	6.1
Example 4	6.6	5.6
Example 5	6.8	6.5
Comparative Example 1	4.7	4.6
Comparative Example 2	8.7	4.1
Comparative Example 3	5.3	5.4
Comparative Example 4	4.7	3.7

Conclusion: By comparing and analyzing the evaluation results of the Examples and Comparative Examples in Table 1, it can be seen that the addition of a honeysuckle extract within the scope of the Examples has an effect of enhancing the characteristic flavors compared with Comparative Examples 1 and 2, without leading to a problem of the characteristic flavors being too prominent and affecting the overall flavors of the product.

Compared with the Examples, the results of Comparative Examples 3 and 4 show that the addition of a probiotic, inulin and xylo-oligosaccharide can enhance the overall characteristic flavors and tastes of the product. From the analysis of the results of Comparative Example 2, it can be seen that the adjustment of the amount of the honeysuckle extract also affects the tastes of

the product. The honeysuckle room-temperature zero-sucrose yogurts prepared in the Examples of the present disclosure are better in overall sensory suitability and flavor preference over the Comparative Examples.

The room-temperature zero-sucrose yogurts prepared in Examples 1 to 5 and Comparative Examples 1 to 4 were analyzed through centrifugal water loss rate and shelf life stability tests at 25°C and 37°C. The results were shown in Table 2.

Table 2 Product texture and stability results

Groups	Centrifugal water loss rate (%)	Observation of storage at room temperature 25°C	Observation of storage at 37°C
Example 1	4.85%	Fine texture state, no syneresis in 6 months	Slight syneresis at the bottom in 3 months
Example 2	5.08%	Fine texture state, no syneresis in 6 months	Slight syneresis at the bottom in 3 months
Example 3	5.69%	Fine texture state, no syneresis in 6 months	Slight syneresis at the bottom in 3 months
Example 4	5.35%	Fine texture state, no syneresis in 6 months	Slight syneresis at the bottom in 3 months
Example 5	5.12%	Fine texture state, no syneresis in 6 months	Slight syneresis at the bottom in 3 months
Comparative Example 1	6.03%	Slight syneresis at the bottom in 5 months	Slight syneresis at the bottom in 3 months
Comparative Example 2	7.45%	Slight syneresis at the bottom in 3 months	Obvious syneresis at the bottom in 2 months
Comparative Example 3	6.24%	Slight syneresis at the bottom in 4 months	Slight syneresis at the bottom in 2 months
Comparative Example 4	9.03%	Slight syneresis at the bottom in 2 months	Obvious syneresis at the bottom in 2 months, slight protein flocculation

The results of Table 2 show that the room-temperature zero-sucrose yogurt prepared in Examples 1 to 5 has a low centrifugal water loss rate after centrifugation at 4000rpm for 15 minutes, the product system is relatively stable, and no obvious syneresis or thinning occurs in the system when observed at room temperature or in heat preservation storage;

In the preparation method of Comparative Example 1, the honeysuckle extract was not added, which has a slight effect on the stability of the product; the honeysuckle liquid added in Comparative Example 2 is not within the claimed scope, resulting in that the additives are

unbalanced and cannot form a good stable system. The product shows obvious syneresis after about 2 months of heat preservation storage.

In Comparative Example 4, a prebiotic different from that in the present disclosure was used. Due to the insufficient protection ability of these prebiotic in terms of stability, the product has serious syneresis, and the system collapses after 2 months of room temperature storage.

In Comparative Example 3, the probiotic of Example 5 was not added. The results showed that although the other raw materials were the same, the product smoothness decreased, and the bottom became thinner after being stored at 37°C for 2 months.

The combination of a honeysuckle extract and inulin ensures the function of the room temperature product while balancing the flavors of the product, ensuring the stability during the shelf life, and simplifying the product formula.

Experimental Example 2 Detection and Analysis of Chlorogenic Acid Content

The method for detecting the chlorogenic acid content of the honeysuckle extract was designed with reference to relevant reference documents.

Reference documents:

[1] Ding Min, Wang Liling, Qin Yuchuan, et al. Study on the water extraction process of chlorogenic acid in honeysuckle [J]. Zhejiang Forestry Science and Technology, 2022, 42(2):6.

[2] Wang Yujie, Jie Weiguang, Guo Na, et al. Simultaneous detection of chlorogenic acid and total flavonoids in honeysuckle by high performance liquid chromatography and its extraction process [J]. Cereals&Oils, 2022, 35(2):6.

[3] Zou Rong, You Yuming, Chen Zexiong, Hu Kai, Ran Lie. Effect of drying method on polyphenol components and antioxidant activity of honeysuckle [J]. Food Science, 2016, 37(5):6.

The content of chlorogenic acid in this Experimental Example was tested by high performance liquid chromatography. First, a standard solution was prepared using a chlorogenic acid control substance (standard substance), and the chlorogenic acid standard solution was accurately aspirated to prepare solutions with different concentration gradients for use. A standard curve of chlorogenic acid content was drawn by high performance liquid chromatography under the detection wavelength of 327nm. The same detection method was further used to detect and calculate the chlorogenic acid contents in the honeysuckle liquid after leaching, in the material after fermentation and in the final product.

(1) Effects of process optimization on the chlorogenic acid content in the product

The present methods for extracting honeysuckle comprise: direct leaching, vacuum freeze-drying, and vacuum freeze-drying + leaching. The results in Figure 1 show that the chlorogenic acid content extracted by the combined processes (i.e., vacuum freeze-drying + leaching) is different from those of the other two groups (i.e., direct leaching, vacuum freeze-drying), indicating that the chlorogenic acid content in the honeysuckle extract is improved by combining vacuum freeze-drying and leaching in the present disclosure.

When preparing room-temperature zero-sucrose yogurts, different heating sterilization methods have different effects on the chlorogenic acid content in room-temperature zero-sucrose yogurts. The results in Figure 2 show that when the honeysuckle extract is added during the mixing step, the chlorogenic acid content in the honeysuckle extract is significantly improved after two times of sterilization heat treatment at different degrees. There was no significant difference in the chlorogenic acid content in the samples after UHT heat treatment (sterilization treatment in step (4)) or UHT+pasteurization heat treatment (preheating and sterilization in step (6)), but the chlorogenic acid content in the samples of the two groups increased significantly compared with the extracts before heat treatment, indicating that the processing of the room-temperature zero-sucrose yogurt further promoted the release of the effective components of the honeysuckle extract.

The results of Figure 3 show that the chlorogenic acid content in the samples showed a significant downward trend when the fermentation conditions were adjusted in Comparative Example 6 or the preheating treatment before sterilization was deleted in Comparative Example 7, indicating the importance of the process conditions in the Examples.

(2) Effect of adding a probiotic on the chlorogenic acid content in the product

In the present disclosure, a probiotic (*Bifidobacterium longum* BBMN68) was added to the yogurt starter to participate in the fermentation. The comparative analysis of the Examples with Comparative Examples 3 and 5 shows that the addition of *Bifidobacterium longum* BBMN68 can better promote the release of chlorogenic acid from the honeysuckle extract (Figure 3). Comparison of the results of Example 2 and Example 5 shows that the amount of probiotic is related to the chlorogenic acid content of the final product, which can enhance the fat-reducing effect of the product.

Experimental Example 3 Detection of short-chain fatty acids in products

In this Experimental Example, the contents of short-chain fatty acids were detected in the prepared room-temperature zero-sucrose yogurt product based on the formula of Example 5. The results were shown in Figure 4. The detection method refers to the following documents:

[1]. Wu Xufang, Zhang Yangdong, Zheng Nan, et al. Research progress on the composition, physiological function and detection technology of short-chain fatty acids in milk [J]. Journal of Animal Nutrition, 2022, 34(04): 2148-2155.

[2]. Fatty acid profile of milk for determining reproductive status in lactating Holstein Friesian cows. Hawar M. Zebari; S. Mark Rutter; Emma C.L. Bleach. Animal Reproduction Science, 2019.

The detection and analysis of short-chain fatty acids show that, by adding a prebiotic in the formula of the present disclosure, Examples 4 and 5 are more effective in increasing the production of short-chain fatty acids during the fermentation process compared with Comparative Example 4, which to a certain extent improves the efficacy of the product in fat loss and weight control.

Experimental Example 4 Weight Regulation Effect

Several male SPF Wistar rats weighing 140-170g were used in the animal housing of laboratory, which was a barrier system + IVC cage, with a temperature of 20-25°C and a relative humidity of 45%-65%. There were 10 rats in each group. The blank group was fed with ordinary feed (gavage with normal saline), the model group was fed with high-fat feed (gavage with normal saline), and the experimental group was gavaged with fermented milk (10 g/kg per day) and fed with high-fat feed. The weight changes of rats were observed on the 7th day after gavage in each group, and the gavage lasted for 12 weeks.

At the beginning of the experiment, the difference in weight between the model group and the blank group should exceed 20% of the average weight. The experimental groups were divided into different groups with different formulas, and the test samples were orally administered. The weight and food intake were measured twice a week. At the end of the experiment, the weight changes were calculated and the body fat percentage was detected (using nuclear magnetic resonance related equipment). The detection method refers to the following documents:

[1]. Li Chenyang. Development and application of non-destructive analysis method of mouse body composition [D]. Dalian Polytechnic University, 2018.

[2]. Choi B, Park S, Lee D, et al. Green coffee bean extract improves obesity by decreasing body fat in high-fat diet-induced obese mice [J]. Asian Pacific Journal of Tropical Medicine, 2016, 9 (07): 616-624.

In this Experimental Example, the weight regulation effect of the product was evaluated, and the results were shown in Figure 5. The results in Figure 5 show that the weight of rats in each

group was increased to varying degrees. There was a significant difference between the model group and the blank group, indicating that the obesity model was successfully established; weight gain was significantly reduced in each Example group in compared with the model group and the Comparative Example groups. There was a significant difference between Example 5 and Example 4, but both were able to exert a good effect of weight regulation. The difference between the Comparative Example groups was not significant, the effect thereof was weaker than that of the Example groups although there was a significant difference between the effect of Comparative Example group and that of the model group. The weight gain of Example 5 was not significantly different from that of the blank group, indicating that the weight control effect was good. Example 5 also achieved a reduction in weight gain compared to Comparative Example 1.

In this Experimental Example, the effect of the product on the body fat percentage of rats was evaluated. The results were shown in Figure 6. The body fat percentage of rats in each group was reduced to varying degrees. There was no significant difference between Example 4, Example 5 and the blank group, indicating that the products in the Examples have a good effect on improving body fat; the body fat percentage in Comparative Example groups was reduced compared to that in the model group, however, the effect was not significant, and thus there was no significant difference between the Comparative Example group and the model group. Among them, the body fat percentage in Comparative Example 1 was between that in the blank group and in the model group, indicating that the zero-sucrose yogurt product itself has a certain effect on weight control. Compared with Comparative Example 1, Example 5 also achieved a reduction in body fat percentage.

Experimental Example 5 Distribution of Intestinal Flora of Rats

In this Experimental Example, the intestinal flora of rats were detected and analyzed, and the method included the following steps: different groups of rats were gavaged with test samples, and 6 rats were selected from each group. At the end of the test, all fresh feces within 5 hours were collected and stored in a centrifuge tube with a lid, and the total mass and feces quantity were recorded, and stored at -80°C for flora determination. The flora determination method was performed by 16s rDNA sequence test. The testing method refers to the following reference documents:

[1]. WEI J, ZHAO Y, ZHOU C, et al. Dietary Polysaccharide from *Enteromorpha clathrata* Attenuates Obesity and Increases the Intestinal Abundance of Butyrate-Producing Bacterium, *Eubacterium xylanophilum*, in Mice Fed a High-Fat Diet[J]. *Polymers (Basel)*, 2021,13(19).

[2]. TANG S, ZHONG R, YIN C, et al. Exposure to High Aerial Ammonia Causes Hindgut Dysbiotic Microbiota and Alterations of Microbiota-Derived Metabolites in Growing Pigs[J]. Front Nutr, 2021,8: 689818.

The results of rat intestinal flora determination are shown in Tables 3 and 4.

Table 3 Distribution changes of rat intestinal flora in the Examples and Comparative Examples (phylum level)

relative ratio	blank group	Model group	Example 4	Example 5	Comparative Example 1	Comparative Example 3	Comparative Example 4	Comparative Example 5
Firmicutes	11±1.75 ^a	32±1.15 ^b	12±1.09 ^a	8±0.87 ^c	17±1.45 ^d	13±1.38 ^d	21±0.93 ^d	15±1.66 ^a
Bacteroidetes	10±0.25 ^a	4.5±0.15 ^b	6.5±0.30 ^c	7.5±0.27 ^d	5±0.10 ^e	5±0.16 ^e	6.25±0.23 ^c	5±0.44 ^b
Actinobacteria	0.25±0.02 ^a	1.25±0.05 ^b	0.3±0.03 ^a	0.35±0.05 ^a	0.525±0.02 ^c	0.55±0.01 ^c	0.5±0.01 ^c	0.5±0.02 ^c
Proteobacteria	0.25±0.02 ^a	1.125±0.01 ^b	0.5±0.05 ^c	0.475±0.03 ^c	0.875±0.05 ^d	0.825±0.01 ^d	0.825±0.03 ^d	0.875±0.04 ^d

Table 4 Distribution changes of rat intestinal flora in the Examples and Comparative Examples (species level)

Relative Ratio	Blank group	Model group	Example 4	Example 5	Comparative Example 1	Comparative Example 3	Comparative Example 4	Comparative Example 5
Bifidobacterium	24±1.45 ^a	12±0.55 ^b	38±1.48 ^c	42±1.24 ^c	30±0.40 ^a	34.5±0.45 ^c	27±0.98 ^a	30.8±0.46 ^a
Lactobacillus	18±2.51 ^a	4±0.84 ^b	16.4±0.74 ^a	17±0.88 ^a	13±0.32 ^c	14±0.47 ^a	14.6±1.05 ^a	14.4±0.91 ^a
Bacteroides	45±1.50 ^a	10±0.85 ^b	30±1.25 ^c	35±1.40 ^d	16±0.85 ^e	16±1.06 ^e	15±0.55 ^e	15±0.86 ^e
Eckermanella	4±0.25 ^a	1±0.11 ^b	10±0.96 ^c	17±1.22 ^d	3.8±0.71 ^a	5±1.05 ^a	4.2±0.45 ^a	3.5±0.53 ^a
Roseburia	21±2.25 ^a	7±0.55 ^b	11±1.15 ^c	14±0.75 ^c	7±0.24 ^b	9±0.64 ^d	8±1.02 ^b	7±0.28 ^b

Note: different letters (i.e., a, b, c and d) indicate differences between different groups ($p < 0.05$), and groups marked with the same letters indicate no significant differences between these groups.

The results of rat intestinal flora determination show that, at the phylum level, the abundances of the Firmicutes, Actinobacteria and Proteobacteria were increased in model group, and was significantly decreased in Comparative Examples as compared with that in model group, but there were still differences between the abundances in Comparative Examples and that in Examples; in the Example groups, the results of Actinobacteria and Firmicutes were similar to those in the blank group; the abundances of the Bacteroidetes in model group and in Comparative Example groups were significantly decreased, which showed an upward trend in the Example groups.

At the species level, the abundances of three bacteria (Bifidobacterium, Lactobacillus and Bacteroides) were significantly increased in the Example group, which were significantly different from those in the Comparative Example group and the model group; Eckermanella and Roseburia bacteria related to weight control were also significantly increased in the Example

group, which to a certain extent explained that the growth of beneficial intestinal bacteria and fat-reducing related strains was promoted in the Examples.

Example 6 Detection of short-chain fatty acids in rat feces

In this Experimental Example, the content of short-chain fatty acids in rat feces were detected. Figure 7 shows the detection results of acetic acid in rat feces after feeding with the room-temperature zero-sucrose yogurt of the Examples and the Comparative Examples; Figure 8 shows the detection results of propionic acid in rat feces after feeding with the room-temperature zero-sucrose yogurt of the Examples and the Comparative Examples; Figure 9 shows the detection results of butyric acid in rat feces after feeding with the room-temperature zero-sucrose yogurt of the Examples and the Comparative Examples. The detection method refers to the following documents:

[1]. MACHIELS K, JOOSSENS M, SABINO J, et al. A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis[J]. Gut, 2014,63(8): 1275-1283.

[2]. XIAO S, LIU C, CHEN M, et al. *Scutellariae radix* and *coptidis rhizoma* ameliorate glycolipid metabolism of type 2 diabetic rats by modulating gut microbiota and its metabolites[J]. Appl Microbiol Biotechnol, 2020,104(1): 303-317.

The results of Figures 7 to 9 show that, as compared with the model group, the contents of short-chain fatty acids (acetic acid, propionic acid, butyric acid) in the intestine of rats in the Example groups increased significantly, and the results of the Example groups were significantly different from those of the blank group and the Comparative Example groups, indicating that the products of the Example groups have a good effect on the production of short-chain fatty acids in the intestine of rats and on the weight control.

Finally, it should be noted that the above Examples are only used to illustrate the technical solutions of the present disclosure, but not to limit them; although the present disclosure is described in details with reference to the above Examples, ordinary technicians in the field would understand that the technical solutions recorded in the above Examples can be modified, or some of the technical features therein can be replaced, while such modifications or replacements do not make the essence of the corresponding technical solutions deviate from the spirit and scope of the exemplified technical solutions of the present disclosure.

I/We claim:

1. A method for preparing yogurt, comprising using a honeysuckle extract, a prebiotic and a probiotic *Bifidobacterium longum* BBMN68 at a ratio of (2-10g): (1-6g): ($1 \times 10^{7-8}$ CFU, e.g., 1×10^7 CFU or 1×10^8 CFU) per 100g of raw materials, wherein the prebiotic is inulin or xylo-oligosaccharide, and the yogurt is preferably room-temperature zero-sucrose yogurt.

2. The preparation method of claim 1, wherein the honeysuckle extract is obtained by a method comprising: pre-freezing and freeze-drying honeysuckle to obtain honeysuckle powder, dissolving and extracting the honeysuckle powder in an insulated tank in sterile water at a ratio of 20g : 1L to 30g : 1L at 65-75°C for 10-20 min, which is then filtered to obtain the honeysuckle extract.

3. The preparation method of claim 2, comprising, after fermentation and before sterilization of the yogurt, preheating the yogurt to 50-55°C and maintaining for 5-10 minutes.

4. The preparation method of any one of claims 1-3, comprising fermenting a raw milk with a stabilizer, a sweetener, the prebiotic, a whey protein and the honeysuckle extract with *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Bifidobacterium longum* BBMN68 to obtain the yogurt, wherein the stabilizer is high-esterified pectin, and the sweetener is erythritol or xylitol.

5. The preparation method of claim 4, wherein the raw materials comprise 80-100 parts by weight of the raw milk, 2-10 parts by weight of the honeysuckle extract, 0.01-10 parts by weight of the sweetener, 0.5-2 parts by weight of the whey protein, 0.1-0.5 parts by weight of the high-esterified pectin, 1-6 parts by weight of the prebiotic, $1 \times 10^{7-10}$ CFU/100g of effective viable bacteria of *Lactobacillus bulgaricus*, $1 \times 10^{7-10}$ CFU/100g of effective viable bacteria of *Streptococcus thermophilus*, and $1 \times 10^{7-10}$ CFU/100g of effective viable bacteria of *Bifidobacterium longum* BBMN68.

6. The preparation method of claim 5, comprising:

(1) filtering the raw milk through a reverse osmosis membrane to obtain a retained solution and a permeate;

(2) preheating the retained solution obtained in step (1) to 45-55°C, adding the sweetener, the whey protein, the prebiotic, and the honeysuckle extract, stirring, homogenizing, sterilizing, and cooling the same;

adding *Streptococcus thermophilus*, *Lactobacillus bulgaricus* and *Bifidobacterium longum* BBMN68 and fermenting at 40-45°C for 4-8 hours to obtain a pH of 4.25-4.35, demulsifying and cooling the same to obtain a first material;

(3) heating the permeate separated in step (1) to 50-55°C, adding the stabilizer, stirring, homogenizing, sterilizing, and cooling the same to obtain a second material;

(4) statically mixing the first material obtained in (2) and the second material obtained in (3), which is then preheated to 50-60°C and maintained for 2-5 minutes, heated to 65-80°C for 15-60 seconds for sterilization, cooled to 20-25°C, aseptically homogenized at 20-30 bar, and aseptically filled to obtain the room-temperature zero-sucrose yogurt.

7. The preparation method of claim 6, wherein, in step (2), the homogenizing before fermentation is carried out at 55-65°C, 150-160 bar, the sterilizing before fermentation is carried out at 120-130°C for 5-10 seconds, and the cooling before fermentation is carried out at 40-42°C, and the cooling after demulsifying is carried out at 20-25°C.

8. The preparation method of claim 6, wherein, in step (3), the homogenizing after adding the stabilizer and stirring is carried out at 55-65°C, 150-160bar, the sterilizing is carried out at 110-120°C for 15s, and the cooling is carried out at 20-25°C.

9. The yogurt prepared by the method of any one of claims 1 to 8.

10. Use of the yogurt of claim 9 in the preparation of a health product for fat reduction and body shaping.