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Study on the Application of Nanochitin for Salt Reduction in Vietnamese Pork Paste (Gio Lua)

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Abstract: Nanochitin is a preparation derived from chitin through a "fragmentation" process down to nanometer size. Compared to chitin, nanochitin exhibits several novel properties such as high solubility, the ability to carry an electric charge, and interaction with certain anions in NaCl solution. Owing to these characteristics, nanochitin has found expanded applications in various fields such as environmental science, medicine, and food technology. This paper presents the results of a study on the application of nanochitin for salt reduction in food products. The experimental research utilized nanochitin, produced by a hydrolysis-ultrasound combined method, with an average size of 228 nm. At a concentration of 80 µg/ml, nanochitin enhanced the salty taste of 0.65% NaCl solution to a level equivalent to that of 0.69% NaCl solution. In trials with Vietnamese pork paste (Gio lua), using nanochitin at 0.109 g/kg of meat enabled a reduction of 0.545 g of salt, corresponding to 5.8% of the total salt content, while still maintaining the same salty taste as the control sample. Other sensory attributes showed no detectable changes. These results suggest a potential application of nanochitin in reducing dietary salt intake, contributing to addressing the current issue of excessive salt consumption.

Keywords: *Nanochitin, Solubility, Ultrasound, Enzymatic Hydrolysis, Animal Safety Testing.*

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I. INTRODUCTION

Chitin is a polysaccharide abundantly found in nature, serving as the primary component of the shells of shrimp, crabs, and other crustaceans. Structurally, chitin exists in the form of fiber bundles, interlaced between amorphous and crystalline regions. Chitin binds with proteins and minerals to form flat layers called "chitin–protein planes", which then twist and stack to form a Bouligand structure [1, 2]. Due to this stable structure, chitin is insoluble in water and therefore has limited applications.

Nanochitin can be produced from chitin with nanometer-scale dimensions using various methods such as chemical, biological, physical, and combined approaches. With its nanometric size, nanochitin exhibits much higher solubility than chitin. It carries a positive charge and thus can interact with anions such as Cl^-. Therefore, in NaCl solution, the interaction of nanochitin with Cl^- increases the mobility of Na^+ ions, thereby enhancing the perception of saltiness. As a result, nanochitin can be used as a saltiness-enhancing agent [3–5].

In modern society, advancements in science and technology, along with increased productivity in agriculture and livestock, have led to an abundant food supply. Excessive consumption of food, along with habitual taste preferences, has caused a significant portion of the population to consume too much salt in their diets, leading to increased risks of cardiovascular diseases and hypertension.

This situation has been warned about in many countries around the world, such as the United States, Japan, Belgium, Brazil, Malaysia, etc. [4, 6–8]. In Vietnam, recent studies have also reported excessive salt intake. The average daily salt consumption of Vietnamese people is 9.4 g/person/day, nearly twice the recommended level by WHO [9, 10]. This situation is alarming, and solutions to reduce salt intake in the population's diet are urgently needed [10].

Due to its relation to taste and eating habits, salt reduction in the diet cannot be implemented abruptly according to recommended levels. In this context, a certain "substance" that can "deceive" the sense of taste, allowing people to consume less salt while still feeling satisfied with the saltiness, would be extremely meaningful in promoting dietary salt reduction strategies. In this regard, nanochitin is an excellent choice.

With the aim of using nanochitin to reduce salt in food, our study investigates and evaluates the salt-reducing potential of nanochitin in Vietnamese pork paste (Gio lua). These results will serve as a basis for applying salt reduction to other types of food, gradually addressing the issue of excessive salt consumption, thereby contributing to disease prevention and improving public health.

II. MATERIALS AND METHODS

A. *Materials*

The nanochitin used in this study is an experimental product developed under the project titled "Research on the preparation of nanochitin from shrimp shell chitin for application in salt reduction in food processing," conducted by the research team of Nguyen Thi Cha, Assoc. Prof. Dr. Ho Phu Ha, and Dr. Nguyen Tien Thanh from 2021 to 2026.

Accordingly, nanochitin was produced by a hydrolysis method combined with ultrasonication. Raw chitin was first cleaned and then hydrolyzed using an acid catalyst combined with enzymes. The hydrolysate was subsequently purified, the solvent removed, and the mixture subjected to ultrasonication. After ultrasonication, the product was collected by freeze-drying. The resulting nanochitin had an average particle size of 228 nm and a solubility of 78.4%.

The experimental ingredients for the Vietnamese pork paste (gio) product, including meat, salt, fish sauce, etc., were sourced from food ingredient suppliers that meet the standards of Vietnam's food regulatory authorities.

B. *Research Methods*

➢ *Zeta Potential Determination of Nanochitin*

Nanochitin was dissolved in distilled water at concentrations ranging from 50 to 150 µg/ml. The zeta potential of each sample was then measured. The measured zeta potential values were used as the basis for evaluating the potential of nanochitin to enhance saltiness.

➢ *Zeta Potential Determination of Nanochitin-NaCl Solution Mixtures*

To investigate the interaction between nanochitin and NaCl in solution, we measured the zeta potential of mixtures consisting of nanochitin solutions with high zeta potential, to which NaCl solution was added. The zeta potential values of the mixtures served as the basis for selecting the appropriate concentration of nanochitin to determine its threshold effect in enhancing saltiness.

➢ *Determination of the Threshold effect in Enhancing Saltiness*

A 0.65% NaCl solution was prepared. Nanochitin was added to the NaCl solution at concentrations determined from zeta potential measurements to create test samples. The 0.65% NaCl solution without nanochitin served as the control. Both test and control samples were evaluated for saltiness using a sensory ranking test. Based on the test results, the concentration of nanochitin that enhances the saltiness of the 0.65% NaCl solution was determined.

➢ *Determination of the Saltiness Enhancement level of Nanochitin*

NaCl solutions were prepared at concentrations of 0.61%, 0.65%, and 0.69%. Nanochitin at the concentration determined from the threshold effect experiment was added to the 0.65% and 0.61% NaCl solutions to create test samples. These samples were evaluated for saltiness using a sensory ranking test. The order of saltiness levels among the samples indicated the saltiness enhancement capability of nanochitin at the tested concentration.

➢ *Application of salt Reduction in Vietnamese pork paste (gio) Products*

The salt reduction experiment was carried out based on the production process of a manufacturing facility as follows: As shown in Figure 1, fresh lean pork was trimmed of tendons and cut into large pieces, typically mixed with approximately 15% pork fat. After cutting, the meat was coarsely ground and mixed with auxiliary ingredients and seasonings such as fish sauce, salt, etc., then finely ground. At the end of this phase, the ingredients and seasonings were evenly mixed, resulting in a smooth, fine, elastic meat paste known as "gio song" (raw pork paste). The paste was then portioned according to requirements and wrapped in banana leaves or PE bags to form cylindrical shapes with a diameter of about 12–13 cm. These were steamed at boiling temperatures for 50 to 60 minutes, then cooled to obtain the final Vietnamese pork paste (gio lua) product.

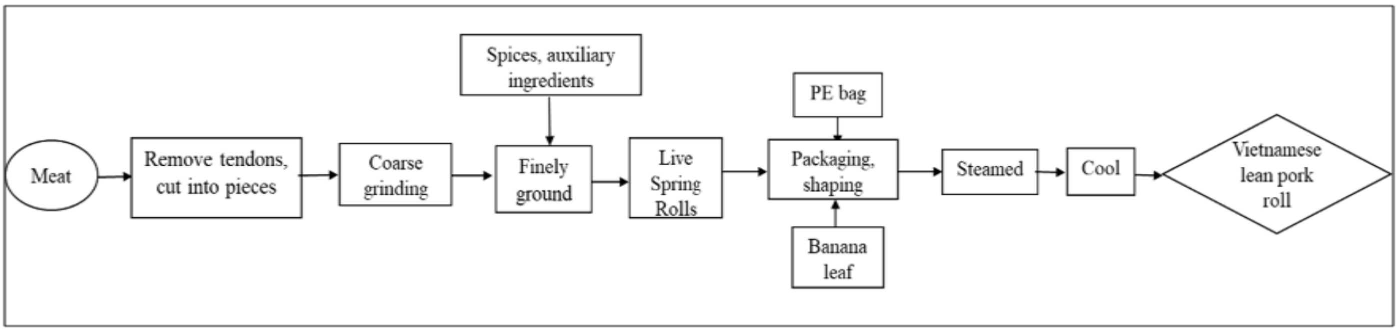

Fig 1 Flowchart of Vietnamese Pork Paste (Gio Lua) Production Process

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The experimental samples were partially reduced in NaCl content and supplemented with nanochitin. Both the experimental and control samples were evaluated for saltiness and other properties using the scoring method.

III. ANALYTICAL METHODS

A. Particle size Determination by laser Diffraction method:

Nanochitin samples were dispersed in water. A monochromatic laser beam from the instrument (model LA-950V2) was directed onto the sample. Based on the relationship between the intensity of the scattered light and the scattering angle, particle size values were obtained for all components in the sample [11].

B. Solubility Determination According to Naiu's method:

A specific amount of nanochitin was dispersed in distilled water. The mixture was centrifuged, and the supernatant was completely separated and then dried to constant weight. Solubility (%) was calculated as the ratio between the mass of solids in the dried supernatant and the initial sample mass [11].

C. Zeta Potential Determination using Zetasizer nano ZS90:

Nanochitin was dispersed in water. When an electric field was applied to the solution, charged particles moved in accordance with their charges. This particle movement caused a shift in the frequency of the scattered light. The instrument analyzed this shift and provided the zeta potential value [11].

D. Sensory Evaluation

The panel consisted of trained experts who regularly assessed products and were trained to evaluate the sensory characteristics of the samples.

E. Ranking test:

Panelists ranked the samples in ascending order of saltiness. In the case of four samples, the ascending order of saltiness was 1, 2, 3, and 4, with 1 being the least salty and 4 being the saltiest. In the case of six samples, the ranking order was 1 to 6, where 1 indicated the least salty and 6 the saltiest. The ranking results were converted into corresponding scores based on the method of Fisher and Yates (1942), followed by analysis of variance (ANOVA), comparison, and conclusion regarding the saltiness levels of the samples in the test series [12].

F. Scoring test:

Panelists were invited to taste and evaluate the intensity of sensory attributes of the product by assigning a score corresponding to a predefined descriptive term as shown in Tables 4 and 6. The results were analyzed using analysis of variance (ANOVA), followed by comparison and conclusion regarding the sensory attributes of the test samples [12].

G. Statistical method

All experiments were repeated three times. The experimental data were subjected to analysis of variance (ANOVA), using Fisher's and Student's t-tests for evaluation.

IV. RESULTS AND DISCUSSION

A. Zeta potential determination of nanochitin solution

Firstly, the study determined the zeta potential of nanochitin solution at different concentrations. The results are presented in Figure 2.

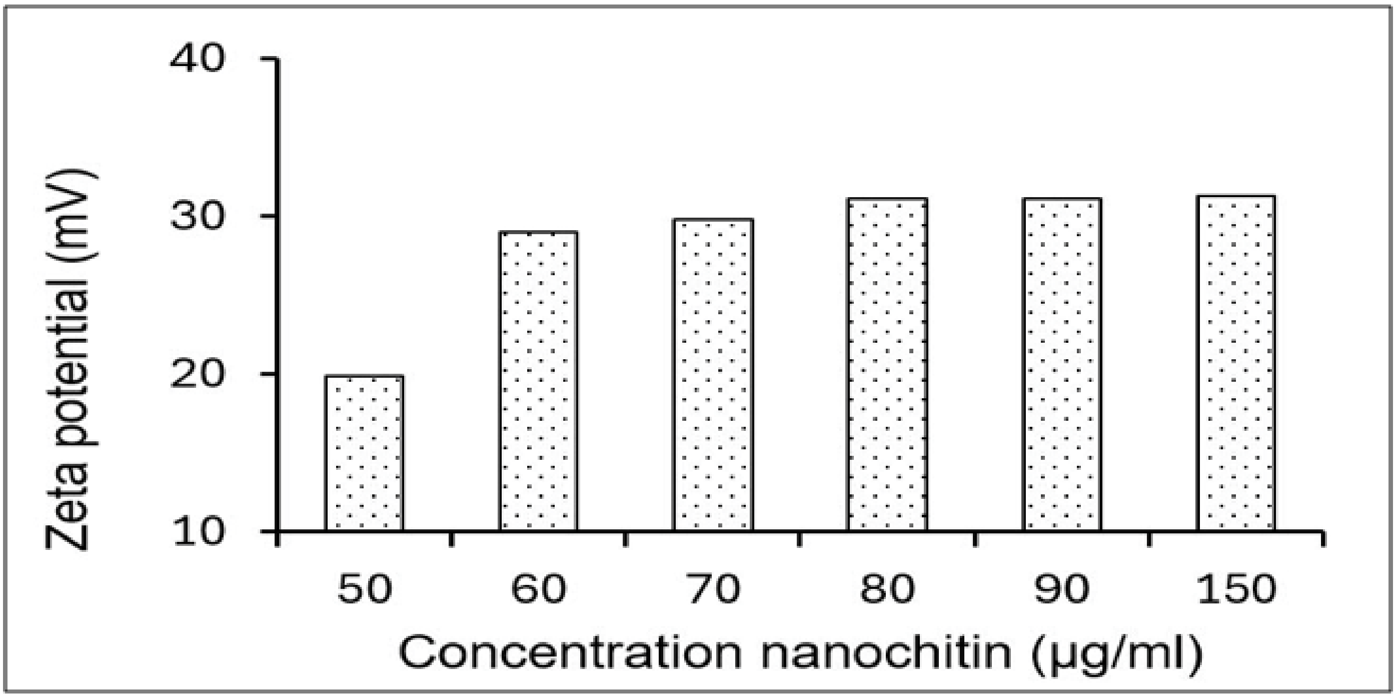

Fig 2 Zeta Potential of Nanochitin Solutions at Different Concentrations

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The results show that the zeta potential of the nanochitin solution increased significantly with concentrations from 50 to 70 µg/mL. From 70 to 150 µg/mL, the zeta potential increased more slowly and reached a high value. Based on this, the concentration range of 70 to 150 µg/mL was selected for further study on the changes in zeta potential when NaCl was added to the nanochitin solution.

B. Zeta potential determination of nanochitin–NaCl solution mixtures

NaCl solutions at various concentrations were added to the pure nanochitin solution, followed by zeta potential measurement. The results are shown in Figure 3.

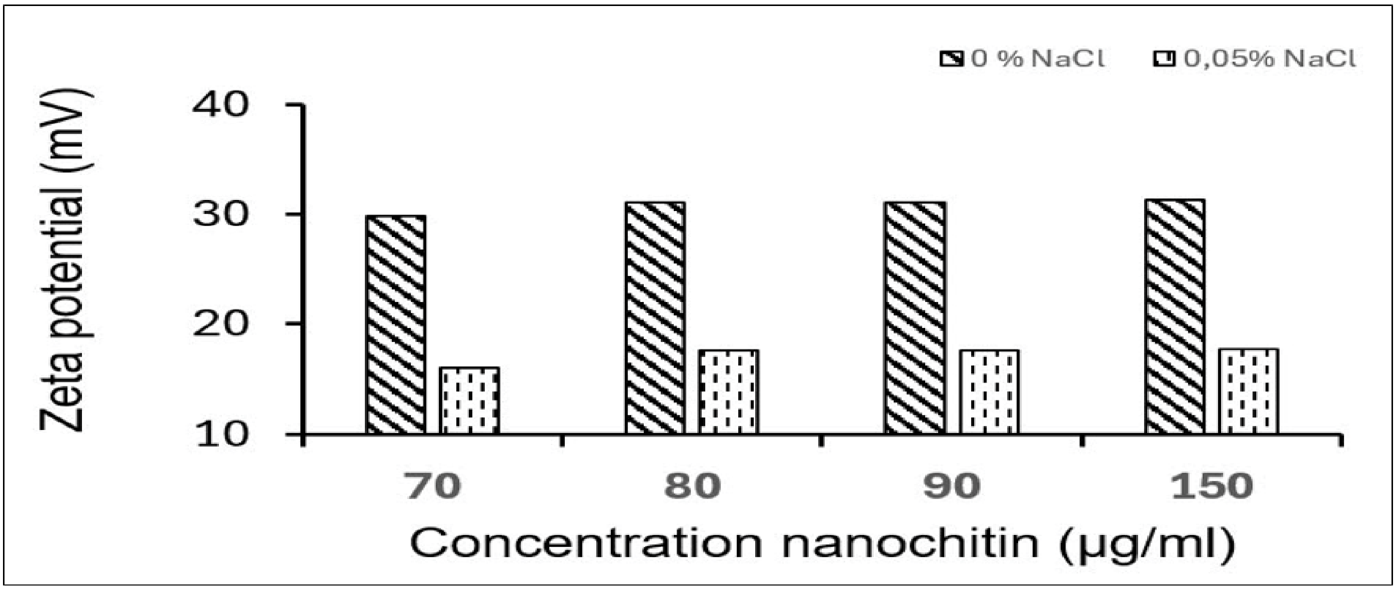

Fig 3 Zeta potential of nanochitin and salt solution mixtures at different concentrations

It was observed that the zeta potential of the mixture decreased compared to the pure nanochitin solution when NaCl was added. This indicates the interaction between Cl^- ions and nanochitin molecules. These results suggest that nanochitin has the potential to enhance the mobility of Na^+ ions, thereby increasing the salty taste perception. The findings are consistent with those reported by some authors, such as Jiang et al. [4] and Tsai et al. [13].

C. Determination of the threshold for saltiness enhancement by nanochitin

The experimental samples consisted of NaCl solutions supplemented with nanochitin, along with control samples, prepared as follows:

- Sample A_0: NaCl 0.65%
- Sample A_1: NaCl 0.65% + nanochitin 70 µg/mL
- Sample A_2: NaCl 0.65% + nanochitin 80 µg/mL
- Sample A_3: NaCl 0.65% + nanochitin 90 µg/mL

Saltiness was evaluated using a sensory ranking test. The converted scores were subjected to analysis of variance (ANOVA), and the results are presented in Table 1.

Table 1 Saltiness levels of the samples

Sample	**A_0**	**A_1**	**A_2**	**A_3**
Mean	0.57^a	0.76^a	-0.67^b	-0.67^b

- Samples with different superscript letters indicate significant differences at the $\alpha = 5\%$ level.
- The results showed that samples A_0 and A_1 were not significantly different at the $\alpha = 5\%$ level.
- Samples A_2 and A_3 were also not significantly different at the $\alpha = 5\%$ level.
- Samples A_2 and A_3 were saltier than samples A_1 and A_0.

This indicates that nanochitin at a concentration of 80 µg/mL enhanced the saltiness of the 0.65% NaCl solution. When the nanochitin concentration was increased to 90 µg/mL, the saltiness-enhancing effect was similar to that observed at 80 µg/mL. This result is consistent with previous studies. It aligns with the findings reported by Jiang et al. [4], Hsueh et al. [14], and Tsai et al. [13], which suggested that nanochitin has the ability to enhance saltiness. At a concentration of 80 µg/mL, nanochitin enhanced the saltiness of a 0.3% NaCl solution.

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D. Determination of saltiness enhancement level in NaCl solution

Experimental samples consisting of NaCl solutions at different concentrations supplemented with nanochitin were prepared as follows:

- Sample P_1: NaCl 0.61%
- Sample P_2: NaCl 0.65%
- Sample P_3: NaCl 0.69%
- Sample P_4: NaCl 0.65% + nanochitin 80 µg/mL
- Sample P_5: NaCl 0.65% + nanochitin 100 µg/mL
- Sample P_6: NaCl 0.61% + nanochitin 75 µg/mL

Saltiness was evaluated using the sensory ranking test. The converted scores were subjected to analysis of variance (ANOVA), and the results are presented in **Table 2**.

Table 2 Saltiness levels of the samples

Sample	P_1	P_2	P_3	P_4	P_5	P_6
Mean	1.27^a	0.42^b	-0.55^c	-0.87^c	-0.69^c	0.42^b

- Samples with different superscript letters indicate significant differences at the $\alpha = 5\%$ level.
- The results in **Table 2** indicate that sample P_1 had the lowest saltiness.
- Samples P_2 and P_6 were not significantly different.
- Samples P_3, P_4, and P_5 were not significantly different.

This means that nanochitin at a concentration of 80 µg/mL enhanced the saltiness of the 0.65% NaCl solution to a level equivalent to that of a 0.69% NaCl solution. Increasing the nanochitin concentration to 100 µg/mL did not further enhance saltiness perception. At a concentration of 75 µg/mL, nanochitin enhanced the saltiness of a 0.61% NaCl solution to a level equivalent to a 0.65% NaCl solution.

In our study, the results indicated that nanochitin enhanced the saltiness of NaCl solutions at concentrations of 0.61% and 0.65%, which are typical salt levels found in human foods. These findings provide a basis for calculating the substitution rate of NaCl in the salt-reduction application experiment.

- *Application of salt Reduction in pork paste Product*

The pork paste samples were prepared according to the procedure described in Section 2.2. The experimental samples were formulated with reduced salt based on the results of the experiment in Section 3.2.4, specifically as follows:

- Sample Ho: 3 g NaCl
- Sample H1: 2.455 g NaCl
- Sample H2: 2.455 g NaCl + 0.109 g nanochitin
- Other components in all three samples were consistent with the technological formulation.

The pork paste products were evaluated for quality using sensory methods. Saltiness was assessed using a scoring test. Color, flavor, and texture were evaluated using a hedonic test with the following scoring scale:

Table 3 Saltiness intensity scoring scale for pork paste product

Saltiness Intensity	Score	Saltiness Intensity	Score
No saltiness	0	Moderately salty	3
Very low saltiness	1	Slightly salty	4
Slightly low saltiness	2	Very salty	5

The sensory panel scores were compiled, subjected to analysis of variance (ANOVA), and the results are as follows:

Table 4 Mean Saltiness Scores of the Experimental Samples

Sample	Ho	H1	H2
Saltiness	2.9^a	1.9^b	2.8^a

Table 5 Scoring scale for evaluating the intensity of color, flavor, and texture attributes of pork paste products

Sensory Attribute	Score	Sensory Attribute	Score
Extremely dislike	1	Somewhat like	6
Strongly dislike	2	Like	7
Dislike	3	Strongly like	8
Somewhat dislike	4	Extremely like	9
Neither like nor dislike	5		

The sensory evaluation scores from the panel were compiled and analyzed using analysis of variance (ANOVA), and the results are as follows:

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Table 6 Mean Hedonic Scores for Sensory Attributes of the Samples

Sample	**Ho**	**H1**	**H2**
Color	5.8^b	5.9^b	5.7^b
Flavor	6.8^c	6.6^c	6.9^c
Texture	6.8^d	6.7^d	7.0^d

Amples with different superscript letters indicate significant differences at the α = 5% level.

The results presented in the tables above show that the addition of 0.109 g nanochitin (equivalent to 1.16% of the total amount of NaCl required per 1 kg of meat) into the technological formulation of pork paste (sample H2) allows for a reduction of 0.545 g NaCl (equivalent to 5.8% of the total NaCl required per 1 kg of meat), while the product still achieves saltiness equivalent to that of the control sample (Ho). Other sensory attributes such as color, overall flavor, and texture remained unchanged compared to the control. The experimental results on Vietnamese pork paste also demonstrate that the application of nanochitin for salt reduction in food is feasible.

V. CONCLUSION

The results of this study show that nanochitin, produced by acid hydrolysis combined with ultrasonication, had an average particle size of 228 nm and a solubility of 78.4%. It was effective in enhancing the salty taste of a 0.65% NaCl solution to a level equivalent to that of a 0.69% NaCl solution at a concentration of 80 µg/mL.

In the pork paste application, with 0.109 g nanochitin added per kg of meat, it was possible to reduce 0.545 g NaCl—equivalent to 5.8% of the total salt content—while still achieving the same level of saltiness as the control sample. Other sensory attributes showed no noticeable differences.

These findings suggest that nanochitin has potential application in reducing salt content in other food products such as surimi, sausages, etc., contributing to product diversification while helping reduce overall salt intake in the human diet.

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