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# STORAGE STABILITY STUDIES ON VITAMIN A FORTIFIED SUNFLOWER (*Helianthus annuus*) OIL

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***Abstract:*** Fortification of foods is a well sighted path for solving nutritional problems in developing society. Common vegetable oil such as sunflower oil was utilized for fortification with Vitamin A. The objective of this study was to fortify the sunflower oil with vitamin A as per World Food Program (2011) guideline and to test the stability of vitamin A during different storage, packaging and deep fat frying conditions. The fortified oil was analyzed for a period of 5 months for its physic-chemical properties under various study conditions. The result of this study showed that vitamin A added to oil stored at room temperature under dark condition was stable for the entire study period. The retention of vitamin A in deep fat fried sunflower oil was found to be 85%, 68%, 49%, 38%, 19% and 7% for first, second, third, fourth, fifth and sixth frying, respectively. Further from the study was understood that there was no significant difference in the physic-chemical properties of the fortified oil during the study period. As a whole, the results of this study clearly revealed that Vitamin A added to refined oil remains stable during commonly adopted storage conditions while there is a significant reduction in vitamin A content during frying cycle.

***Key words****: fortification, physic-chemical properties sunflower oil, storage, vit. A*

## INTRODUCTION

Deficiency of vitamin A is a common health complication in many parts of the world [1]. Vitamin A insufficiency continues to be a major public health nutritional dispute in India. The prevalence of Bitot's spot, the objective sign of clinical vitamin A deficiency (0.8%) was higher than the figures endorsed by the WHO (0.5%), indicating the public health implication in rural pre-school children of India [2]. Analysis of blood samples revealed that the overall median vitamin A level was16.8 mg/dL, and ranged

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from a low 9 $mg \cdot dl^{-1}$ in Madhya Pradesh to a high 20.1 $mg \cdot dl^{-1}$ in Tamil Nadu. About 62% of children in general, had vitamin A levels of <20 $mg \cdot dl^{-1}$, indicating sub-clinical vitamin A deficiency (*VAD*). The proportion of children with sub-clinical *VAD* was significantly higher among 3-5 year children (63.1%) compared to 1-3 years (59.6%) [3]. The median intake of vitamin A (124 $\mu g \cdot CU^{-1} \cdot day^{-1}$) was grossly deficient as compared to *RDA* 600 $\mu g \cdot CU^{-1} \cdot day^{-1}$ in all the States [4]. A consequence of severe vitamin A deficiency, especially in children, is ocular lesions leading to xerophthalmia and blindness (*WHO,* 1982). Acute and subclinical deficiency of vitamin A is also known to increase the risk of respiratory infections and diarrhea [5] and thereby influence morbidity and mortality rate in infants [6]. The considerable cause of vitamin A deficiency are incompetent dietary intake of the preformed retinol or precursors of vitamin A, increased vitamin A requirement in certain physiological or pathological conditions, inadequate absorption, or loss of intestinal contents in diarrhea [7]. The three main mediation approaches across micronutrient malnutrition are: direct supplementation of micronutrients, dietary improvements and micronutrient fortification of common foods [7]. Fortification with vitamin A sounds to be most adequate approach for the prevention and control of vitamin A deficiency in various parts of the world [8]. It becomes very significant to select an appropriate carriage which is most suitable for fortification with vitamin A. The use of vegetable oils is increasing very rapidly in all regions of India. Oil is the most suitable vehicle for vitamin A fortification and efforts to fortify vegetable oil with retinyl palmitate have been well-established at a low cost. The oil matrix protects against the oxidation of vitamin A during storage, improves stability of the retinol, and facilitates the vitamin's absorption by the body [9]. Sunflower oil provides significant health benefits and supplies more vitamin E than any other vegetable oil. Since sunflower oil has less saturated fat it lowers cholesterol more significantly which reduces the risk of many heart problems [10]. Sunflower oil contains the highest percentage of poly unsaturated fatty acid (71%) with the predominant presence of linolenic acid which makes this oil suitable for use as a salad oil [11]. The objective of this study was to evaluate the stability of vitamin A fortified in sunflower oil during various conditions of storage, packaging material and during deep fat frying of food.

## MATERIAL AND METHODS

Particular brand of refined sunflower oil manufactured in specific date was purchased from local market and used throughout the study. Pure retinyl palmitate was purchased from Nicholas Piramal India limited, Thane and used for the fortification. All chemicals used for analysis were of analytical grade and purchased from *SGS* private limited, Chennai.

*Vitamin A fortification.* A large batch of vitamin A fortified sunflower oil was prepared by heating the commercial oil to 45-50°C [12]. The recommended dosage [13] of retinyl palmitate at the rate of 30 $IU \cdot g^{-1}$ of sunflower oil was added by constant stirring to ensure complete mixing of vitamin A with the sunflower oil.

*Storage study analysis*. One liter fortified oil was packed in two different packaging materials viz, *PET* bottle and nylon pouch (commercially accepted) and stored at room temperature under normal light and dark Conditions. Required quantity of control and fortified oil was drawn from each packaging material analyzed for its various physico-chemical properties viz, moisture content, refractive index, color, saponification value,

acid value, free fatty acid value, iodine value, and peroxide value as per the methods described by *FSSAI* (2012) [14].

*Deep fat frying and vitamin A retention*. Deep frying of papadum was performed using fortified sunflower oil to check the Vitamin A retention after frying. Totally six frying were carried out in two cycles. First cycle was performed with a representative portion of Vitamin A-fortified sunflower oil heated in a stainless steel frying pan and three portions of papadum (15g) were fried. The left out oil was stored for three days and second cycle was repeated in the same manner. After each frying, an aliquot of frying oil (approximately 100 ml) was drawn for the analysis of Vitamin A and other physico-chemical properties. The practical significance of this trial was to determine the retention of Vitamin A during repeated use of the same oil for frying foods during common household practice and during extreme situations.

*Quantification of vitamin A.* The analysis of vitamin A in oil and in food samples was carried out according to the method of *HPLC* or high pressure liquid chromatography [15]. The method consisted of weighing aliquots of oil, carrying out saponification and extraction of the lipid fraction, evaporating ether extract, and dissolving the residue in n-hexane, and injecting the final extract in the chromatograph. The conditions used for *HPLC* analysis were as follows: (a) stationary phase: Lichrosorb Si 60.5 m, length 12.5 cm and internal diameter 4 mm; (b) moving phase: n-hexane containing 2% of isopropanol (isocratic); (c) flow rate: 1 ml·min$^{-1}$; (d) injection volume: 10-50 ~1; (e) pressure: about 40 bar; (f) detection: by fluorescence (excitation: 325 nm and emission: 480 nm). The amount of vitamin A in samples was calculated by measuring the area under the peak of the sample and comparing it with the area under the peak of the known standard of vitamin A, which was made by saponification of pure all-trans-retinyl acetate in cotton seed oil (*USP* capsule), and used for external calibration.

*Statistical analysis*. Data collected during the study period was statistically analyzed using *SPSS* 20.0 for windows. The mean, standard deviation, coefficient of variation in percent (*CV* %) and the test of significance were performed.

## RESULTS AND DISCUSSION

*Stability of Vitamin A in fortified sunflower oil during storage.* Fortified sunflower oil sealed in nylon pouch and stored under normal light and dark condition, retained 97 and 100% vitamin A respectively for a period of 5 months. On the other hand fortified sunflower oil packed in *PET* bottles retained 98.2 and 100% vitamin A respectively, for the same condition. Several studies were reported on fortification of Vitamin A in different food products also confirms the study results [16] [17]. The stability of vitamin A in different vegetable oils was studied and found that stability of vitamin A decreases on exposure to light. The content of added vitamin A to the soybean oil which was protected against light was unaltered up to six months. The outcome of the quality assessment of the fortified sunflower oil during storage in the different packaging material and storage conditions are given in Tabs 1, 2, 3, 4.

The fortified oil stored in nylon pouch under light condition has peroxide value between 0.63 to 6.43 meq·kg$^{-1}$, IV was 130-133.86, free fatty acid value was between 0.03-0.10%, acid value was between 0.050-0.085 mgKOH·g$^{-1}$ and saponification value was between 190-194 mgKOH·g$^{-1}$ and for the oil which was stored under dark condition has peroxide value between 0.63-4.02 meq·kg$^{-1}$, IV was 130-133.16, free fatty acid value

was between 0.03-0.08% acid value was between 0.050-0.07 mgKOH·g$^{-1}$ and saponification value was between 190-193.61 mg KOH/g. The fortified oil stored in *PET* bottle under light condition has peroxide value between 0.63 to 3.24 meq·kg$^{-1}$, IV was 130-133.62, free fatty acid value was between 0.03-0.10%, acid value was between 0.050-0.072 mgKOH·g$^{-1}$ and saponification value was between 190-192.71 mgKOH·g$^{-1}$ and for the oil which was stored under dark condition has peroxide value between 0.63-2.87 meq·kg$^{-1}$, IV was 130-132.69, free fatty acid value was between 0.03- 0.08%, acid value was between 0.050-0.060 mg KOH·g$^{-1}$ and saponification value was between 190-191.84 mg KOH·g$^{-1}$.

*Table 1. Storage stability studies of sunflower oil (pouch light)*

| *No* | *Period of storage* | *Color* | *Moisture content (%)* | *Rf* | *AV (mgKOH·g$^{-1}$)* | *FFA (%)* | *PV (mEq·kg$^{-1}$)* | *SV (mgKOH·g$^{-1}$)* | *IV* | *Vit. A (%)* |
|---|---|---|---|---|---|---|---|---|---|---|
| *1* | *Before fortification* | *7* | *0.03* | *1.466* | *0.05* | *0.03* | *0.63* | *190.00* | *130.00* | *Nil* |
| *2* | *After fortification* | *7* | *0.03* | *1.466* | *0.05* | *0.03* | *0.65* | *190.32* | *131.37* | *100* |
| *3* | *First month* | *7* | *0.03* | *1.466* | *0.052* | *0.05* | *0.74* | *191.24* | *132.92* | *100* |
| *4* | *Second month* | *7* | *0.03* | *1.466* | *0.055* | *0.07* | *1.67* | *192.42* | *133.86* | *100* |
| *5* | *Third month* | *7* | *0.03* | *1.468* | *0.061* | *0.08* | *3.67* | *193.62* | *134.12* | *99* |
| *6* | *Fourth month* | *7* | *0.03* | *1.468* | *0.07* | *0.08* | *5.04* | *193.96* | *134.34* | *98* |
| *7* | *Fifth month* | *7* | *0.03* | *1.468* | *0.085* | *0.10* | *6.43* | *194.00* | *134.39* | *97* |
| *8* | *Mean* | *7* | *0.03* | *1.467* | *0.054* | *0.06* | *1.205* | *191.83* | *133.86* | *100* |
| *9* | *Standard deviation* | *0* | *0.00* | *0.00099* | *0.012* | *0.025* | *2.2567* | *1.59* | *1.54* | *1.22* |
| *10* | *CV (%)* | *0* | *0.00* | *13.21* | *21.23* | *11.24* | *78.36* | *21.32* | *12.58* | *18.9* |

*FFA- Free Aftty Acid, $R_f$- Refractive Index, AV- Acid Value, PV- Peroxide Value, SV- Saponification Value, IV- Iodine Value*

*Table 2. Storage Stability studies of sunflower oil (pouch dark)*

| *No* | *Period of storage* | *Color* | *Moisture content (%)* | *Rf* | *AV (mgKOH·g$^{-1}$)* | *FFA (%)* | *PV (mEq·kg$^{-1}$)* | *SV (mgKOH·g$^{-1}$)* | *IV* | *Vit. A (%)* |
|---|---|---|---|---|---|---|---|---|---|---|
| *1* | *Before fortification* | *7* | *0.03* | *1.466* | *0.05* | *0.03* | *0.63* | *190.00* | *130.00* | *Nil* |
| *2* | *After fortification* | *7* | *0.03* | *1.466* | *0.05* | *0.03* | *0.65* | *190.32* | *131.37* | *100* |
| *3* | *First month* | *7* | *0.03* | *1.466* | *0.051* | *0.05* | *0.94* | *191.12* | *132.36* | *100* |
| *4* | *Second month* | *7* | *0.03* | *1.466* | *0.053* | *0.05* | *1.92* | *191.96* | *132.96* | *100* |
| *5* | *Third month* | *7* | *0.03* | *1.468* | *0.059* | *0.05* | *2.84* | *193.26* | *133.07* | *100* |
| *6* | *Fourth month* | *7* | *0.03* | *1.468* | *0.063* | *0.07* | *3.59* | *193.54* | *133.16* | *100* |
| *7* | *Fifth month* | *7* | *0.03* | *1.468* | *0.07* | *0.07* | *4.02* | *193.61* | *133.16* | *100* |
| *8* | *Mean* | *7* | *0.03* | *1.467* | *0.052* | *0.05* | *1.43* | *191.54* | *132.66* | *100* |
| *9* | *Standard deviation* | *0* | *0.00* | *0.00099* | *0.0073* | *0.0163* | *1.3346* | *1.42971* | *1.05534* | *0* |
| *10* | *CV (%)* | *0* | *0.00* | *7.38* | *12.22* | *28.33* | *59.35* | *16.52* | *19.36* | *0* |

The FFA, AV, IV, SV and PV got increased for all the samples including control. All the physic-chemical values of fortified oil were within the limits prescribed by World Food Program guidelines for the entire study period. The reason for this increase

in all these parameters may be due to the absorption of moisture from the atmosphere, oxidation reaction, heat and light [18]. The free fatty acid values obtained during the entire period of storage was below the maximum value of 0.15% as recommended by World Food Program (2011) [13]. The IV is an index of instauration which gives the molecular weight of their fatty acid composition. The highest level of PV was found in the sample stored in pouch under normal light condition. However, the values obtained were within the acceptable limit of 10 meq·kg$^{-1}$ [18]. The low PV indicated that the oil had lower susceptibility to oxidative rancidity and is suitable to be stored for some more time without any appreciable deterioration. Other parameters like SV, moisture content, refractive index were also within the limits of 188-194 mgKOH·g$^{-1}$ oil, 0.2%, 1.461-1.468 respectively [13]. Subjective examination of the stored oil sample indicated no alteration in the organoleptic properties such as taste, smell, and color of fortified oil.

*Table 3. Storage stability studies of sunflower oil (pet light)*

| *No* | *Period of storage* | *Color* | *Moisture content (%)* | *Rf* | *AV (mgKOH·g$^{-1}$)* | *FFA (%)* | *PV (mEq·kg$^{-1}$)* | *SV (mgKOH·g$^{-1}$)* | *IV* | *Vit. A (%)* |
|---|---|---|---|---|---|---|---|---|---|---|
| *1* | *Before fortification* | *7* | *0.03* | *1.466* | *0.05* | *0.03* | *0.63* | *190* | *130* | *Nil* |
| *2* | *After fortification* | *7* | *0.03* | *1.466* | *0.05* | *0.03* | *0.65* | *190.32* | *131.37* | *100* |
| *3* | *First month* | *7* | *0.03* | *1.466* | *0.053* | *0.04* | *0.68* | *190.78* | *132.94* | *100* |
| *4* | *Second month* | *7* | *0.03* | *1.466* | *0.055* | *0.05* | *0.88* | *191.54* | *133.01* | *100* |
| *5* | *Third month* | *7* | *0.03* | *1.467* | *0.06* | *0.07* | *1.72* | *192.58* | *133.49* | *99* |
| *6* | *Fourth month* | *7* | *0.03* | *1.467* | *0.068* | *0.07* | *2.12* | *192.63* | *133.54* | *98* |
| *7* | *Fifth month* | *7* | *0.03* | *1.467* | *0.072* | *0.08* | *3.24* | *192.71* | *133.62* | *97* |
| *8* | *Mean* | *7* | *0.03* | *1.467* | *0.054* | *0.05* | *0.78* | *191.16* | *132.975* | *100* |
| *9* | *Standard deviation* | *0* | *0.00* | *0.00053* | *0.008* | *0.0206* | *0.949* | *1.08071* | *1.21780* | *1.215* |
| *10* | *CV (%)* | *0* | *0.00* | *7.83* | *15.84* | *27.23* | *83.34* | *12.52* | *23.58* | *18.92* |

*Table 4. Storage stability studies of Sunflower Oil (pet dark)*

| *No* | *Period of storage* | *Color* | *Moisture content (%)* | *Rf* | *AV (mgKOH·g$^{-1}$)* | *FFA (%)* | *PV (mEq·kg$^{-1}$)* | *SV (mgKOH·g$^{-1}$)* | *IV* | *Vit. A (%)* |
|---|---|---|---|---|---|---|---|---|---|---|
| *1* | *Before fortification* | *7* | *0.03* | *1.466* | *0.05* | *0.03* | *0.63* | *190* | *130* | *Nil* |
| *2* | *After fortification* | *7* | *0.03* | *1.466* | *0.05* | *0.03* | *0.65* | *190.32* | *131.37* | *100* |
| *3* | *First month* | *7* | *0.03* | *1.466* | *0.05* | *0.03* | *0.65* | *190.45* | *131.98* | *100* |
| *4* | *Second month* | *7* | *0.03* | *1.466* | *0.051* | *0.05* | *0.95* | *191.08* | *132.18* | *100* |
| *5* | *Third month* | *7* | *0.03* | *1.466* | *0.055* | *0.06* | *1.23* | *191.67* | *132.43* | *100* |
| *6* | *Fourth month* | *7* | *0.03* | *1.466* | *0.06* | *0.07* | *2.04* | *191.75* | *132.62* | *100* |
| *7* | *Fifth month* | *7* | *0.03* | *1.466* | *0.06* | *0.07* | *2.87* | *191.84* | *132.69* | *100* |
| *8* | *Mean* | *7* | *0.03* | *1.466* | *0.0505* | *0.05* | *0.8* | *190.705* | *132.08* | *100* |
| *9* | *Standard deviation* | *0* | *0.00* | *0* | *0.0044* | *0.0186* | *0.8958* | *0.71129* | *0.85345* | *0* |
| *10* | *CV (%)* | *0* | *0.00* | *7.83* | *13.128* | *25.83* | *53.27* | *7.84* | *35.87* | *17.89* |

Statistical result depicts that CV (%) among the parameters with peroxide value being the highest; refractive index value was very least while color and moisture content

showed no variation. However there was similarity in the peroxide value in all the containers. There was similarity in CV (%) in parameters such as color, refractive index value, acid value and moisture content. However they were no significant differences in parameters such as free fatty acid, saponification value and iodine value (Tab 5). All these results were compared with triplicates with ±SD values.

*Table 5.Chemical properties of oils stored different containers and storage conditions*

| *Parameter* | *f-value* | *Test of significance* |
|---|---|---|
| *Color* | *0.00* | *s* |
| *Moisture content* | *0.00* | *s* |
| *Refractive index* | *0.03* | *s* |
| *Acid value* | *0.07* | *s* |
| *Free Fatty acid* | *0.15* | *ns* |
| *Peroxide value* | *0.05* | *s* |
| *Saponification Value* | *0.173* | *ns* |
| *Iodine Value* | *1.00* | *ns* |
| *Vitamin A* | *Not aplicable* | *Not aplicable* |

*Table 6. Stability studies of sunflower oil during deep fat frying*

| *No* | *Period of storage* | *Color* | *Rf* | *AV ($mgKOH \cdot g^{-1}$)* | *FFA (%)* | *PV ($mEq \cdot kg^{-1}$)* | *SV ($mgKOH \cdot g^{-1}$)* | *IV* | *Vit. A (%)* |
|---|---|---|---|---|---|---|---|---|---|
| *1* | *Before frying* | *7* | *1.466* | *0.05* | *0.05* | *0.63* | *190* | *128.21* | *100* |
| *2* | *First frying* | *7* | *1.467* | *0.104* | *0.06* | *4.34* | *190.46* | *129.53* | *85* |
| *3* | *Second frying* | *7* | *1.467* | *00.13* | *0.06* | *5.55* | *190.82* | *12.70* | *68* |
| *4* | *Third frying* | *7* | *1.467* | *0.22* | *0.06* | *7.99* | *191.56* | *129.70* | *49* |
| *5* | *Mean* | *7* | *1.467* | *0.117* | *0.06* | *4.945* | *190.64* | *130.16* | *68* |
| *6* | *Standard deviation* | *0* | *0.0005* | *0.062* | *0.0045* | *2.6445* | *0.57121* | *0.73699* | *14.71* |
| *7* | *CV (%)* | *0* | *17.83* | *12.38* | *77.84* | *56.37* | *57.23* | *56.38* | *59.36* |

*Table 7. Stability studies after 3 days storage*

| *No* | *Period of storage* | *Color* | *Rf* | *AV ($mgKOH \cdot g^{-1}$)* | *FFA (%)* | *PV ($mEq \cdot kg^{-1}$)* | *SV ($mgKOH \cdot g^{-1}$)* | *IV* | *Vit. A (%)* |
|---|---|---|---|---|---|---|---|---|---|
| *1* | *First frying* | *7* | *1.467* | *0.206* | *0.07* | *8.23* | *191.72* | *13.22* | *38* |
| *2* | *Second frying* | *8* | *1.467* | *0.31* | *0.07* | *8.56* | *191.86* | *13.29* | *19* |
| *3* | *Third frying* | *8* | *1.467* | *0.35* | *0.08* | *9.01* | *191.97* | *130.34* | *7* |
| *4* | *Mean* | *7* | *1.467* | *0.31* | *0.07* | *8.56* | *191.86* | *130.16* | *19* |
| *5* | *Standard deviation* | *0.578* | *0* | *0.0616* | *0.005* | *0.3203* | *0.10243* | *0.07889* | *12.82* |
| *6* | *CV (%)* | *57.38* | *21.82* | *22.38* | *25.86* | *26.68* | *23.23* | *38.63* | *28.39* |

*Stability of vitamin A in oil during Cooking*. The *HPLC* results revealed that Vitamin A added to sunflower oil was reduced to less than 50% after 3 repeated frying and that it took about 6 repeated frying to destroy the most of the fortified Vitamin A (Tabs 6 and 7). Previous studies on fortification of vitamin A in vegetable oil reported

that the biological value of vitamin A fortified vegetable oil is reduced by about half after frying of foods [16].

## CONCLUSIONS

The result of this study clearly indicates that the stability of vitamin A palmitate in vegetable oils is satisfactory during its storage and frying. Fortification of common vegetable oils with vitamin A is therefore feasible. The processing and distribution of vegetable oils is well organized and fortification can easily be implemented and is cost effective. Vegetable oils are well liked and relatively inexpensive sources of fat. Refined oil has no cholesterol and is rich sources of other naturally occurring nutrients such as polyunsaturated fatty acids and vitamin E. The average amount of vegetable oil consumed is about 30 g per person per day [19] which if fortified at the rate of 30 $IU \cdot g^{-1}$ can contribute up to 900 IU of vitamin A per day, respectively.

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## STABILNOST VITAMINA A PRI SKLADIŠTENJU I KORIŠĆENJU OBOGAĆENOG SUNCOKRETOVOG ULJA (*Helianthus annuus*)

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***Sažetak:*** Obogaćivanje hrane je dobar način za rešavanje prehrambenih problema u društvima u razvoju. Obična biljna ulja, kao što je suncokretovo, upotrebljena su za obogaćivanje vitaminom A. Cilj ove studije bio je da suncokretovo ulje obogati vitaminom A prema uputstvu Svetskog programa hrane (2011) i da testira stabilnost vitamina A u različitim uslovima skladištenja, pakovanja i prženja. Obogaćeno ulje je analizirano u period od 5 meseci. Rezultati su pokazali da je dodati vitamin A bio stabilan tokom celog perioda skladištenja na sobnoj temperaturi u tamnoj prostoriji. Retencija vitamina A pri prženju u dubokoj masnoći bila je 85%, 68%, 49%, 38%, 19% i 7% od prvog do šestog prženja, redom. Nije bilo značajne razlike u fizičko-hemijskim osobinama obogaćenog ulja. Ukupni rezultati studije su jasno pokazali da vitamin A dodat u rafinisano ulje ostaje stabilan tokom skladištenja u uobičajenim uslovima, dok tokom prženja u dubokoj masnoći dolazi do značajnog smanjenja sadržaja vitamina A.

***Ključne reči****: obogaćivanje, fizičko-hemijske osobine suncokretovog ulja, vitamin A*

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