

SHELF-LIFE PREDICTION OF SEASONING POWDER MADE FROM WHOLE FERMENTED FISH (PEDA) BY USING ARRHENIUS METHOD

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ABSTRACT

Ready to use seasoning powders in markets are commonly based on meat extract or spices. Such seasoning powder made from fish or fish products is rarely found, whereas the raw material has great potential. This research aimed to produce seasoning powder as flavor enhancer for foods which was made from whole fermented fish ('peda'). The product was stored for 8 weeks at two different temperatures, i.e. chilling (7°C) and room temperature (28°C) with aluminum foil packaging. The change of product profile was evaluated and the shelf-life of product was predicted. 'Peda' was processed by addition of clove and cinnamon. After fermentation, 'peda' was then milled into paste. After that onion and garlic were added to the paste and the mixture was then dried. Measuring parameters used for seasoning powder were histamine content, water content, total mesophilic microbe and sensory evaluation using hedonic scale for color, texture and odor. Shelf-life prediction was carried out using Arrhenius method. Research result showed that there was an increase of histamine content during eight weeks storage. The lowest and highest level of histamine was found during storage at chilling temperature, i.e. 1.311 (control) and 20.144 mg/100g (7th week) respectively, whereas at room temperature there were 5.459 (1st week) and 19.845 mg/100g (7th weeks), respectively. However, the increase was still below the un-safety level. Water content and total mesophilic microbe also increased during storage. Water content ranged between 6.3% to 9.8% (at chilling temperature) and 6.3% to 10.5% (at room temperature). Total mesophilic microbe ranged between 2.1×10^3 cfu

to 8.7×10^5 cfu (chilling temperature) and 2.86×10^3 cfu to 1.5×10^6 cfu (room temperature). **Arrhenius method** was used to predict the **shelf-life of seasoning powder** because temperature is the main factor which influenced the quality of foodstuff. **Shelf-life** prediction based on histamine content, water content and total mesophilic microbe at 224 days (32 weeks), 70 days (10 weeks) and **91 days** (13 weeks) respectively. During storage, response of panelists on color, texture and odor of product was evaluated. The result showed that seasoning powder made from 'peda' was still acceptable **during** 8 weeks storage.

Key Words: 'peda', seasoning powder, herbs and spices, shelf-life.

INTRODUCTION

Ready to use seasoning powders in markets are commonly based on meat extract or spices. Such seasoning powder made from fish or fish products is rarely found, whereas the raw material has great potential, for example, fermented fish product that can be developed as seasoning powder for various menus. Besides that, fish products based on fermented fish are well known and processed through local and indigenous technology like **peda** or fermented whole fish. The problem, which occurs oft by such process, is the formation of histamine which can cause allergy and even poisoning.

Histamine poisoning case with symptoms such as itching in face and neck, headache, vomiting, nausea, diarrhea, usually rapidly occurred. **Avoiding** against poisoning is necessary by inhibition the histamine formation during processing of fishery products. Once histamine is formed, it is difficult to destroy it. Therefore, the most important thing is to avoid the decarboxylation process by inhibition of enzyme histidine decarboxylase (HDC)-activity. HDC takes important role to change free histidine into histamine (1H-imidazol-4-ethanamin) through decarboxylation.

One of many ways to avoid the formation of histamine is the application of materials which possess antimicrobial properties. Previous research **proved** that the use of clove and cinnamon could inhibit the activity of HDC, so automatically the formation of histamine could be inhibited (Mahendaradatta and Adiansyah, 2007; 2008). Besides that, **spices** and herbs are used also in menu food products as flavor enhancer.

By using clove **and** cinnamon to inhibit the HDC activity, 'peda' can be further **process** in seasoning powder which is safe for consumption and provide special odor for various menus.

METHODOLOGY

Materials and chemicals

Materials used in this research were short-bodied mackerel (*Rastrelliger neglectus*), table salt, cloves, cinnamon, garlic and onion. All materials used were bought from fish market and traditional market in Makassar. Chemicals used **in** this research were histamine dihydrochloride, trichloro acetic acid, n-butanol, NaOH, NaCl, n-heptane, HCl, 4-nitrobenzen diazonium tetrafluoroborat. These chemicals were bought from chemicals distributors in Makassar.

Methods

A. Product Quality

Seasoning fish-paste powder **was** evaluated for its histamine content (Mahendradatta and Schwedt, 1998), total bacterial (Fardiaz, 1989), **water content** (Horwitz, 1980) during eight weeks storage. The sensory properties of products (color, texture and odor) were evaluated by a 10 panelists using a five-point scale (5-excellent, 4-good, 3-acceptable, 2-doubtful, 1-unacceptable) (Larmond, 1970). Samples graded above point **3** were considered to be acceptable for consumption.

B. Prediction of shelf life

Shelf life of seasoning powder was predicted using Arrhenius method (Syarief and Halid, 1989)

Analysis Procedure

A. Histamine content

Ten g of each sample **was** homogenized with 25 ml **of** 5% trichloro acetic acid (TCA) then centrifuged by 4000 rpm for 10 minutes. The supernatant was decanted in 50 ml measured flask. Residue was homogenized once more with 5% of TCA and then centrifuged. The supernatant **was** combined and filled with 5% **of**

TCA till mark and filtered. One ml filtrate was transferred to centrifuge tube containing 5 ml n-butanol, 0.25 ml NaOH 5mol/l and 0.75 g NaCl. The tube was shaken for 3 minutes and centrifuged for 10 minutes at 4000 rpm. All organic phase was **transferred** to the second tube containing 2.5 ml NaOH 0.1 mol/l saturated-NaCl. After shaken and centrifuged at the same **way** described previously, 4 ml organic-phase are transferred to the third tube containing 2.5 ml HCl 0.1 mol/l and 7.5 ml n-heptane. The solution was shaken and centrifuged. Acidic-phase was ready **for** analysis. One ml 0.2% 4-nitrobenzene diazonium tetrafluorborat was added to 1 ml of acidic-phase and 1ml 10% of $\text{Na}_2\text{CO}_3 \cdot \text{H}_2\text{O}$ solution will be added. After 5 minutes the absorbance was measured on 470 nm.

B. *Arrhenius Method (Syarief and Halid, 1989)*

Seasoning powder **was** divided into 2 parts and was stored under different temperature, i.e. 7°C and 28°C with room and refrigerator humidity 85.6% and 76% respectively. Observation on the change of product characteristic was conducted every week from the day before storage (control) until eighth week. Product quality measured can **be seen** in point A. Measurement result during storage was then tabulated, regressed and two regression equations has been drawn for two different conditions. From that Arrhenius equation, parameter score has been provided, i.e. $1/T$ (°K) and $\ln K$. Furthermore, $1/T$ **was** plotted toward $\ln K$ and Arrhenius equation as well as the change of product characteristic every week was drawn. Arrhenius equation $K = K_0 \cdot e^{-E/T}$1)

Note:

K_0 = constant pre exponential or absolute rate (undependable on temperature)

E_a = activation energy of change reaction of quality characteristic (cal/mol)

R = constant of ideal gas (1.986 cal/mol°K)

T = absolute temperature (°C + 273)

Prediction of shelf-life of seasoning powder was carried out by using semi empirical approach with order zero reaction. Determination of reaction order was based on constant correlarion score of equation. The shelf-life prediction **was** measured using formula:

$$T = (A_c - A_0) / K$$

Note:

A_t = standard quality of **product** characteristic

A_i = product characteristic at initial condition

K = rate of the change of product quality

Data Processing

For histamine content, **the** absorbance measured was plotted into standard curve. The histamine content is calculated by multiplying the value with correction factor from sample preparation step, sample **clean up** step and standard solution (Mahendradatta, 1997). Analysis was **carried** out in triplicate. Other data were processed as quantitatively descriptive.

RESULT AND DISCUSSION

Product Quality

Result showed **that** there was an **increase** of histamine content, water content and total bacteria for 8 weeks **storage**. Compared to **fish** with white **flesh**, the higher level of histamine is found in fish with dark flesh. It is related **to** the high **level** of free histidine in dark flesh. The increase of histamine content (Fig. 1) **agreed** with the increase of total bacteria (Fig.2) during storage.

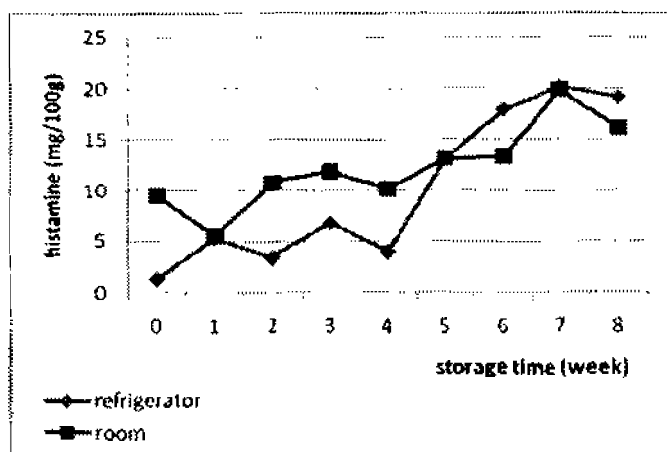


Figure 1. Histamine content during 8 weeks storage

Based on histamine content analysis, it showed that histamine content at 8th week under room temperature and chilling temperature was 16.0877mg/100g and 19.168 mg/100g, respectively. The increase was still under safety limit of histamine content, i.e. 50mg/100g.

Processing of whole fermented fish powder involved bacteria. Some bacteria can support the formation of histamine through decarboxylation process from free histidine into histamine. Although there was an increase of water content, but it did not exceed critical limit 12%.

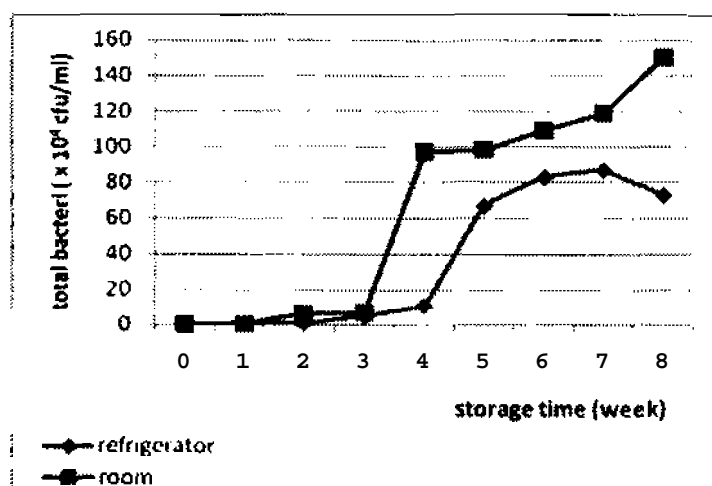


Figure 2. Total bacteria during 8 weeks storage

Raw material used to produce seasoning powder was 'peda' or whole fermented fish. 'Peda' was made by fermentation process which involved some bacteria. Bacteria which are important in histamine formation included *Morganella morganii*, *Klebsiella pneumoniae* and some *Alvei*. These bacteria could grow well in fermented fish and could produce histamine (Ward and Cameron, 1991).

Total mesophilic bacteria have been determined in this research, however, the optimum temperature for growth is 37°C and according to Hadiwiyo (1993), the optimum temperature where the enzyme and microbe have the great activity is 30 - 70°C. Beutling

(1996) said that the maximum activity of HDC enzyme is at temperature 55°C. Although there is an increase of histamine content during storage, its level is still below the safety limit, less than 50mg/100g.

The addition of spices into seasoning powder gave effect on inhibiting the histamine formation. It was due to the bioactive compound of spices which have antimicrobial activity (Hirasa and Takemasa, 1998). According to Wendakoon and Sakaguchi (1995), clove and cinnamon were able to inhibit the growth of bacteria and formation of amine compound at level 1%. Mahendradatta (2005) reported that spices combination of clove and cinnamon that was applied to cook fish had the best sensory score of panelist compared to other combinations consist of ginger and tamarind.

Essential oil of spices possessed antibacterial properties. Making of seasoning powder from 'peda' was carried out by adding onion and garlic which have function as flavour and antimicrobial agents.

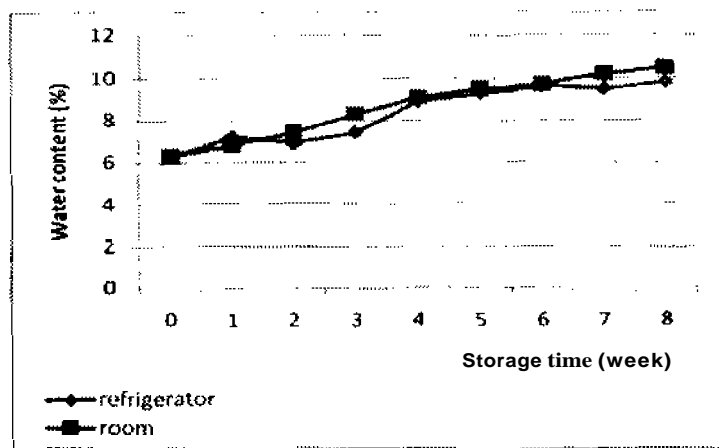


Figure 3. Change of water content during 8 weeks storage

Water content increased during eight weeks storage (Fig. 3). The increase was due to the hygroscopic properties of product. For hygroscopic product, temperature and humidity are very important factors (Syarief *et al.*, 1989). The increase of humidity will be

followed by the increase of water content **and** it will affect the quality of product.

Sensory evaluation of seasoning powder based on color, texture and odor **can be seen** at Figure 4. Sensory score ranged between 3.1 to 3.5 for color; 3.1 to 3.7 for texture; and 2.75 to 3.5 for odor. It means that until eight week storage, seasoning **powder** made from 'peda' was still **accepted** by the **panels**, except the odor, because it scored below 3.

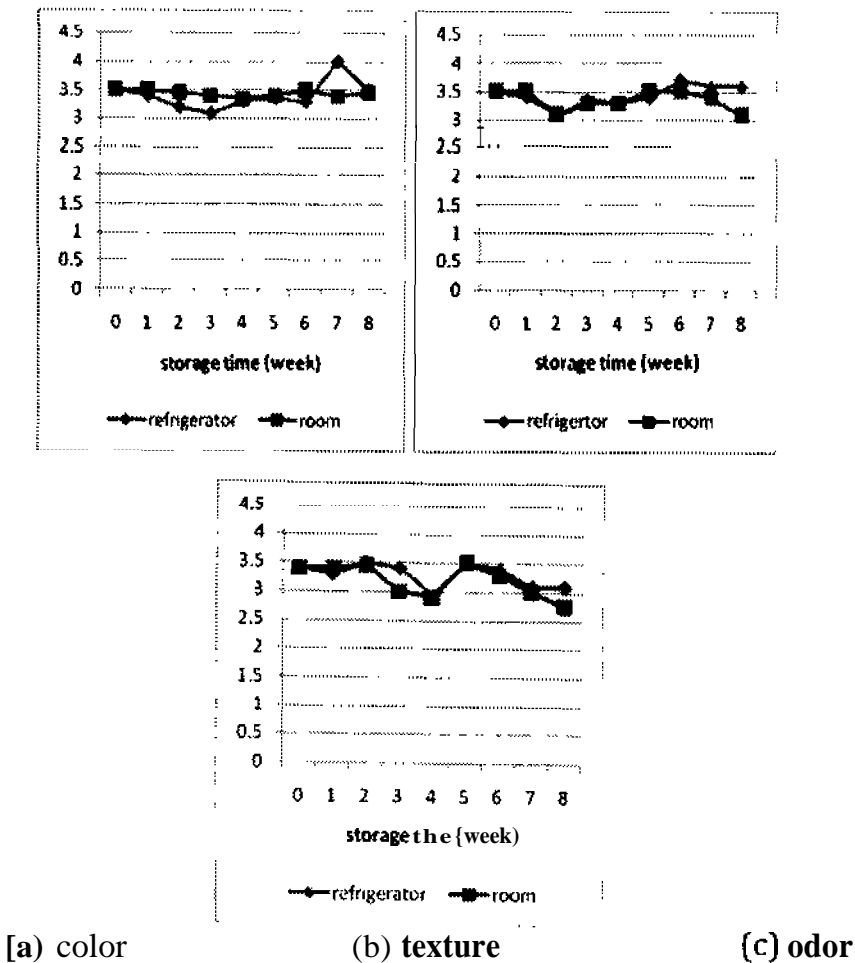


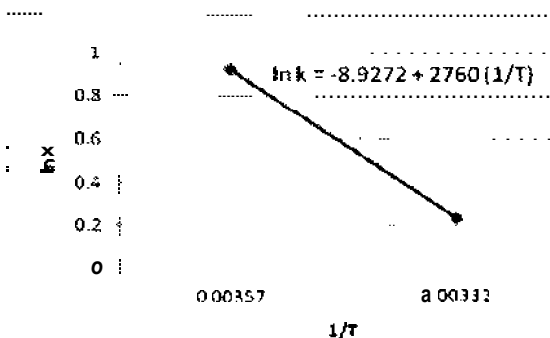
Figure 4. Change of sensory acceptance during 8 weeks storage

Preference level of panels towards color decreased at room temperature then increased at sixth week and the decreased again until the **end of storage** time. Whereas at chilling temperature, preference level of panels decreased **and then** increase at seventh and eight week. The preference score **ranged** between good until acceptable. Seasoning powder colored brown and possessed special odor from spices added.

Prediction of Shelf Life

The food industry **has a** great need to obtain, in a relatively short time, the necessary information for determining the shelf-life of its products. It has a very important impact on handling of the products' storage, distribution and shelf-life dating (Mizrahi, 2000).

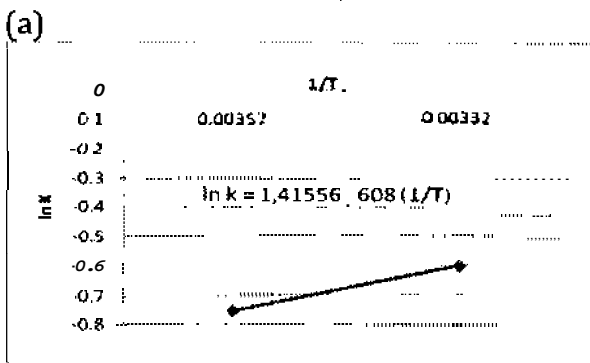
Based on measurement of histamine content, water content and total mesophilic microbe resulted from every observation week at **each** temperatures, an equation of linear relationship with curve slope k (constant of histamine increase) was drawn. Another linear **regression** of $\ln k$ and each storage temperature (in $^{\circ}\text{K}$) was then drawn.



The increase of histamine at each temperature based on the curve of linear relationship as follows,

$$y = -8.889106 + 2.525005x$$

($r = 0.8548$; $K_1 = 2.525005$)
Room temperature (28°C)



$y = 7.133257 + 1.266188x$
($r = 0.712$; $K_2 = 1.266188$)
Curve slope from each temperature ($\ln k$) was related with storage ($1/T$) in $^{\circ}\text{K}$ and plotted into curve of temperature and $\ln k$

and formed linear equation $\ln k = -8,89272 + 2760 (1/T)$.

The cut of curve at y -

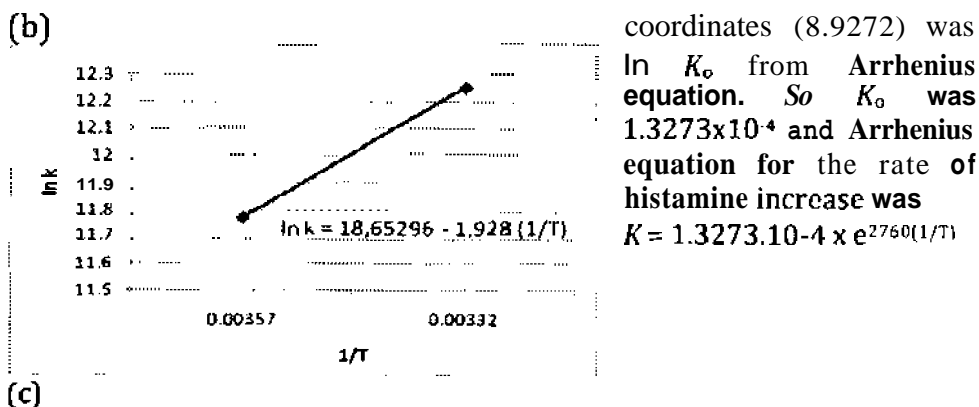


Figure 5. Linear equation on the changes of (a) histamine content, (b) water content and (c) total mesophilic microbe

From this equation, the rate of histamine increase was calculated as follows: chilling temperature: 2.5244 mg/100g sample weekly and room temperature: 1.2662 mg/100g sample weekly. By using Arrhenius equation order zero, the prediction of product shelf-life could be determine through equation $T = (A_c - A_0)/K$, so that shelf-life of seasoning powder made from 'peda' was 32 weeks or 224 days at room temperature and 19 weeks or 133 days at chilling temperature.

The same calculation was applied to the measurement result of water content and total mesophilic microbial. The increase of water content at each temperature based on the curve of linear relationship was as follows,

Chilling temperature (7°C): $y = 6.4422 + 0.47x$ ($r = 0.9007$; $K_1 = 0.47$)

Room temperature (28°C): $y = 6.4578 + 0.5467x$ ($r = 0.9702$; $K_2 = 0.5467$)

Curve slope from each temperature ($\ln K$) was related with storage ($1/T$) in °K and plotted into curve of temperature and $\ln K$ and formed linear equation linear

$\ln K = 1.41556 - 608(1/T)$ [Figure Sb). The cut of curve at y-coordinates (1.41556) was $\ln K_0$ from Arrhenius equation. So K_0 was 4.119 and Arrhenius equation for the rate of histamine increase was $K = 4.119 \times e^{-608(1/T)}$. From this equation, the rate of histamine increase was calculated as follows: chilling temperature : 0.470% sample weekly and room temperature : 0.547% sample weekly.

By using Arrhenius equation order zero, the prediction of product shelf-life could be determined through equation $T = (A_c - A_o)/K$, so that shelf-life of seasoning powder made from 'peda' was 10 weeks or 70 days at room temperature and 12 weeks or 84 days at chilling temperature.

The change of total mesophilic microbial at each temperature based on the curve of linear relationship was as follows;

Chilling temperature (7°C): $y = -153542.44 + 129365.33x$ ($r = 0.794$; $K_1 = 129365.33$)

Room temperature (28°C): $y = -182257.33 + 209482.67x$ ($r = 0.889$; $K_2 = 209482.67$)

Curve slope from each temperature ($\ln K$) was related with storage ($1/T$) in °K and plotted into curve of temperature and $\ln K$ and formed linear equation linear

$\ln K = 18.65296 - 1928(1/T)$ (Figure 5c). The cut of curve at y-coordinates (18.65296) was $\ln K_0$ from Arrhenius equation. So K_0 was 126147195.3 and Arrhenius equation for the rate of histamine increase was $K = 126147195.3 \times e^{-1928(1/T)}$. From this equation, the rate of histamine increase was calculated as follows: chilling temperature: 1290114.15 colony/ml sample weekly and room temperature : 209399.67 colony/ml sample weekly.

By using Arrhenius equation order zero, the prediction of product shelf-life could be determine through equation $T = (A_c - A_o)/K$, so that shelf-life of seasoning powder made from 'peda' was 13 weeks or 91 days at room temperature and 21 weeks or 147 days at chilling temperature.

Table 1 showed the all tabulated data from calculation based on histamine content, water content and total mesophilic microbial at chilling and room temperature. Based on calculated data, the shortest time was taken, so that seasoning powder made from 'peda' could be stored until 10 weeks or 70 days.

Table 1. Calculated data for shelf-life prediction

Parameters	E	Ko	R	T	$K = \frac{K_0 \cdot e^{-E/RT}}$	$T = \frac{(A_c - A_0) \cdot K}{R}$	T	$K = \frac{K_0 \cdot e^{-E/RT}}$	$T = \frac{(A_c - A_0) \cdot K}{R}$
Histamine	5481.36	$1.3273 \cdot 10^4$	1.986	301°K	1.2662	32 weeks	280°K	2.525	19 weeks
Water content	1207.488	4.119	1.986	301°K	0.547	10 weeks	280°K	0.47	12 weeks
Total mesophilic microbe	3829.008	126147195.3	1.986	301°K	209399.67	13 weeks	280°K	129365.33	21 weeks

CONCLUSION

1. Histamine content, total microbe and water content increased during eight weeks storage of seasoning powder made from 'peda'.
2. Seasoning powder made from 'peda' can be stored in aluminium foil until 10 weeks at room temperature.
3. Based on sensory evaluation, this product was still accepted until 8 weeks storage (during research schedule), except its odor.

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