

Article

# Characteristics of Synbiotic Drink from Jicama (*Pachyrhizus erosus* L.) Tubers and Sunflower (*Helianthus annuus* L.) Seeds Extract

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## **Abstract**

Synbiotic drinks are fermented beverages composed of ingredients that encompass prebiotics and probiotic bacteria. A study was conducted to develop a synbiotic drink using Jicama tubers extract and sunflower seeds extract along with the probiotic bacteria including *Lactobacillus casei* FNCC-0090, *Lactobacillus acidophilus* FNCC-0051, *Bifidobacterium breve* BRL-131, and *Bifidobacterium bifidum* BRL-130. The objective of this research was to assess the impact of varying proportions of Jicama tubers extract (Jc) and Sunflower seeds extract (Sf), as well as fermentation duration on the characteristics of synbiotic drinks. The experimental design employed a factorial completely randomized design with two factors: the proportions of Jicama and sunflower seed extract (75:25, 50:50, and 25:75) and fermentation time (8, 12, 16, and 20 h). Data from the study were analyzed using ANOVA (P<0.05), and significant differences between treatments were assessed by DMRT. The optimal treatment was determined at the 50:50 proportion of Jicama and sunflower seed extract, with an 20 h fermentation time, yielding a synbiotic drink characterized by total LAB of 11.34 log CFU/mL, total lactic acid of 1.29%, N-amino of 4.67 %, antioxidant activity of 33.5%, viscosity of 37.08 cP, inulin content of 1.03%, and vitamin E content of 0.58%.

Keywords: Synbiotic; prebiotic; probiotic; Jicama; Sunflower.

## 1. Introduction

A synbiotic drink is a fermented beverage, enriched with a combination of prebiotics and probiotics [3;4]. **Probiotics** are live microorganisms that, when administered in sufficient quantities, confer advantages to the host such as alleviate constipation and lower the risk of colon cancer [35;38], whereas prebiotics are indigestible substances that promote bacterial proliferation [1;2]. Therefore, synbiotic drinks is recognized as a category of functional food products that have recently gained popularity. According to Market Analysis Report (2025), the global functional food market size was estimated

at USD 280.7 billion in 2021 and continuously increased until 2024 at USD 357.7 billion. This category is projected to reach USD 586.1 billion by 2030, growing at a CAGR of 8.5% from 2022 to 2030. Nevertheless, the majority of fermented products, including synbiotic beverages, are dairy-based and are unsuitable for certain groups of people, such as individuals with milk protein allergies, lactose intolerance, vegetarians, and vegans, or those with sustainability, lifestyle, and dietary considerations [39].

In order to produce synbiotic drinks without the inclusion of dietary sources, prebiotics from plants are required to support the growth of probiotic bacteria, such as inulin [8;9]. Inulin, a fructose polymer, is water-soluble, indigestible by digestive enzymes, yet fermentable by colonic microorganisms. Inulin is commonly found in roots, such as Jicama tubers. The inulin content in jicama tubers is reported as 6.512%, with a filtrate content of 4.41% [12]. In contrast, [13] found that the water extract of jicama tubers contains approximately 12.322%±0.733% of inulin. Additionally, jicama tubers boast a sugar content of 2.13±0.11% and a starch content of 9.04±0.11%, which also serving as a carbon source for the growth of lactic acid bacteria [14].

In addition of utilizing Jicama tubers, the production of synbiotic drink may also incorporate nuts and/or seeds. One of the seeds that has high nutrition compound is sunflower seed. Sunflower seeds contain a substantial amount of protein, specifically 18.70%, which higher to the amount of protein in some nuts such as pecan nut of 9.0%, pine nut of 13.7%, hazelnut of 14.5-15.2%, macadamia nut of 7.55-8.58%, chestnut of 1.63%, and walnut of 14.4-16.0%. The protein of sunflower seeds is also comparable to the amount of protein in cashew nut of 17.5-19.0% and pistachio of 19.4-22.1% [15;40]. The protein in sunflower seeds serves as a metabolic substrate for lactic acid bacteria, contributing to their growth. Furthermore, the inclusion of sunflower seeds in the synbiotic drink represents a novel advancement and adds value due to their vitamin E content in the form of tocopherol alpha-tocopherol), (containing 98% functions as an antioxidant [16;17]. As per the USDA Nutrient Database (2019), sunflower seeds exhibit the highest vitamin E ( $\alpha$ -tocopherol) levels among nuts and seeds, measuring at 35.17 mg/100g. Therefore, the mixture of sunflower seeds with jicama tubers could have a mutual complementary effect between probiotic survival and nutritional advancement of synbiotic drink produced.

Synbiotic drinks are typically produced by employing lactic acid bacteria that serve as probiotics. In this study, the probiotic bacteria including *Lactobacillus casei* FNCC-0090, *Lactobacillus acidophilus* FNCC-0051, *Bifidobacterium breve* BRL-131, and *Bifidobacterium bifidum* BRL-130 were utilized. The selection of these probiotic strains is based on the findings of [5], which showed that inulin from lesser yam tubers can stimulate the growth of some probiotics including *Bifidobacterium breve* BRL-131, *Bifidobacterium bifidum* 

BRL-130, and *Lactobacillus casei* FNCC-90 during a 72 h fermentation time. Moreover, it is known that inulin derived from lesser yam tubers may affect intestinal microbiota. In Sprague Dawley rats, the quantity of *Bifidobacterium* and *Lactobacillus* is potentially enhanced while the level of *E. coli* is diminished. In addition, lesser yam inulin elevated the levels of SCFA (acetate, propionate and butyrate) produced [7]. Furthermore, [6] also exhibited that the probiotic bacteria such as *Lactobacillus acidophilus* FNCC-051 had superior utilization of inulin compared to *Lactobacillus fermentum* S21209, *Lactobacillus plantarum* 1-S27202, *Pediococcus pentosaceus* 1-A38, and *Lactobacillus rhamnosus* R23.

Moreover, the characteristics of synbiotic drinks also can be influenced by the proportion of the ingredients used and fermentation duration. An inadequate amount of the ingredient added may result in suboptimal growth of lactic acid bacteria [41]. Excessive fermentation time results in synbiotic drinks with a low pH, leading to an overly sour taste [19]. Conversely, an insufficient fermentation duration will result in a stable pH, low total acidity, and restricted proliferation of lactic acid bacteria. It is showed that the optimal treatment for fermented salak (Salacca zalacca) probiotic drink, using L. casei strain Shirota, involves an 8 h fermentation time. In contrast, the optimal treatment for a probiotic drink made from dates, fermented with Lactobacillus casei FNCC 0090 and Lactobacillus plantarum FNCC 027, requires a 20 h fermentation time [20]. Therefore, appropriate treatment is essential to produce synbiotic drinks with optimal physicochemical and microbiological properties that are well-received by consumers [18].

According to those several previous studies related to synbiotic drinks, there have never been any reports about synbiotic drinks from jicama tubers enriched with sunflower seed extract. Therefore, this research aimed to assess the impact of varying proportions of jicama tubers and sunflower seed extract, as well as different fermentation times on the characteristics of the synbiotic drink, including the growth of lactic acid bacteria, total lactic acid, viscosity, N-amino content, antioxidant activity, inulin content, and vitamin E levels.

# 2. Material and Method

# 2.1. Materials and Equipment

The materials utilized in the formulation of synbiotic beverages for this study comprised jicama tubers of a local variety acquired from a traditional market in Surabaya; sunflower seeds of a local variety obtained from the Lumajang Regency Agriculture Service; in addition to glucose, CMC (Carboxy Methyl Cellulose), and tap water. The criteria for selecting jicama tubers included the necessity for maturity, the absence of physical defects, and uniformity in size, specifically ranging from 200 to 400 g per tuber. Only tubers that were healthy, intact, and free from bruising were selected. Tubers exhibiting evidence of microbial decay or significant moisture loss were excluded. Furthermore, only light grayish-brown sunflower seeds of uniform size and weight (approximately 20-30 mg per seed) were utilized. The additional materials employed comprised purified cultures of Lactobacillus casei FNCC-0090, Lactobacillus acidophilus FNCC-0051, Bifidobacterium breve BRL-131, and Bifidobacterium bifidum BRL-130, sourced from the Food and Nutrition Center Laboratory at Universitas Gadjah Mada, Yogyakarta, Indonesia.

Additionally, the materials that were used for the analysis of each parameter in this study comprised 2,2 Bipiridin 0.07%, FeCl<sub>3</sub> 0.02%, ethanol 96%, NaOH 0.1 N, PP indicator, Formaldehida 40%, DPPH (1,1 diphenyl-2-picryhydrazil) 400 μM, the bacterial growth media including MRS Agar (Oxoid), MRS Broth (Oxoid), and Sodium Chloride (Merck) along with distilled water obtained from the Food Analysis and Microbiology Laboratory in the Food Technology Department of the Faculty of Engineering at Universitas Pembangunan Nasional Veteran Jawa Timur, Indonesia.

The apparatus utilized in this study comprises UV-Vis spectrophotometer (SCITEK SP-UV5100, China), autoclave (HIRAMAYA HVA-110), incubator (BIOBASE), colony counter (BIOLABTEK), biosafety cabinet (LABTEK), HPLC, magnetic stirrer, analytical balance (KERN), a digital balance, beaker glass, test tubes, measuring cups, petri dishes, knives, blender, and filter cloth.

## 2.2. Experimental design

This study utilized a completely randomized design involving a factorial arrangement that included two factors, with three replications assigned to each treatment condition. Factor I involved the ratios of Jicama and sunflower seed extract (75:25, 50:50, and 25:75), whereas Factor II corresponded to the duration of fermentation (8 h, 12 h, 16 h, and 20 h). The chosen proportion quantities were designed to identify the ideal combination between inulin sourced from jicama and proteins obtained from sunflower seeds, which promotes the development of microbial and bioactive compounds. Moreover, the selected fermentation durations evaluate the improvement of microbial activity and the synthesis of metabolites to determine the optimal time period that enhances microbial growth and the generation of advantageous compounds, while simultaneously preventing excessive acidification. The data obtained were analyzed using ANOVA (p<0.05), and when significant differences among treatments were identified, additional analysis was performed using Duncan's Multiple Range Test (DMRT) at a confidence level of 95%.

## 2.3. Research procedure

Jicama tubers were peeled, rinsed, and subsequently cut into small pieces. The tubers were mashed with a ratio of tubers to water of 1:2. The mixture was filtered to obtain the extract. Furthermore, sunflower seeds were cleaned, and water was added with a ratio of seeds to water of 1:5. The seeds were then ground using a blender to obtain sunflower seeds slurry. The slurry was subsequently heated to a temperature of 70°C and then filtered using a filter cloth to acquire sunflower seeds extract. Jicama tubers and sunflower seeds extract in the proportion of 75:25, 50:50, and 25:75 v/v, were mixed with 2% (w/v) glucose and 0.75% (w/v) CMC. This mixture was pasteurized at 80°C for 15 min, followed by cooling to a temperature between 35-40°C. Additionally, bacteria, specifically Lactobacillus casei FNCC-0090, Lactobacillus acidophilus FNCC-0051, Bifidobacterium breve BRL-131, and Bifidobacterium bifidum BRL-130 at a concentration of 7% (v/v) were added in a ratio of 1:1:1:1. The incubation of synbiotic drinks was carried out for 8, 12, 16, and 20 h at 37°C.

### 3. Results and Discussion

The synbiotic drink produced in this research was analyzed and measurized for total lactic acid bacteria (LAB), total lactic acid (titrimetric method with an phenolphthalein indicator), N-amino content (formol titration method analysis), viscosity using viscometer, antioxidant activity (DPPH method analysis), inulin (Spectrofotometric analysis in 560 nm) and vitamin E content (Spectrofotometric analysis in 520 nm).

# 3.1. Total Lactic Acid Bacteria (LAB)

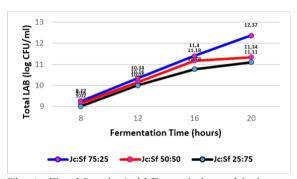


Fig. 1. Total Lactic Acid Bacteria in synbiotic drink.

Fig. 1 exhibits that the addition of jicama tubers extract (Jc) and sunflower seeds extract (Sf) in different proportion of 75:25, 50:50, and 25:75 results the various total LAB during 8 to 20 h fermentation period ranging from log 9.27 to 12.37 CFU/mL, log 9.06 to 11.34 CFU/mL, and log 9.02 to 11.11 CFU/mL, respectively. It indicates that an increase in the proportion of jicama tubers extract and a decrease in the proportion of sunflower seeds extract result in an enhancement of the total LAB. This can be attributed to the presence of inulin in jicama tubers, which stimulating the growth of LAB [13;20]. Inulin, as a carbon source, is hydrolyzed by Lactobacillus, providing energy for growth, cell reproduction, and activities during fermentation period [21]. Furthermore, several types of Bifidobacteria also produce the extracellular enzyme inulinase, which hydrolyzes inulin into fructose that subsequently utilized as an energy source for bacteria to growth [1]. This is consistent to [22], which shows that the prebiotic inulin can promote the proliferation of probiotic bacteria, resulting in augmentation of the probiotic bacteria population.

Similarly, the prolonged fermentation times also shows in an increased population of LAB. This finding indicates that extended fermentation times enable LAB to effectively breakdown nutrients in the medium into simpler components, including lactic acid, CO<sub>2</sub>, H<sub>2</sub>O, and energy. The generated energy is subsequently employed for synthesis, leading in an overall increase in total cell count and a heightened presence of LAB in the synbiotic drink [7;20]. This observation is also supported by [19;20], which demonstrated that the longer fermentation times provide the greater time for LAB to metabolize nutrients, such as inulin and sugars into lactic acid and organic acid.

### 3.2. Total Lactic Acid

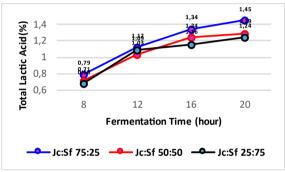


Fig. 2. Total Lactic Acid in synbiotic drink.

Fig. 2 demonstrates that the addition of jicama tubers extract (Jc) and sunflower seeds extract (Sf) in different proportion of 75:25, 50:50, and 25:75 results the various total lactic acid during 8 to 20 h fermentation period ranging from 0.79 to 1.45%, 0.71 to 1.29%, and 0.68 to 1.24%, respectively. It shows that an increase in the proportion of jicama tubers extract and a decrease in the proportion of sunflower seeds extract lead to an elevation of total lactic acid in the synbiotic drink. This trend is ascribed to the concentration of jicama tubers extract, which leads to heightened quantities of inulin, starch, and total sugar, serving as carbon sources for metabolism of LAB. As a result, bacterial proliferation increases (Fig 1.), which ultimately resulting in elevated lactic acid production [22].

Furthermore, an extended fermentation duration also results in an increased total lactic acid concentration in the synbiotic drinks. This finding aligns with [23], which reported that an increase in total acid occurs in conjunction with the duration of fermentation. This shows that an extended fermentation duration results the LAB

more opportunity to metabolize inulin, starch, and sugar into lactic acid, leading to a higher proliferation of probiotic bacteria, which in turn reveals in increased acid production and a reduction in pH [20;22]. [21] also exhibited that the breakdown of prebiotic by probiotic bacteria generates a byproduct in the form of lactic acid.

## 3.3. Content of N-Amino

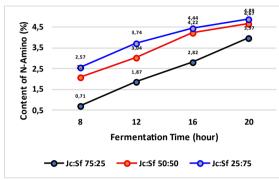


Fig. 3. Content of N-Amino in synbiotic drink.

In Fig.3, the addition of jicama tubers extract (Jc) and sunflower seeds extract (Sf) in different proportion of 75:25, 50:50, and 25:75 results the various total lactic acid during 8 to 20 h fermentation period ranging from 0.71 to 3.97%, 2.1 to 4.67%, and 2.57 to 4.89%, respectively. This data indicates that a reduced proportion of jicama tubers extract, combined with an increased proportion of sunflower seed extract, leads to a larger production of N-amino acids during fermentation. This phenomenon is due to the protein concentration in sunflower seeds, measured at 18.70%, which exceeds the protein level in jicama tubers, measured at 1.23% [15]. Therefore, a greater proportion of sunflower seeds extract contributes to a higher N-amino Furthermore, the analysis of raw content. materials indicates that the N-amino concentration in sunflower seed extract is 0.70%, whereas in jicama tubers extract is 0.23%. The lower N-amino concentration in the raw materials compared to the synbiotic beverage products is expected due to the breakdown of protein by LAB during fermentation, vielding molecules such amino acids.

Moreover, an extended fermentation duration promotes the production of a higher N-amino content in the synbiotic drinks. The extended fermentation duration may provide LAB with increased opportunity to hydrolyze proteins into

amino acids, leading to heightened N-amino levels [25]. The elevation of N-amino levels indicates the amounts of amino acids produced. Furthermore, amino acids, resulting from protein degradation, function as growth factors for bacteria and are employed in the synthesis of proteins and enzymes into amino acids, peptides, and other metabolic byproducts [16;23].

Amino acids, especially N-Amino in synbiotic products, enhance nutritional value by supplying required and non-essential building blocks for human metabolism, hence improving the protein bioavailability of nitrogenous and substances. Additionally, these amino acids can also serve as precursors for flavor chemicals, such as glutamate, which imparts umami taste, or branched-chain amino acids that produce aromatic aromas, thus enhancing the sensory profile of the beverage [42]. Moreover, the interaction of amino acids with other constituents may influence the texture, viscosity, and stability of the synbiotic beverage, thereby indirectly affecting consumer acceptance and shelf life [43].

## 3.4. Viscosity of Synbiotics Drink

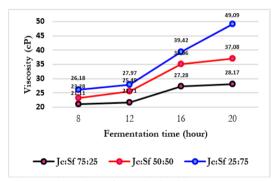


Fig. 4. Viscosity of synbiotic drink.

Fig. 4 reveals that the incorporation of jicama tubers extract (Jc) and sunflower seeds extract (Sf) in several different ratios of 75:25, 50:50, and 25:75 yields various viscosities across the fermentation period of 8 to 20 h, including 21.11 to 28.17 cP, 23.28 to 37.08 cP, and 26.18 to 49.09 cP, respectively. The findings indicate that an increased proportion of sunflower seed extract and extended fermentation duration, along with a reduced proportion of jicama tubers extract, yield a higher viscosity in the synbiotic drink. This occurrence is a consequence of a greater concentration of sunflower seed extract added, which contributes additional protein or amino

acids to the synbiotic drink, resulting in protein coagulation, thus leading to a clumped texture during extended fermentation due to elevated lactic acid production. [27]. This observation aligns with [28], which states that elevated acidity can induce protein aggregation, hence increasing product viscosity. This finding is also comparable to Patent for A Ready to Drink Plant Based Baverage, which specified that the recommended viscosity range for ensuring good flow and drinkability is 20-40 cP.

Viscosity is considered as an essential indicator of mouthfeel characteristics, including thickness, creaminess, smoothness, mouthcoating. The augmentation of viscosity typically amplifies the sensations of creaminess and smoothness, as well as perceived quality, which are highly valued by consumers in products such as synbiotic beverages [44]. Moreover, the inadequate viscosity may be linked to heightened syneresis, which is interpreted as a defect and lowers product attractiveness [45]. Nevertheless, the excessively increased viscosity may adversely affect sensory characteristics, leading to an undesirable mouthfeel and texture, which can result in diminished acceptability [46].

## 3.5. Antioxidant Activity

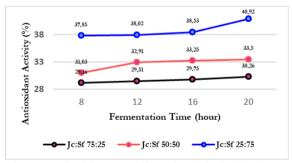


Fig. 5. Antioxidant Activity of synbiotic drink.

Fig. 5 demonstrates that the incorporation of jicama tubers extract (Jc) and sunflower seeds extract (Sf) in varying ratios of 75:25, 50:50, and 25:75 yields various antioxidant activities throughout an 8 to 20 h fermentation period, measuring at 29.16 to 30.26%, 31.03 to 33.5%, and 37.85 to 40.92%, respectively. The findings indicate that an increased proportion of sunflower seeds extract and extended fermentation duration, along with a reduced proportion of jicama tubers extract, enhance the antioxidant activity of the synbiotic drinks. It may be correlated to the larger

antioxidant activity of the raw ingredients that will consistently influence the final product. The analysis of raw materials reveals that the antioxidant activity of jicama tubers extract is 6.45%, while that of sunflower seeds extract is 42.12%. Consequently, synbiotic beverages containing a greater concentration of sunflower seeds extract demonstrate enhanced antioxidant activity. The enhanced antioxidant activity of sunflower seeds extract is due to the presence of vitamin E, which acts as an antioxidant [29;30].

Furthermore, an extended fermentation duration results in an increased amount of total lactic acid in the synbiotic beverage, hence improving the antioxidant activity of the product. This corresponds with the findings of [31], which revealed that extended fermentation results in enhanced proliferation of LAB. As a result, the ability of bacteria for transforming glucose into main metabolites (lactic acid) and secondary metabolites (polyphenols) is enhanced. The transformation of sugar into lactic acid by LAB synergistically supplies H+ ions to free radicals, hence enhancing primary antioxidant activity. Lactic acid comprises α-hydroxy acids (AHA), which serve as antioxidants and are commonly employed in the formulation of cosmetics and food product. In conjunction with lactic acid, probiotic bacteria also generate secondary metabolites that function antioxidants [33].

phytochemicals, Antioxidant including vitamin E derived from natural food sources, are crucial in the prevention and treatment of chronic diseases induced by oxidative stress. These compounds also confer additional health benefits, such as anticancer, anti-aging, and protective effects against cardiovascular diseases, diabetes mellitus, obesity, and neurodegenerative disorders [47;48]. However, antioxidants are recognized as unstable compounds during storage, particularly when subjected to temperatures over 35°C, ultraviolet light, and oxygen exposure. Therefore, the utilization of cool, dark, and oxygen-restricted storage, preferably with encapsulation protective packaging, is crucial for optimizing stability and shelf life [49;50].

# 3.6. Inulin and Vitamin E Content

The synbiotic drink consisting of a 50:50 ratio of jicama tubers extract (Jc) to sunflower

seed extract (Sf), fermented for 20 h, was determined as the most optimal treatment, subsequently evaluated for inulin and vitamin E concentrations by using spectroscopic analysis. Fig 6 indicates that the inulin content derived from the analysis of the raw extract and the synbiotic drinks after fermentation is 1.73% and 1.03%, respectively. The reduction in inulin content after fermentation is due to its enzymatic hydrolysis by Lactobacillus and Bifidobacterium species, which utilize inulin as a primary carbon source for their proliferation [1;21]. This degradation process is also closely associated with the increase in LAB observed, as illustrated in Fig 1. Moreover, as noted in [34], a high-quality synbiotic product is considered to contain 1-3% inulin regarding its physiological characteristics. Thus, the combination of jicama tubers extract and sunflower seeds extract in a 50:50 ratio, fermented for 20 h, meets the standards for a synbiotic beverage.

Moreover, the vitamin E concentration derived from the analysis of the raw extract and the synbiotic drink after fermentation is 0.98% and 0.58%, respectively. The reduction of vitamin E content following fermentation may be attributed to the process applied to the production of the synbiotic beverage. According to [36], vitamin E (Alpha-tocopherol) exhibits instability in the presence of oxidation and elevated temperatures. Thus, it tends to diminish after processing, storage, and utilization in specific food products. Nevertheless, vitamin E exhibits a degree of resistance to acid, allowing it to endure under acidic conditions (low pH).

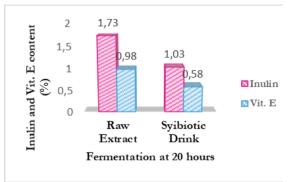


Fig. 6. Inulin and Vitamin E content in symbiotic drink.

## 4. Conclusions

The study revealed that a 50:50 mixture of jicama tubers and sunflower seeds extract yielded

the optimal synbiotic drink, exhibiting a total LAB of 11.34 log CFU/mL, lactic acid concentration of 1.29%, N-amino content of 4.67%, viscosity of 37.08 cP, antioxidant activity of 33.55%, inulin content of 1.03%, and vitamin E levels of 0.58%. The results indicate a well-balanced product characterized by probiotic viability, prebiotic substrate, physical properties, protein content, and antioxidant effectiveness. This plant-based functional beverage may serve as a lactose-free, healthy for the gut, antioxidant-rich substitute for probiotic drinks. The inulin in jicama tubers and the bioactive compounds in sunflower seeds offer nutritional and physiological advantages, enhancing their commercial value in the functional food industry. Nevertheless, the stability and viability of probiotic cultures during storage have been evaluated specifically under controlled laboratory conditions, which may not accurately reflect the issues associated with industrial-scale production, shipping, and shelf life. Subsequent research should focus on optimizing fermentation conditions, microencapsulation methods, and storage stability, in addition to conducting in vivo evaluations and consumer acceptance assessments to support the functional efficacy and enhance the commercialization prospects of the product.

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