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Research Article

Development of plant-based meat analogue

Siddhesh Mishal¹; Shraddha Kanchan¹; Pravin R. Bhushette,¹✉ Sachin K Sonawane¹✉

¹Food Science and Technology, School of Biotechnology and Bioinformatics, D. Y. Patil Deemed to be University, Level 5, Plot No. 50, CBD Belapur, 400614, Navi, Mumbai, India

Abstract

Plant-based meat analogue is a mimicked product that looks like animal meat along with nutritional qualities, beneficial health effects, and sensory characteristics. The main objective of this study was to formulate a meat analogue with similar nutritional and sensory characteristics as that of animal meat, which can potentially help with the shortcoming of meat to fulfil the demand. The process of one factor optimization optimized each selected ingredient of the meat analogue. Final base ingredients for meat analogue were selected, and the developed product was evaluated sensory using a nine-point hedonic scale. Desired treatment was selected based on the maximum index score obtained from nine points hedonic scale for ingredient optimization of jackfruit (26%), soy protein (26%), meat flavor (3%), and wheat gluten (4.3%). Physicochemical analysis of optimized plant-based-meat analogue was also conducted. The moisture content of the meat analogue was about 48.73%, the fat content - 2.46%, pH of raw product - 6%, cooking yield of product - 90.25%, diameter reduction of analogue - 3.86% and thickness reduction - 11.83%.

Keywords: Plant-based meat analogue, jackfruit, soya protein

Abbreviations: OA – overall acceptability; PBMA - plant-based meat analogue; WAC – water absorbed content

✉Corresponding author: Pravin R. Bhushette, Sachin K Sonawane, Food Science and Technology Department, School of Biotechnology and Bioinformatics, D. Y. Patil University, Level 5, Plot No. 50, CBD Belapur, 400614, Navi, Mumbai, India, E-mail: pravinbhushette@gmail.com; sac007s@gmail.com

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Introduction

Recently, there have been increased studies of plant-based meat analogue as an alternative protein source to substitute the traditional animal-based food in various parts of the world. This may be due to the rising world population and limited natural resources, causing animal meat production to be an unsustainable practice (Dekkers et al. 2018a). According to consumer studies, consumers tend to choose meat alternative which accurately mimics the desired sensory attributes of conventional meat (Hoek 2010). Consumers interested in meat alternatives are people who are concerned about their well-being and health, as well as those who are aware of the environmental, social, and ethical ramifications of eating animal meat (Apostolidis and McLeay 2016).

The actual meat analog content is a protein that accounts for 50-95% of dry matter. The structural organization of plant-based meat analogue also depends on properties of proteins like gelation, solubilizing capabilities, and the ability to retain liquid (Dekkers et al. 2018a). Moreover, different types of proteins can impact the final product's appearance, nutrition, flavors, and health effects. Hence, one of the critical aspects of developing a plant-based meat analog is selecting a suitable protein source. Although many plant-based proteins are used for meat analogue production, soy protein, peas, and wheat gluten are commonly used for meat analog production. Due to their low-cost reasons and possession of the ability to give the desired texture and other characteristics as animal meat (Kyriakopoulou et al. 2018). It is listed that blending 30% wheat gluten mixed with soy protein produced a meat analogue with physical properties like animal meat (Chiang et al. 2019).

Mushroom is another option used in meat analogue manufacturing as it is rich in sulfur-containing amino acids that help to attain meat flavor (Kumar et al. 2017). Further, the physiological and nutritional quality of the meat can be enhanced by exploring new protein sources and replacing it partially or completely with the traditional alternatives. One such suitable ingredient for this purpose is jackfruit. Jackfruits are similar to meat in terms of texture and characteristics and are preferred by consumers looking for vegan products (School 2017).

Therefore, this study aimed to develop an optimal composite of plant-based protein and other ingredients to produce a plant-based meat analogue with desired meat-like properties. The developed meat analogue's physicochemical qualities, such as hydration time and sensory characteristics, were investigated. Base ingredients concentration was also optimized for the development of meat analogue. The results of this study could minimize the production load from the meat industry by increasing the uses of underutilized agricultural sources such as jackfruit to produce healthy meat analogue.

Materials and Methods

Materials

Fresh jackfruit was purchased from the local market vendor of Diva, Thane. Texturized soy (Nutrela soy) was purchased Locally. While ingredients like shiitake mushroom, nutritional yeast extract, chickpea protein, wheat gluten, maltodextrin, guar gum, carrageenan, citric acids were purchased from Urban platter and Blue-bird. Food-grade Ascorbic acid was purchased from SRL chemicals. Vegetarian meat-based flavor mixture was obtained from Vista Processed Foods Pvt Ltd company Talaja, district: Panvel. Others ingredients like salt, oil, and starch were also purchased from the local market.

Pre-treatment

The jackfruit was first washed under running tap water to remove unwanted residues present on the surface. The small pieces of jackfruit were obtained by traditional method, i.e., cutting by using a knife. Before cutting, knife and hands were oil to cut jackfruit and remove the latexes and separate the pulp from the core. Washed the pulp under running tap water kept the jackfruit pulps in 0.02 mg of KMS solution to avoid the browning reaction (Galvez et al. 2013). After removing pulps from KMS solution, pulp was boiled in water at atmospheric pressure for 35 minutes to remove latex. The excess amount of water from the boiled pulp was drained to avoid the pulps turning mushy and partially kept in a cold-water container to cool the pulps. The pulps were spread on cotton cloths to drain the traces of water. Stored the partially dried pulps in a closed container and kept in the freezer till the following process.

Hydration rate and time for texturized soy and mushroom

The hydration time and rate are very important to determine the quality of texturized soy protein and mushroom. Ten grams of soy protein or shiitake mushroom was soaked in 50 ml of water at room temperature. During hydration, the weight for both ingredients was constantly observed every 2 minutes to achieve full hydration, which provides soft textured soy protein and shiitake mushroom. The time was recorded, and the hydrated sample was weighed to calculate the hydration rate using a similar method (Roberts et al. 2010).

Hydration rate was calculated as:

$$\text{Hydration rate} = \frac{\text{final weight} - \text{initial weight}}{\text{hydration time}} \quad (1)$$

Plant-based meat analogue preparation

The development of meat analogue was carried out by sensory evaluation, where various trials were carried out for ingredients concentration optimization in analogue product formation. Initial meat analogue was prepared with composition as shown in Table 1. In this study meat patties and nuggets formation process was used for meat analogue formation with modification (Ahirwar et al. 2015; Kurni et al. 2019; Rosli et al. 2011). Texture soy protein was hydrated by soaking in water for 15 min and ground in a food processor for 20 s. Shiitake Mushrooms was also hydrated by soaking for 5-8 min and ground for 30 seconds to make a fine paste. Raw jackfruit fibers (pulp) were ground for 15 s in a food processor for fine paste. Ground texture soy protein was mixed in dough making food processor with jackfruit fiber and shiitake mushroom paste for 2 min. In the mixture, coconut oil was added and blended for 35 s. Chickpea protein flour, wheat gluten and refined flour were added to the mix and blended the mixture for 2 min. Salt, garlic powder, guar gum, carrageenan, yeast extract, flavor, natural color was added and mixed for 1 min. The mixture was filled in a cookie-cutter to provide a definite structure and shape. The initial weight of the raw mixture was weighed and the thickness and diameter (length and width) were also measured to maintain appropriate physical properties in the process. The raw plant-based meat analogue patties were kept in a freezer at 4°C until further process. Steamed the raw

product for 25 min without creating pressure and fried the product on the low gas flame for 4 min with oil.



Figure 1. Developed plant-based meat analogue

Table 1. Initial ingredient formulation of meat analogue

Ingredients	Amount (g/100g)
Ground jackfruit pulp	26
Ground texturized soy	26
Ground mushroom	17.14
Wheat gluten	4.3
Coconut fat	8.57
Gram flour	2.86
Refined flour	4.3
Garlic powder	0.03
Flavor mixture	3
Yeast extract	3.72
Salt	1.14
Guar- gum	0.86
Carrageenan	0.43
Colour	1.43
Ascorbic acid	0.29

Sensory evaluation

The sensory evaluation was conducted using a 9-point hedonic scale, and the panel comprised 10 semi- trained panels from the food science and technology department. The inclusion criteria for semi-trained panels were: 1) 18-30 years, 2) healthy, 3) did not smoke, 4) did not drink alcohol, and 5) did not have color blindness.

Proximate analysis

Proximate composition of developed plant-based meat analogue carried out by standard AOAC method (AOAC 1995).

Determination of moisture content

The moisture content of the meat analogue was estimated using the AOAC method. To determine the moisture content, 10 g of the whole sample was taken in the Petri plate/ dish, dried in a hot air oven at 110°C, then cooled in a desiccator and weighed. The process of heating and cooling was repeated until a constant weight was achieved. (MC = moisture content)

$$MC (\%) = \frac{\text{initial weight} - \text{final weight}}{\text{weight of sample}} \times 100 \quad (2)$$

Determination of fat content

Soxhlet extraction was used to determine the crude lipid/fat by petroleum ether on a Soxhlet extraction system. A total of 10 gram of the sample was put into pre- weighed thimbles and plugged with a wad of cotton. The material was extracted with 180 ml of petroleum ether for 3 hours without interruption by gentle heating in a Soxhlet apparatus. After the extraction, the solvent was evaporated. The extraction mixture was removed and placed in an oven for drying at 105°C, and weight was taken. Fat content was calculated as:

$$\text{Crude lipid or crude fat (\%)} = \frac{W_3 - W_1}{W_2} \times 100 \quad (3)$$

Where,

W₁ = container weight,

W₂ = sample weight and

W₃ = lipid and container weight

Determination of Ash content

Ash defines inorganic content as the total amount of mineral content within the food, which is left over after burning organic matter like fat and protein. 10 grams of sample was taken into the crucible of known weight, which was then dried and kept in a muffle furnace at 600°C for 5-6 h (Ojwang et al. 2018). Ash content was determined using the formula,

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100 \quad (4)$$

Physical characteristics of plant-based meat analogue

Determination of pH

The pH of plant-based meat analogue patties was determined (Troutt et al. 1992) using digital pH meter (Equiptronics, India) equipped with a combined glass electrode in the homogenized mixture. The pH meter was calibrated before measuring the sample. The readings were taken in triplicates and expressed as units of pH.

Determination of cooking yield

The cooking yield of plant-based meat analogue (PBMA) patties was determined by measuring the weight of patties for each treatment and calculating the difference in weight of patties before and after cooking (El-Magoli et al. 1996).

$$\text{Cooking yeild (\%)} = \frac{\text{Weight of cooked PBMA}}{\text{Weight of precooked PBMA}} \times 100 \quad (5)$$

Diameter and thickness reduction (%)

Change in plant-based meat analogue patties diameter was determined by using the following equation.

$$\text{Diameter reduction (\%)} = \frac{D_1 - D_2}{D_1} \times 100 \quad (6)$$

Where,

D₁ = Diameter of raw analogue patties

D₂ = Diameter of cooked analogue patties

$$\text{Thicknes reduction (\%)} = \frac{T_1 - T_2}{T_1} \times 100 \quad (7)$$

Where,

T₁ = Thickness of raw analogue patties

T₂ = Thickness of cooked analogue patties

Statistical analysis

The data collected on sensory evaluation was statically analyzed by using IBM SPSS version 20 statistics software. The analysis of variance (ANOVA) was used to analyze sensory evaluation results with the significant difference among the sample for an accepted level or percentage of jackfruit pulp, texturized soy, wheat gluten, and flavor mixture in product formation. Observed the significant difference, and the value was analyzed with post hoc test Ducan multiple range test. P < 0.05 shows the significant difference.

Results and Discussion

Hydration rate time for texturized soy and mushroom

Hydration properties include the amount of water absorbed (WAC) by texturized soy protein and shiitake mushroom. Oversoaking causes loss of prominent flavor, whereas under soaking leads to hard texture. The water absorption rate per minute for textured soy protein as measured in the current study was 0.437 for the hydration time of 15 min (Table 2). While the absorption rate per minute for shiitake mushroom was 3.055 for the hydration time of 8 min uneven water distribution between soy protein isolate and gluten had also been reported earlier. This can be due to differences in polymer-water affinity and cross-link density between two phases, but this uneven water distribution is essential for obtaining a meat-like structure (Dekkers et al. 2018b).

However, increasing or reducing the soaking time with warm water (above 50°C) led to a loss of umami flavor. Hydration temperature is the most important factor followed by hydration time. The hydration experiments at 50°C by other researchers did not show much final reduction even at longer hydration or soaking time (4 - 16 h). Maximum activity of semi-purified soybean phytase was shown at 60°C (Buckle 1986); however soaking at 60°C causes some of the protein to denature (Beleia et al. 1993).

WAC (Water absorption capacity) is an important parameter that indicates the product quality, which contributes to the texture of the product after hydration (Lin et al. 2002; Köhn et al. 2015; Ning and Villota 1994). It was reported that texturized protein has higher WAC than freeze-dried meats, which may be due to protein types, protein-water interactions, water-water interactions, or structural characteristics (Samard and Ryu 2019).

Table 2. Hydration rate and time for texturized soy and mushroom

Ingredients	Hydration time, min	Final weight, g	Hydration rate, g/min
Texturized soy protein	15±1	32.755	0.437
Shiitake mushroom	8±1.10	34.447	3.055

Values are indicated as mean ± standard deviation.

Optimization of base ingredients for plant-based meat analogue

The plant-based meat analogue was optimized using ingredients like jackfruit (unripe), chickpea flour, mushroom, yeast extract, refined flour, wheat gluten, oil, and other components (listed in Table 1). The control sample of meat analogue was prepared in the optimization by varying one ingredient and keeping other elements constant, which helps to understand the significant effect of the ingredient in the development of meat analogue.

Effect of jackfruit pulp (JF) on the sensory attributes of meat analogue

The plant-based meat analogue was made by varying jackfruit pulp concentration from 22- 28% and keeping other ingredients like chickpea flour, mushroom, yeast extract, refined flour, wheat gluten, oil constant. Incorporation of 22-28% unripe jackfruit pulp into meat analogue showed desirable changes in appearance, texture, mouthfeel, aftertaste, and overall acceptability (Table 3) by keeping other ingredients like chickpea flour, mushroom, yeast extract, refined flour, wheat gluten, oil, and other components constant. The meat patty made without jackfruit was used as a control to judge the quality of the meat patty. Although the jackfruit flavor was absent irrespective of the concentrations, the meaty flavor and texture were formed due to the addition of other contents. A significant ($p > 0.05$) difference was observed in all the samples for appearance, texture, flavor, mouthfeel, aftertaste, overall acceptability characteristics. The color of the patties was brownish-yellow to dark brown due to the Maillard reaction. Variation in batter also affected the formation of a fibrous structure in analogue. The sensory panel found the samples with 25% and 26% more acceptable. Increasing or reducing the concentration of unripe jackfruit pulp affected the textural characteristics such as chewiness, binding, juiciness, hardness, and softness.

Table 3. Sensory attributes of meat analogue formulated with different percentage of unripe jackfruit (JF)

JF (%)	Appearance	Texture	Flavor	Mouthfeel	Aftertaste	OA
Control	7.2±0.8 ^e	7.7± 0.21 ^d	7.6± 0.24 ^c	8.1±0.36 ^f	7.8±0.26 ^e	7.9±0.12 ^e
22%	5.4± 0.52 ^{bc}	4.7 ± 0.48 ^{ab}	5.5± 0.53 ^b	5.4± 0.47 ^{cd}	5.1± 0.2 ^{bc}	5.2± 0.35 ^{ab}
23%	5.8 ±0.2 ^{cd}	4.8 ± 0.42 ^{ab}	5.5± 0.53 ^b	5.6± 0.23 ^{bc}	5.2± 0.1 ^{bc}	5.1± 0.10 ^{ab}
24%	6.2 ±0.63 ^{de}	5.3± 0.51 ^b	6.0±0.36 ^b	5.7± 0.44 ^{de}	5.3±0.29 ^c	5.9± 0.25 ^b
25%	6.5 ±0.53 ^{de}	6.6± 0.48 ^c	5.7± 0.48 ^b	5.8± 0.33 ^{de}	5.8±0.42 ^d	6.0±0.15 ^{cd}
26%	6.7 ±0.48 ^e	7.0 ±0.0 ^c	6.0± 0.25 ^b	6.0±0.0 ^d	5.9±0.32 ^d	6.3±0.19 ^d
27%	5.5 ±0.12 ^{ab}	4.3 ± 0.48 ^a	4.3 ± 0.48 ^a	4.7±0.48 ^{ab}	4.6±0.52 ^b	4.5±0.37 ^a
28%	5.2 ±0.48 ^a	4.6 ± 0.52 ^{ab}	4.6 ± 0.52 ^a	4.5±0.49 ^a	4.1±0.32 ^a	4.1±0.32 ^{ab}

Values are indicated as mean ± standard deviation.

The tenderness of the meat was improved with the increase in concentration.

However, all other factors were affected at 27% and 28% of unripe jackfruit. Hence, the incorporation of 26% unripe jackfruit pulp was most desired for the production of meat analogue.

Effect of texturized soy on the sensory attributes of meat analogue

Texturized protein plays a critical role in developing texture, juiciness, and flavor in the

development of meat analogue. The meat analogue prepared with a constant ingredient like unripe jackfruit pulp, chickpea flour, yeast extract, refined flour, wheat gluten, oil, and other ingredients and varying the texturized soy concentration shows a significant influence on the quality of meat analogue (represented in Table 4). The sample prepared without texturized protein were received a lower score in texture, juiciness, and flavor.

Table 4. Sensory attributes of meat analogue formulated with different percentage of texturized soy protein

Texturized Soy concentration	Texture	Juiciness	Flavor	Mouthfeel	Aftertaste	OA
Control	5.2±0.8 ^e	4.1± 0.21 ^d	4.6± 0.24 ^c	4.1±0.36 ^f	4.8±0.26 ^c	4.9±0.12 ^e
20%	5.61±0.31 ^a	4.55±0.53 ^a	5.0±0.2 ^a	5.0±0.5 ^a	4.83±0.22 ^a	4.79±0.87 ^a
22%	5.34±0.26 ^b	5.02±0.63 ^b	5.1±0.1 ^a	5.0±0.12 ^a	5.0±0.35 ^b	5.06±0.5 ^b
24%	6.37±0.26 ^c	6.32±0.19 ^c	5.36±0.16 ^b	5.43±0.13 ^b	5.51±0.99 ^c	5.77±0.82 ^c
26%	7.0±0.53 ^d	6.94±0.96 ^d	7.0±0.18 ^c	6.0±0.32 ^c	6.0±0.49 ^d	6.47±0.48 ^d
28%	7.2±0.4 ^d	7.1±0.75 ^d	6.0±0.25 ^c	6.0±0.26 ^c	6.0±0.28 ^d	6.5±0.5 ^d

Values are indicated as mean ± standard deviation.

Samples with 26% and 28% texturized soy protein caused improved texture and juiciness, but it also showed some effects on flavor, mouthfeel, and taste (Table 4). Increasing the concentration resulted in the masking of umami flavor in meat analogues. Although lower concentration doesn't affect the flavor, it affects the chewiness of the analogue. An equal ratio of texturized soy protein and jackfruit pulp led to a

homogenous mixture, providing better stability and shape retention of the batter. Therefore, 26% of texturized soy protein was accepted for analogue development.

Effect of flavor on the sensory attributes of meat analogue

The presence of simple sugar and sulfur-containing amino acids, which leads to the

entrapment of components in the precursor in water and lipid as they are soluble, does not affect the appearance and texture properties of the meat analogue. Still, it does improve flavor and aroma generation by Maillard reaction, which leads to the entrapment of components in the precursor in water and lipid as they are soluble. The change is partly related to the flavor mixture concentration of some substances, such as spices and yeast extract, which had a strong sulfur flavor before. The processing is projected to be partially similar

to meat because of free amino acids and carbohydrates in the emerging analogue, which helps increase the meaty flavor. In this study, the flavor concentration was varied by keeping other ingredients unripe jackfruit pulp, texturized soy protein, mushroom, chickpea flour, yeast extract, refined flour, wheat gluten, oil constant. The difference in flavor concentration did not affect the appearance and textural properties, but it improved aroma generation (Table 5).

Table 5. Sensory attributes of meat analogue formulated with different percentage of flavor concentration

Flavor (%)	Flavor	Aroma	Mouthfeel	Aftertaste	OA
Control	5.0±0.1 ^a	4.4±0.18 ^a	5.1±0.2 ^a	5.3±0.21 ^a	5.5±0.69 ^a
2%	5.4±0.11 ^b	5.8±0.23 ^b	5.9±0.35 ^c	6.1±0.2 ^c	6.2±0.11 ^b
3%	5.9±0.13 ^c	6.0±0.1 ^b	6.0±0.12 ^d	6.1±0.2 ^c	6.2±0.08 ^b
4%	6.4±0.18 ^d	6.4±0.13 ^c	5.6±0.20 ^b	5.6±0.12 ^b	6.2±0.14 ^b

Values are indicated as mean ± standard deviation.

Effect of wheat gluten on the sensory attributes of meat analogue

The meat analogue made with unripe jackfruit pulp, texturized soy protein, mushroom, chickpea

flour, yeast extract, refined flour, oil, and other ingredients are kept constant by varying the wheat gluten concentration. The control sample was made without wheat gluten.

Table 6. Sensory attributes of meat analogue formulated with different percentage of wheat gluten.

Wheat gluten (%)	Texture	Mouthfeel	Aftertaste	OA
Control	7.0 ± 0.2 ^a	6.1 ± 0.3 ^a	6.52 ± 0.04 ^b	6.52 ± 0.04
1.43%	7.0 ± 0.2 ^a	6.1 ± 0.3 ^a	6.52 ± 0.04 ^b	6.48 ± 0.04 ^a
2.86%	7.15± 0.15 ^b	6.3 ± 0.09 ^b	6.52 ± 0.04 ^b	6.6 ± 0.12 ^b
4.3%	7.39± 0.73 ^c	6.4 ± 0.18 ^c	6.27 ± 0.07 ^a	7.0 ± 0.12 ^c
5.71%	7.39±0.73 ^c	6.4± 0.18 ^c	6.27 ± 0.07 ^a	7.0 ± 0.12 ^c

Values are indicated as mean ± standard deviation.

However, increasing the amount of wheat gluten incorporation from 1.43% to 2.86%, 4.3%, and 5.71% results in some positive texture, mouthfeel, aftertaste, and overall acceptability modifications (OA). The progressive increase in gluten in the product disguised the noticeable odor of mushroom and soy protein. When blended with gluten had a higher concentration acceptance ratio of change, resulting in both pleasant and unpleasant flavors.

Physicochemical characteristics of plant-based meat analogue

The plant-based meat analogue, made with an optimized concentration of 26% of jackfruit pulp, 26% of texturized soy, 17% of mushrooms, gluten-4.3%, coconut fat, -8.57%, gram flour, 2.86%, refined flour- 4.3%, was observed to have the physicochemical characteristics shown in Table 7.

Table 7. Physicochemical characteristics of prepared plant-based meat analogue

Parameters	%
pH	6.0 ± 0.20
Cooking yield	90.25 ± 1.14
Diameter reduction	3.26 ± 0.05
Thickness reduction	11.83 ± 0.76
Moisture content	48.73 ± 2.22
Fat content	2.46 ± 0.54
Protein content	22.32 ± 1.23

Values are indicated as mean ± standard deviation.

In the current study, the pH, fat content, and cooking yield of plant-based meat analogue measured 6%, 2.46%, and 90.25%, respectively. Moisture content is an important parameter because it determines the quality attributes like physical characteristics, nutritional composition, and shelf life of the developed product. The moisture content was observed to be 48.73%, and a similar value was obtained in another study by (Ranjan Kumar 2012) in optimizing gluten levels.

Conclusions

Meat analogue was prepared by optimization of the ingredients and their acceptance. The incorporation of jackfruit pulp (26%), texturized soy protein (26%), vegetarian meat-based flavor (3%), and other ingredients improved the textural properties. The addition of (4.3%) wheat gluten produced fibrous texture in meat analogue. To accept the developed plant-based meat patty, sensory (organoleptic) evaluation with white meat (chicken) and lamb meat patty is needed. Because the vegetable protein lacks specific characteristics like to complete amino acid profile. Nutritional evaluation of the developed meat showed that it could be a good source of protein that can help replace meat in the future. There is a significant difference in the processing and production procedures used to create new products. The addition of components and their concentration must be regulated by food legislation. Due to differences in nutritional quality and sensory qualities, plant-based meat analogues cannot strongly impact retail markets.

More modification is needed to develop plant-based meat analogue products to improve the product.

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