

THE STUDY AND ANALYSIS OF APIS DORASATA HONEY COLLECTED FROM WARDHA AND GADCHIROLI DISTRICTS OF VIDHARBHA IN MAHARASHTRA**P.D. Khandelwal¹, G.N. Paliwal² and V.S.Zade³**^{1,3}Government Vidarbha institute of science and humanities, Amravati (MS), India²Centre for Bee Development, Nalwadi, Wardha, (MS), India*¹pujaysharma0990@gmail.com, ²gopalpaliwal23@gmail.com, ³zvarsha27@gmail.com

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ABSTRACT

The present study evaluated physicochemical composition of some honey isolated from the natural combs of *Apis dorsata* from Wardha and Gadchiroli districts in Vidarbha region of Maharashtra state in India. The physico-chemical analysis of honey samples was carried out as per the standard procedure detailed in booklet (IS: 4941-1994). The Summer and winter honey samples' parameters like total sucrose, true fructose, true glucose, optical density, moisture, minerals, acidity, aniline chloride test and Fiehe's tests have been studied to determine any notable fluctuation in the basic quality of the honeys gathered from different location and different sessions.

The results indicated that, *A. dorsata* honey in principle qualifies all quality parameters as per the Indian Standards (IS), Agriculture Mark (AGMARK) and Food Safety and Standards Act of India (FSSAI) standards. *A. dorsata* honey has internationally acceptable quality with little variation in its physical parameters.

There are slight variations in the overall results however of no much of the significance, might be due to similar or unified source of blossom availability irrespective of the locations. These variations may be addressed by developing targeted honey hunter education programs to improve honey harvesting and storage practices.

So the farmers of the region can add Honey Harvesting as their side business along with their routine farming. It can enhance their source of income leading towards Rural Development; it will help to overcome financial crises of farmers.

Keywords: *Apis-Dorsata, Honey, Analysis, Parameters, Vidharbha, Rural development*

Introduction

Honey is a unique combination of nectar and pollen grains gathered from different flowers by bees. It is produced by honeybees from the nectar of flowering plants, which they collect and transform by combining with their salivary secretions, and deposit, dehydrate and store in the honey comb to ripen (Codex, 2009). The bees produce honey in order to serve it as own food (James, 2009). Besides moisture, fructose and glucose, honey also contains other substances like organic acids, enzymes and solid particles like pollen (Saxena *et al.*, 2010). All these microelements make honey a unique nutritional or medicinal substance (James OO 2009).

The Vidarbha region of Maharashtra state in Centre India, has many forest pockets with a large population *Apis dorsata* bee, yielding forest honey during the summer season Ana Winter session. Hundreds of local traditional

honey collectors gather it and sell it into the local market directly to the consumers. Some of them may adulterate by adding jaggery to the basic quantity of honey far making the big gains. Therefore, the present study was undertaken to test of the purity of honey and authenticate the basic quality of honey as a part of farmer and consumer awareness that ultimately leads to rural development.

Material and Methods**Material**

Fresh honey samples (1 kg each) were directly procured from the honey collectors of summer and winter session of Wardha, and Gadchiroli districts of Maharashtra for undertaking the detailed physicochemical analysis under the present study.

Methods

For the detection of total reducing sugar (TRS), sucrose, fructose - glucose (F/G) ratio, ash, acidity, aniline chloride- and

Fiehe's tests, the quality control manual for honey IS- 4941, 1994 was followed. The details of the methods adopted are as in the below.

I] Physical Parameters

Determination of optical Density of Honey

a. Procedure

Weigh accurately 2 g of honey in a small beaker and dissolve it in distilled water. Make the solution to 10 ml in 10ml measuring cylinder. Adjust the colorimeter with distilled water in a cuvet at (O) absorbance or 100 percent transmittance at 660 nm. Take the honey solution in the cuvet and read the absorbance directly or as the percent transmittance at the same wave length.

b. Calculation

Calculate the optical density by using the following formula, if the colorimeter has been provided with transmittance scale only

Optical density = $2 - \log$ percent transmittance.

II] Biochemical parameters

1. Determination of Non Reducing Sugars (NRS)

a. Reagents-

Stock solution of honey, concentrated hydrochloric acid and sodium carbonate

b. Procedure-

To 100 ml of the stock honey solution add one mililitre of concentrated hydrochloric acid, heat the solution to near boiling and keep aside overnight. Neutralize this inverted honey solution with sodium carbonate and determine the total reducing sugar as described in above.

c. Calculation-

Sucrose, percent by mass = (reducing sugars after inversion, percent by mass) – (reducing sugars before inversion percent by mass) x 0.95.

2. Determination of Total Reducing Sugar (TRS)

a. Reagents

Soxhlet Modification of Fehling's Solution- Prepared by mixing equal volumes of Solution A and Solution B immediately before using.

i) Copper Sulphate Solution (Solution A)

Dissolve 34.639 g of copper sulphate crystals ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in water, dilute to 500 ml and filter through glass wool or filter paper.

Standardization of copper sulphate solution:- Using separate pipettes, pipette out accurately 5 ml of Solution A and 5 ml of Solution B in to a conical flask of 250 ml capacity. Heat this mixture to boiling on an asbestos gauze and add standard invert sugar solution from a burette, about one milliliter less than the expected volume which will reduce the Fehling's solution completely (about 48 ml). Add one milliliter of Methylene blue indicator while keeping the solution boiling. Complete the titration within three minutes, the end point being indicated by change of colour from blue to red. From the volume of invert sugar solution used, calculated the strength (S) of the copper sulphate solution by multiplying the titre value by 0.001 (mg/ml of the standard invert sugar solution). This would give the quantity of invert sugar required to reduce the copper in 5 ml of copper sulphate solution.

ii. Potassium Sodium Tartrate (Rochelle salt) Solution (Solution B):-

Dissolve 173 g of potassium sodium tartarate and 50 g of sodium hydroxide in water, dilute to 500 ml. Let the solution stand for a day and filter.

Hydrochloric Acid –Sp gr 1.18 at 20° C. (approximately 1/2 N).

iii. Standard Invert Sugar Solution

Weigh accurately 0.95 g sucrose and dissolve it in 500 ml of water. Add 2 ml of concentrated hydrochloric acid, boil gently

for 30 minutes and keep aside for 24 hours. Neutralize with sodium carbonate and make the final volume to 1000 ml, 50 ml of this solution contains 0.05 invert sugar.

iv. Methylene Blue Indicator – 0.2 percent in water.

b. Procedure

Place accurately weighed about one gram (W) of the prepared sample of honey into a 250 ml volumetric flask and dilute with about 150 ml of water. Mix thoroughly the contents of the flask and make the volume

to 250 ml with water. Using separate pipettes, take accurately 5 ml each of Solution A and Solution B in a porcelain dishes. Add about 12 ml of honey solution from a burette and heat to boiling over asbestos gauze. Add one milliliter of methylene blue indicator and while keeping the solution boiling complete the titration, within three minutes, the end point being indicated by change of colour from blue to red. Note the volume (H) in ml of honey solution required for the titration.

c. Calculation-

$$\text{Total reducing sugars, percent by mass} = \frac{250 \times 100 \times S}{H \times M}$$

where, S = strength of copper sulphate solution,

H = volume in ml of honey solution required for titration, and

M = mass in g of honey.

3. Determination of Fructose - Glucose (F/G) Ratio

a. Reagents-

Iodine Solution – 0.05 N

Sodium Hydroxide Solution – 0.1 N.

Sulphuric Acid – concentrated. Standard

Sodium Thiosulphate Solution – 0.05 N.

b. Procedure-

Pipette out 50 ml of honey solution in a 250 ml stopper flask. Add 20 ml of iodine solution and 25 ml of sodium hydroxide solution. Stopper the flask and keep in dark for 20 minutes. Acidify with 5 ml of sulphuric acid and titrate quickly the excess of iodine against standard sodium thiosulphate solution. Conduct a blank using 50 ml of water instead of honey solution.

c. Calculations-

$$\text{Approximate glucose, percent by mass (w)} = \frac{(B - S) \times 0.004502 \times 100}{a \times 5}$$

where,

B = volume of sodium thiosulphate solution required for the blank,

S = volume of sodium thiosulphate solution required for the sample, and

A = mass of honey taken for test.

$$\text{Approximate fructose, percent by mass (x)} = \frac{\text{Approximate total reducing sugars} - w}{0.925}$$

$$\begin{aligned} \text{True glucose, percent by mass (y)} &= w - 0.012 \times \text{True fructose, percent by mass (z)} \\ &= \frac{\text{Approximate reducing sugars, percent} - y}{0.925} \end{aligned}$$

True reducing sugars, percent by mass = y + z

$$\text{Fructose / glucose ratio} = \frac{\text{True fructose, percent by mass (z)}}{\text{True glucose, percent by mass (y)}}$$

4. Determination of Acidity

a. Reagents

Standard Sodium Hydroxide solution – 0.05N
Phenolphthalein Indicator Solution – Dissolve 0.5 g of phenolphthalein in 100 ml of 50 percent ethyl alcohol (v/v).

b. Procedure

Take 10 g of the sample in a suitable titration flask and dissolve it in 75 ml of carbon dioxide free water. Mix thoroughly. Titrate against standard sodium hydroxide solution using 4 to 6 drops of carefully neutralized phenolphthalein solution (pink colour of indicator should persist for at least 10 seconds). Determine blank on water and indicator and correct the volume of standard sodium hydroxide solution used.

c. Calculation

$$\text{Acidity (as formic acid), percent by mass} = \frac{0.23 \times V}{M}$$

Where,

V = corrected volume of 0.05 N sodium hydroxide solution required for titration, and
M = mass in g of the sample taken for the test.

5. Determination of Ash

a. Reagents

Honey sample and pure olive oil.

b. Procedure

Weigh accurately 5 to 10 g of the sample in a silica or platinum dish; add a few drops of pure olive oil to prevent spattering, heat carefully over a low flame until swelling ceases. Ignite in a muffle furnace at 600 + 20c till white ash is obtained. Cool the dish in a desiccator and weigh. Incinerate to constant weight.

c. Calculation

$$\text{Ash, percent by mass} = \frac{100(M_2 - M)}{M_1 - M}$$

Where,

M₂ = mass in g of the dish with the ash
M = mass in g of the empty dish, and

M₁ = mass in g of the dish with the material taken for the test.

6. Determination of Fiehe's and Aniline Chloride Tests

Fiehe's Test

a. Reagents

Resorcinol Crystal and Hydrochloric acid

Ether – Sulphuric ether

b. Procedure

Transfer about 5 g of the honey sample into a mortar; mix the honey with 10 ml of ether. Decant the ether in to a porcelain dish. Repeat the extraction to evaporate and collect the extract in the same dish. Allow the extract to evaporate to dry at room temperature and add a large drop of freshly prepared resorcinol solution. The production of cherry red colour appearing instantly indicates a positive reaction. Faint pink colour disappearing after a short time or yellow to salmon pink colours indicate a negative reaction.

Aniline Chloride Test

a. Reagents

Aniline Chloride solution (Aniline and Hydrochloric Acid)

b. Procedure

Place 5 g of the sample in a porcelain dish and add, while stirring 2.5 ml of recently prepared aniline chloride solution. In the presence of commercial invert sugar within one minute, the reagent assumes orange red colour turning dark red. Yellow to salmon shades has no significance.

Result and Observation

The samples of honey were analyzed with reference to the quality and gradation as per the parameters of Bureau of Indian standards (IS: 4941 - 1974). Under this, the physico-chemical tests like specific gravity (rho), moisture, total reducing sugar (TRS), Non Reducing Sugar (NRS), true glucose, true fructose, fructose / Glucose ratio, acidity, ash, Fiehe's test and aniline chloride test were conducted on the honey samples

of summer and winter session gathered from honey collectors.

Bureau of Indian standards (IS: 4941 - 1974)

Analytical specifications mandatory under ARE: 4941-1974 certification for various grades of

extracted honey are given in table1. There are total 3 grades of honey. Therefore, analytical data on *A. dorsata* honey is compared with that of IS: 4941-1974.

Table 1. Analytical specifications for honey under BSI, 1974

Sr. No.	Parameters	Various grades of extracted honey IS: 4941-1974		
		Special	A	Standard
1.	Specific gravity at 27 ⁰ C (min.)	1.41	1.39	1.37
2.	Moisture (max.)	20%	22%	25%
3.	Total reducing sugar by mass % (min.)	70	65	65
4.	Sucrose by mass % (max.)	5	5	5
5.	Fructose to Glucose ratio (min.)	1	1 (0.95)	1 (0.95)
6.	Acidity (max.)	0.2	0.2	0.2
7.	Ash (minerals) (max.)	0.5	0.5	0.5
8.	Aniline chloride Test	-ve	-ve	-ve
9.	Fiehe's Test	-ve	-ve	-ve

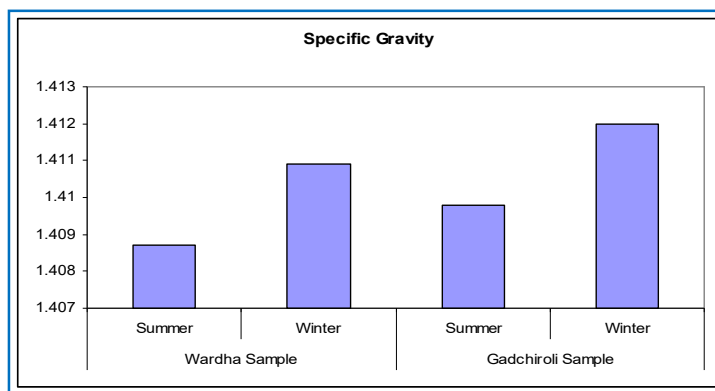
Table .2 :- Physico- chemical analysis of processed honey with reference to the quality parameters IS- processed honey of different flowering seasons

Particulars	Wardha Sample		Gadchiroli Sample	
	Summer	Winter	Summer	Winter
Specific Gravity	1.4087±0.1024	1.4109±0.0104	1.4098±0.0142	1.4178±0.0108
Moisture	19.112±0.1425	20.152±0.1302	19.296±0.125	21.001±0.324
TRS	70.7521±0.5321	69.987±0.5812	71.0134±0.4215	68.976±0.435
NRS	2.4725±0.017	2.701±0.021	2.8086±0.0321	3.1020±0.0210
F/G Ratio	1.7350±0.07154	1.3012±0.0101	1.8012±0.0321	1.4502±0.0125
Acidity	0.17±0.0012	0.15±0.0013	0.11±0.001	0.18±0.0012
Ash	0.25486±0.0020	0.2421±0.0017	0.28104±0.0021	0.301±0.0019
Fiehe's test	Negative	Negative	Negative	Negative
A/C test	Negative	Negative	Negative	Negative

• Specific gravity (SG):

The specific gravity of Wardha honey sample of summer and winter seasons are measured to about 1.4087±0.1024 and 1.4109±0.0104 rho and Gadchiroli honey sample of summer and winter are measured to be 1.4098±0.0142 and 1.4178±0.0108

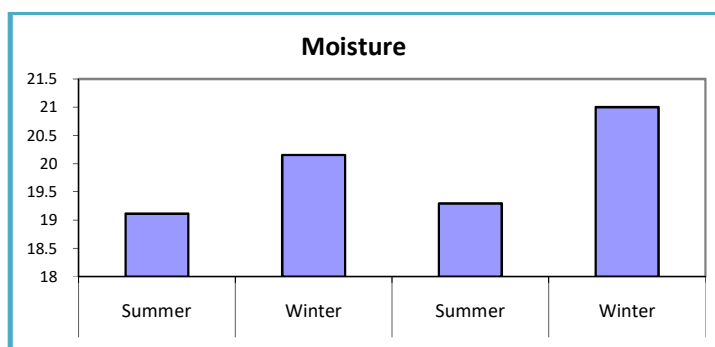
rho respectively, The result shows that the SG is higher for honey of winter season as compared to that of the other seasons. Both winter and summer honeys meet the optimal level of SG (IS: 4941-1974).



• **Moisture (M) at 27⁰ C temp:**

The Moisture in Wardha honey sample of summer and winter seasons are measured to about 19.112±0.1425 and 20.152±0.1302%, and moisture in Gadchiroli summer and winter honey sample is measured to be

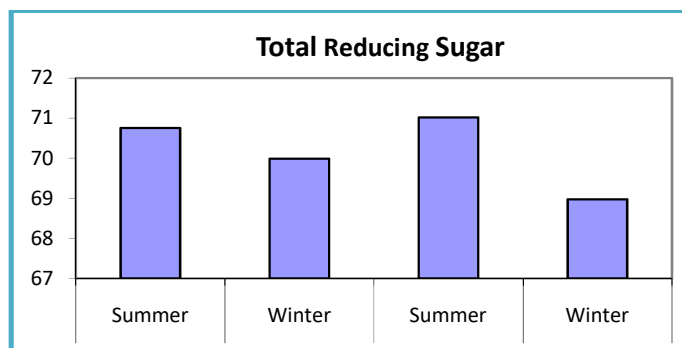
19.296±0.125 and 21.001±0.324 respectively. These results confirmed that all tested samples passes the maximum limit as set by IS & FSSAI.



• **Total Reducing Sugar (TRS):**

The TRS in the samples collected from Wardha and Gadchiroli districts of summer session were measured (in %) to be 70.7521±0.5321 and 71.0134±0.4215 and Winter session is measured to be 69.987±0.5812 and 68.976±0.435 respectively. It also means that summer

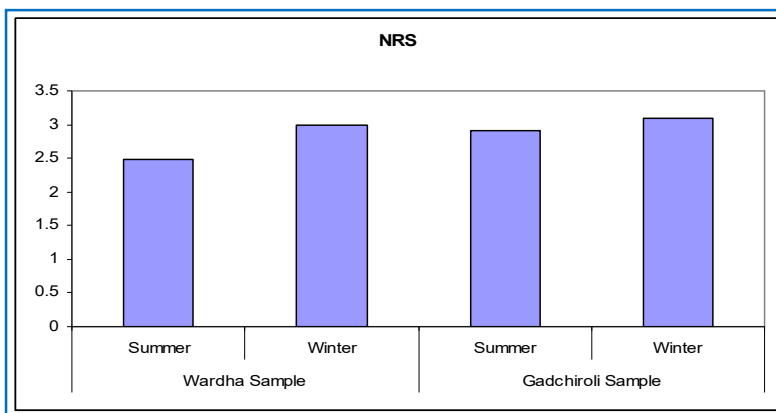
honey showed the highest value of reducing sugars while Winter honey showed the lowest value of reducing sugars. The honey samples from the Winter showed moderate values. The reducing sugars present in the honey samples are represented by fructose and glucose.



Non Reducing Sugar (Sucrose)

Sucrose content of honey samples of summer session from Wardha and Gadchiroli were measured to be 2.4725±0.017 and 2.8086±0.0321 respectively. Reading of winter session

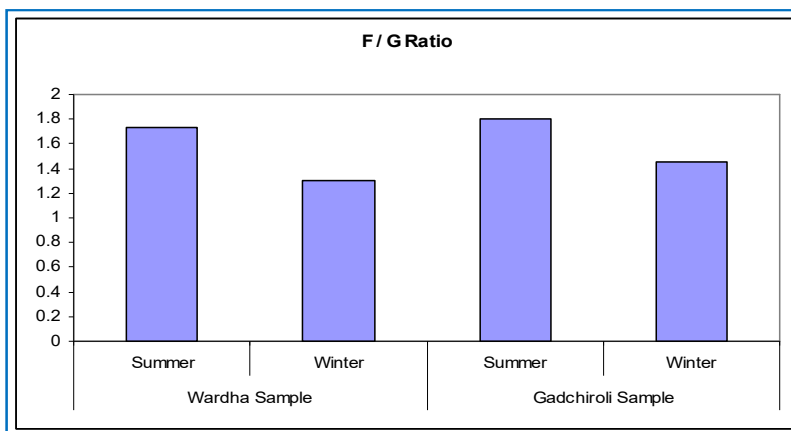
honey sample is measured to be 2.701±0.021 and 3.102±0.0210 respectively. Sucrose content of honey samples of winter session showed higher level than that of summer session of Wardha and Garchiroli districts.



Fructose / Glucose Ratio (%)

The fructose by glucose ratio in the Summer session honey samples of Wardha and Gadchiroli came to 1.7350±0.07154 and 1.8012±0.0321 respectively, while

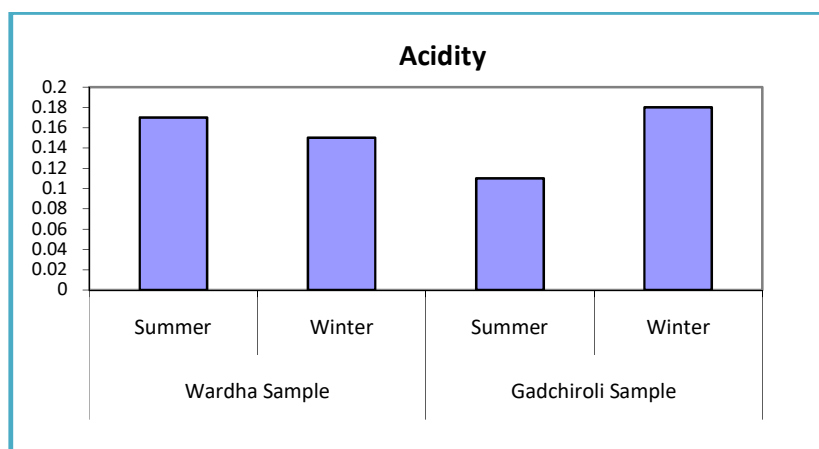
winter session reading of Wardha honey sample came to 1.3012±0.0101 and Gadchiroli honey sample is 1.4502±0.0125. It suggests that honey do not granulate as the ration is above 0.95 in all the samples.



Acidity

The acidity present in summer session honey samples of Wardha and Gadchiroli was measured to be about 0.17±0.0012 and 0.11±0.001 respectively. Likewise winter session honey sample of Wardha and

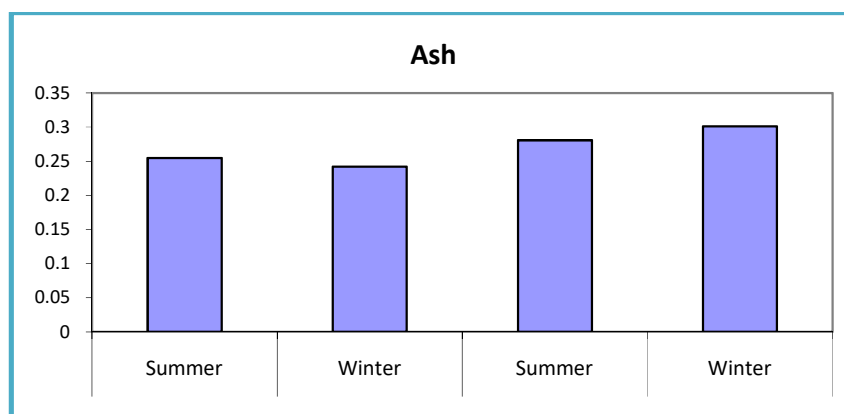
Gadchiroli was measured to be 0.15±0.0013 and 0.18±0.0012. There has been no significant difference recorded as far acidity was concerned in these different honey samples.



Ash Content

The ash (minerals) present in Summer session honey samples of Wardha and Gadchiroli was measured to be about 0.25486 ± 0.0020 and 0.28104 ± 0.0021 respectively, while winter session honey

sample was measured to be about 0.2421 ± 0.0017 and 0.301 ± 0.0019 . There has been no significant difference recorded as far acidity was concerned in these different honey samples.



Aniline chloride Test (ACT)

The ACT test is performed for the detection of commercial sugars (adulteration) in honey. The positive result ensures added jaggery in honey, however, not a single sample collected for analysis tested positive indicating the zero level of adulteration.

Fieh's test

The positive Fieh's test of honey sample confirms the over-heating of honey during processing or older honey. Not a single sample collected for analysis was tested positive confirming the freshness of honey sample.

Conclusions

The present study demonstrates no significant variations in some physico-chemical characteristics of different honey samples of *A. dorsata* bee-species collected during the summer season and winter session from the wardha and Gadchiroli districts of Vidarbha region in Maharashtra state. However, these parameters clearly pass with the standards set by national & international honey certification agencies.

Farmers in the region can adopt Honey Harvesting as their side business as the honey produced by *Apis Dorsata* has more medicinal values and international market

demand that can lead the rural population towards the Rural Development.

So the farmers of the region can add Honey Harvesting as their side business along with their routine farming. It can enhance their source of income leading towards Rural Development; it will help to overcome financial crises of farmers.

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