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Research Article

Efficacy of microwave against alicyclobacillus acidoterrestris in apple Juice

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Abstract: Apple juice inoculated with alicyclobacillus acidoterrestris was treated with microwave at different power and time, and the efficacy of microwave against alicyclobacillus acidoterrestris was investigated. The results showed that alicyclobacillus acidoterrestris quantity decreased with microwave enhancement or time extension. When juice was treated with microwave at higher than 720 W and for longer than 40 seconds, the alicyclobacillus acidoterrestris of apple juice might effectively be prevented. Microwave treatment changed microbial morphology and destroyed cell membrane permeability, and this was one probable reason that led alicyclobacillus acidoterrestris to death..
Keywords: microwave, alicyclobacillus acidoterrestris, apple juice

INTRODUCTION

Apple juice concentrated is one of main agricultural products in world trade, mostly produced by China, America, Poland and Argentina. In recent years, its yield has steadily been increasing. In 2012, China produced 660,000 tons of apple juice concentrated, which accounted for about 60% of the world production [1]. The contamination of alicyclobacillus acidoterrestris is a difficult problem in apple juice processing. Alicyclobacillus acidoterrestris is rod-shaped and gram - positive, forming spore in acid environment. It is heat-resistant, grows well in the wide range of pH 2.5~ 6, and cannot be killed in usual pasteurization[2]. At present, people think that the alicyclobacillus acidoterrestris of apple juice mainly originates from orchard soil. Hippchen isolated bacillus from non acid and heat soil, and the acquired bacillus was remarkably similar to the bacillus isolated from apple juice[3]. In 1984, Cerny also isolated bacillus from rancidity apple juice, considering that bacillus mainly caused the rancidity of apple juice [4].

Apple juice was contaminated during processing owing to the remnant of alicyclobacillus acidoterrestris in raw material and production line. In fruit juice, alicyclobacillus acidoterrestris produces guaiacol and halide phenol such as 2, 6-dibromophenol and 2, 6-dichlorophenoxyacetate, which give off an unpleasant smell similar to bad breath odor. Thus, the commodity value of apple juice is discounted and manufacturers are subject to huge economic loss [5]. At present, some measures including ozone, ultraviolet, and supercritical sterilization were adopted to prevent alicyclobacillus acidoterrestris during apple juice processing[6-8]. However, these applied measures are costly or not efficient on the whole.

Microwave is an electromagnetic wave with the wavelength ranging from one metre to one millimetre. Its sterilization has thermal effect and irradiation effect. Microwave may induce the high-speed rotating friction of polar molecule inside material, and much heat is generated. Accordingly, microorganism is sterilized by thermal effect. In addition, Microwave can penetrate into the inner of microorganisms, damaging to cell membrane, intracellular enzyme or nucleic acid. Thus, microorganism was led to cell death through irradiation effect [9]. In recent years, microwave sterilization has been paid attention to, and is experimentally applied in oil palm, beef, mashed potato, and so on[10-12].

In the experiment, the apple juice inoculated with alicyclobacillus acidoterrestris was treated with microwave and the efficacy of microwave against alicyclobacillus acidoterrestris was investigated. Meanwhile, the influences of microwave on microbial morphology and cell membrane permeability were also researched. Currently, there were few reports about controlling the alicyclobacillus acidoterrestris of apple juice with microwave. Our experimental results might serve as a reference for fruit processing to prevent alicyclobacillus acidoterrestris.

MATERIALS AND METHODS

Strains, chemicals and equipments

Soil was collected from an apple orchard located in Shangyang Village, Dayang Town, Yaodu District, Linfen city, Shanxi Province of China in 2013 October on a fine afternoon. The surface soil under apple trees was dig and sealed with black plastic bag. And then it was quickly carried to food microbiology laboratory and stored in 4 ^{0C}.

Standard alicyclobacillus acidoterrestris ATCC49025 was purchased from Microbial Culture Collection Center of Guangdong Institute of Microbiology, China.

Peptone, glucose, yeast extract, tween 80, and agar were purchased from Aoboxing Bio-techCo. Ltd. (Beijing, China) and malic acid were purchased from Jingke Chemical Co., Ltd. (Wuxi, China). Chemicals used were all of biochemical grade.

WD900Y1SL23-2 Microwave oven, Shunde Galanz Electric Appliance Co., Ltd., China; WYT-II Refractometer, Qingyang Optical Instrument Co., Ltd., Chendu, China; DS–1 Juicer, Shanghai Jingke Instrument Company, China; pHS-3C pH-meter and DDS-307 conductivity meter, Shanghai REX Instrument Factory, China; SHA-C Water-bathing Constant Temperature Vibrator, Jintan Ronghua Instrument Manufacture CO., LTD, China; MC50 Olympus Biological microscope, Guangzhou Ming-Mei Technology Co.，Ltd, China; WGZ-4000AP Turbidimeter, Shanghai Xinrui Instruments Co., Ltd, China; LS-50LD Pressure steam sterilizer, Jiangyin Binjiang Medical Instruments Co., Ltd, China; 5804R Refrigerated centrifuge, Gene Co., Ltd., Germany.

Isolation and identification of alicyclobacillus acidoterrestris

Preparation of K medium

K medium was composed of 0.5% of peptone, 0.1% of glucose, 0.25% of yeast extract, 0.1% of tween 80 and 2.0% of agar. Its pH was adjusted to 3.7 with malic acid. Afterward, the medium was sterilized at 121 ^{0C} for 20 min using pressure steam sterilizer [13].

Isolation of alicyclobacillus acidoterrestris from orchard soil

Cleaned apples were squeezed to acquire juice. The apple juice was filtered with 160 mesh strainer, and then boiled for one minute. Afterward, it was filtered with 0.45μm membrane and diluted to 11.5 brix using de-ionized water. The prepared juice was reserved for next experiment.

50 g of orchard soil was added to 500 ml of de-ionized water; the mixture was shaken up and filtered with 160 mesh strainer. 10 ml of water prepared with soil was added to 500 ml of 11.5 brix juice, and the mixed juice sample was heated at 80 ^{0C} for 15 min. After that, the mixed juice sample was incubated in oscillating shaker at 41^{0C} for 8 hours. 1 ml of incubated juice was incubated in plate and cultured with K medium for 96 h at 41 ^{0C}. Thus, the isolated bacteria were acquired [3].

Identification of alicyclobacillus acidoterrestris

Standard alicyclobacillus acidoterrestris and separated bacteria were respectively coated in plates. And they were cultured at 41 ^{0C} for 96h. Afterward, the isolated bacteria were identified and compared with standard alicyclobacillus acidoterrestris through microbial morphology and gram stain.

Treating apple juice with microwave

In each mixed sample, 80 ml of 11.5 brix apple juice was inoculated with 3 ml of isolated alicyclobacillus acidoterrestris with 6×10^7 /ml bacterium density. The mixture was shaken up. And then it was treated with different microwave power for 40 seconds, or treated with 900 W microwave at different time, respectively. Afterward, the mixture was incubated in oscillating shaker at 41^{0C} for 8 hours.

Determination of microbial quantity

The incubated microbial quantity of apple juice was indicated using turbidity[14]. After alicyclobacillus acidoterrestris was incubated in apple juice for some time, the turbidity of the juice increased with microbial quantity enhancement. The mixture turbidity was directly assayed using turbidimeter.

Observation of microbial morphology

80 ml of 11.5 brix apple juice was inoculated with 3 ml of isolated alicyclobacillus acidoterrestris with 6×10^7 /ml bacterium density. The mixture was shaken up and incubated in oscillating shaker at 41^{0C} for 8 hours. And then it was treated with microwave at 900 W for 40 seconds. After gram staining, microbial morphology was observed through microscopic examination

Determination of alicyclobacillus acidoterrestris membrane permeability

The permeability of alicyclobacillus acidoterrestris membrane is expressed in the relative electric conductivity and determined according to the method described by Diao et al. [15]. After the juices containing alicyclobacillus acidoterrestris were incubated at 41 ^{0C} for 8 h, they were treated with different power microwave for 40 s, alicyclobacillus acidoterrestris were separated by centrifugation at 4500 rpm for 15min. After centrifugation, the supernatant was discarded and 15 ml of de-ionized water was added to the centrifuge tube. The sediment of centrifuge tube was stirred gently with glass rod to form suspension. 30 minutes later, the suspension conductivity was determined as L_1 at 25 ^{0C} using conductivity meter. Subsequently the suspension was boiled for 15 min, cooled to 25 ^{0C}, and evaporating water was supplemented into the suspension. Now, its conductivity was determined as L_2. The permeability of alicyclobacillus acidoterrestris membrane was calculated according to the formula, the relative electric conductivity (%) = $L_1/L_2 \times 100$.

Statistical analysis

Each treatment was repeated 3 times. Data analysis was executed using DPS7.05, and Tukey 's method was used for multiple comparisons. $P<0.05$ served as the criterion of significant difference. The value was expressed with mean±standard deviation.

RESULT AND ANALYSIS

Isolation and identification of alicyclobacillus acidoterrestris

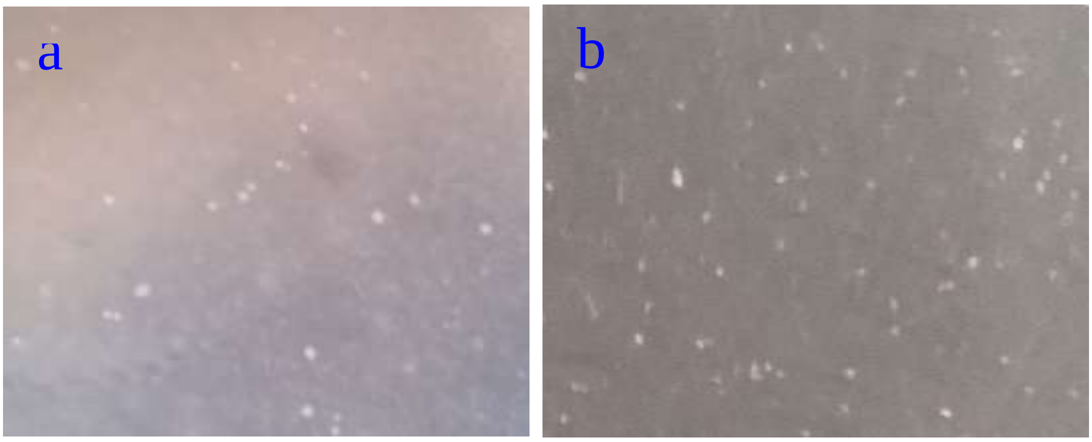

Fig-1: Bacterial colony of (a) standard and (b) Isolated bacterium

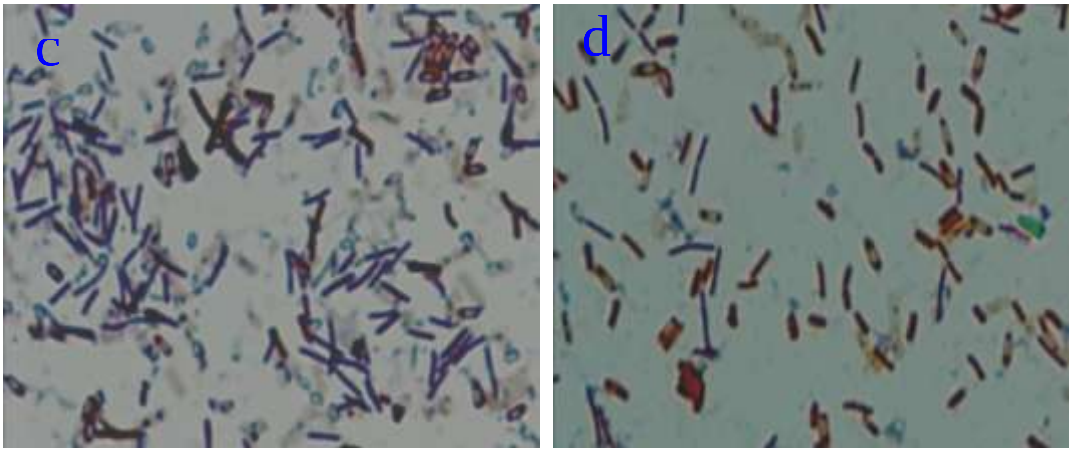

Fig- 2: Gram staining of (c) standard and (d) isolated bacterium

As shown in Figure 1, the bacterial colony of two strains were round and full, milky white and translucent. According to their similar bacterial colony, we preliminarily judged that they were probably the same strain species. Figure 2 showed the results of Gram staining. Two bacteria were all rod-shaped and gram positive, and have oval spores at bacterial secondary end. They were with 0.35~1.1 μm width and 2~6.3 μm length. This characteristic was in accordance with the description of Hippchen who isolated from soil[3]. To sum up, based on the same culture condition, resembling bacterial colony and cell character, and similar descriptions of previous literature, we might judge that the bacterium isolated from orchard soil was the target strain of alicyclobacillus acidoterrestris.

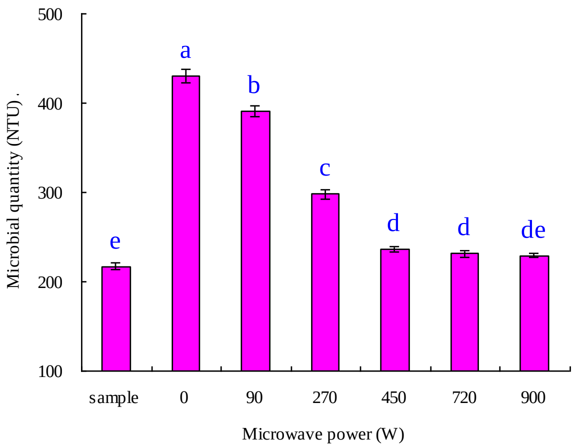

Fig- 3: Effect of microwave power on the microbial quantity of apple juice

Effect of microwave power on the microbial quantity of apple juice

As described in Figure 3, after the apple juice containing alicyclobacillus acidoterrestris was treated with microwave, microbial quantity decreased with microwave power enhancement. The turbidity of untreated juice is 2 times of initial juice through incubation for 8 hours. This suggested that alicyclobacillus acidoterrestris rapidly propagated during incubation processing. While the juice was treated with 90W or 270W microwave, the turbidity decreased compared to untreated juice, but it was still higher than that of initial juice. After the juice was treated with 450, 720 and 900 W microwave, alicyclobacillus acidoterrestris was effectively controlled, and there were no significant differences of microbial quantity among the three levels of microwave power (P>0.05). Especially when the juice was treated with 900 W microwave, alicyclobacillus acidoterrestris was almost no proliferation compared to initial juice (P>0.05).

Effect of microwave treating time on the microbial quantity of apple juice

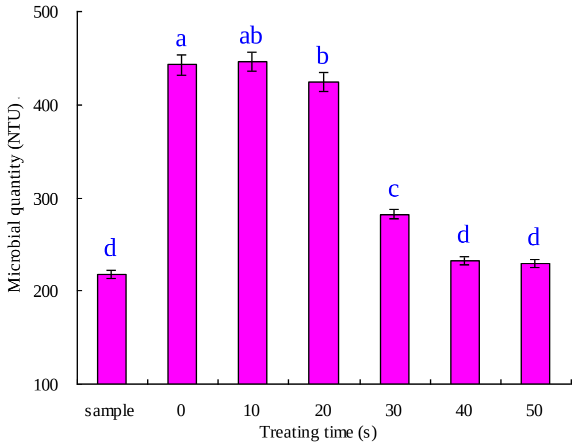

Fig-4: Effect of microwave treating time on the microbial quantity of apple juice

As described in Figure 4, after the juice was treated with 900 W microwave, microbial quantity decreased with treating time extension. There were few effects to sterilize alicyclobacillus acidoterrestris when the juice was treated with microwave for 10 or 20 seconds. Through incubation for 8 h, the microbial quantity of treated juice for 20 seconds was only 4.2% lower than that of untreated juice. Fortunately, when treating time was beyond 40 seconds, alicyclobacillus acidoterrestris was almost completely killed (P>0.05). Therefore, through longer time to treat juice with microwave, the alicyclobacillus acidoterrestris of apple juice might effectively prevented.

Influence of microwave treating apple juice on microbial morphology

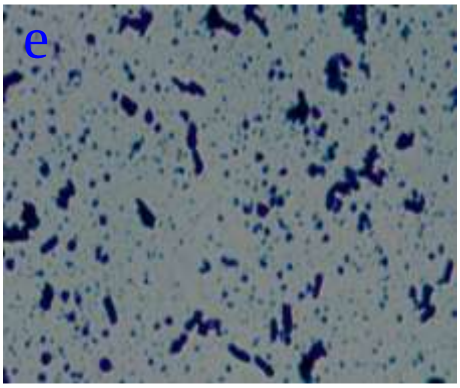

Fig-5: Gram staining of (e) treated isolated bacteria with microwave

Figure 5 demonstrated the change of microbial morphology after alicyclobacillus acidoterrestris was treated with 900 W microwave for 40 s. The cell edge blurred and untidied, and the shape of some cells became irregular compared to untreated isolated bacteria (Figure 2d). So microwave treatment partly changed the microbial morphology, and this probably influenced the normal physiological metabolism of alicyclobacillus acidoterrestris.

Influence of microwave treating apple juice on membrane permeability

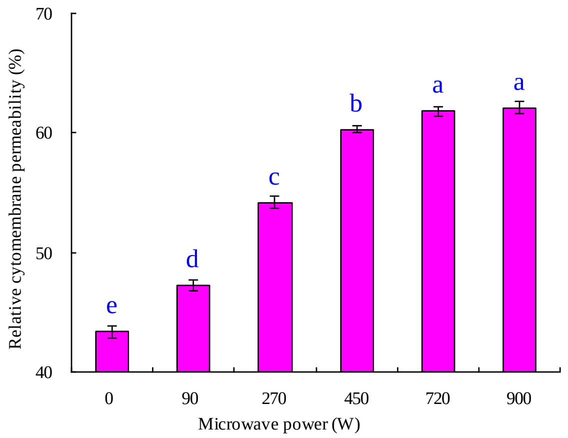

Fig-6: Influence of microwave treatment on microbial membrane permeability

Membrane permeability represents the integrity degree of cell membrane. After cells subject to injury such as heat and radiation, the osmotic adjustment of cell ability membrane is reduced. And membrane permeability increases accordingly[16]. As shown in Figure 6, the membrane permeability of alicyclobacillus acidoterrestris increased with microwave power enlargement. When microwave power ascended to 720W, the membrane permeability reached a maximum. Afterward, the membrane permeability did not increased with microwave power enhancement. Therefore, treating juice with higher than 720W microwave might effectively control alicyclobacillus acidoterrestris.

DISCUSSION

After the alicyclobacillus acidoterrestris of apple juice was treated with microwave, its cell edge blurred and cell shape became irregular. Thus, the normal physiological metabolism of alicyclobacillus acidoterrestris might be affected. Moreover, microwave could destroy cell membrane. Once alicyclobacillus acidoterrestris lost the integrated permeability, its metabolism disordered and was led to death [17]. In this experiment, treating apple juice with higher than 720 W microwave increased the cell membrane permeability, and this greatly damaged the normal metabolism of alicyclobacillus acidoterrestris. Therefore, the microbial quantity of apple juice was almost no increase when the apple juice was treated with microwave beyond 720 W and 40 seconds.

Microwave treating apple juice to control alicyclobacillus acidoterrestris showed intensity and time effects. In shorter time and lower power, the germicidal efficacy of microwave was very limited owing to the weak destruction to alicyclobacillus acidoterrestris. However, in higher microwave power and longer time, much more energy was accumulated in microorganism, or alicyclobacillus acidoterrestris was strongly irradiated. Thus, alicyclobacillus acidoterrestris was seriously damaged [18]. In the experiment, microwave treatment with 900 W for 40 seconds might cause microbial morphology irregular and destroy the membrane permeability, and this was probably one of the reasons that alicyclobacillus acidoterrestris was led to death.

CONCLUSION

Alicyclobacillus acidoterrestris quantity decreased with microwave enhancement or time extension. When juice was treated with microwave at higher than 720 W and for longer than 40 seconds, the alicyclobacillus acidoterrestris of apple juice might effectively prevented. Microwave treatment changed microbial morphology and destroyed cell membrane permeability, and this was one probable reason that led alicyclobacillus acidoterrestris to death.

Acknowledgments

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