

## Comparison of antioxidative potentials of methanol sweet basil (*Ocimum basilicum*) extract and butylatedhydroxytoluene on stability of refined soybean oil.

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### Abstract

Sweet basil leaves were obtained from a backyard garden at Oka in Ondo-City, Nigeria. The leaves were rinsed with water to remove the dirt, air-dried and ground to powdery sample. The leaf sample was then extracted with methanol by adding 20 g of the powdery sample into 200 mL of methanol and left it for 72 hours. The extract was obtained by concentrating the filtrate from the mixture in a rotary evaporator. The extract was dosed at varying concentration (200-1000 ppm) to refined soybean oil (RSBO). Another set up was made by dosing RSBO with 400 ppm butylatedhydroxytoluene (BHT). A control which contained no additive (0 ppm) was also set up. The oil was stored in a transparent plastic bottle for five months. The antioxidative potential of methanol extract of sweet basil (MESB) at varying concentrations (200-1000 ppm) was compared with 400 ppm BHT on RSBO by monthly monitoring their refractive index (RI), free fatty acid (FFA), acid value (AV) and peroxide value (PV) for five months. There was no remarkable difference in RI of RSBO containing additives and that of the control sample. The mean value of FFA, AV and PV of RSBO containing MESB were lower than the RSBO containing 400 ppm BHT over the five months of storage. There were significance difference ( $P < 0.05$ ) in average FFA, AV and PV of RSBO containing MESB, BHT and RSBO without additive. It is therefore noticed that MESB is superior to BHT in combating both hydrolytic and oxidative rancidity of RSBO.

**Keywords:** Refined soybean oil, Sweet basil, Stability, Methanol extract, Antioxidants.

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### Introduction

Refined soybean oil is an edible oil obtained from soybean seeds and the oil contains more unsaturated fatty acid than saturated fatty acid hence it is a liquid at room temperature [1]. The crude soybean oil is often obtained from soybean seeds through solvent extraction using n-hexane [2]. The crude oil is being processed to refined oil by subjecting it to degumming, neutralization, bleaching and deodorization [3]. The refined oil has a superb organoleptic and nutritive qualities hence it is relatively more expensive than most of the commonly available edible oils like palm oil, palm kernel oil, groundnut oil etc. Being an edible oil, refined soybean oil is also prone to deterioration during storage and the deterioration is termed rancidity which can be hydrolytic and/or oxidative. An indiscriminate consumption of rancid oil by humans has been found to be a major cause of heart related diseases such as heart failure, stroke, hypertension, thrombosis, obesity, atherosclerosis etc. [4-7].

Consequently, there is a need to preserve edible oils from going rancid by addition of additives which will delay the onset of both hydrolytic and oxidative rancidity. Most of these additives are antioxidants that reduce oxidative damage caused by highly reactive chemicals (free radicals) that attack oil molecules. Common synthetic antioxidants such as propyl gallate, butylatedhydroxyanisole, butylatedhydroxytoluene, tertiarybutylatedhydroxyquinone etc. have been found effective

in prolonging the shelf life of edible oils but they are also being found to be carcinogenic, toxic, mutagenic, costly and not readily available [8-10]. Due to the foregoing reasons, the usage of these synthetic antioxidants as additives or preservatives is being banned in the international market [11,12]. This necessitates the rousing interest of food chemists, nutritionists and scientists in seeking for alternative sources that are cheaper and safer from plant origin by using plant extracts as additives in combating vegetable oil rancidity during storage [13-17].

Sweet basil originated from India and it is botanically known as *Ocimum basilicum*. It is a culinary herb which prominently features in Italian cuisine of Taiwan. It is an annual plant which requires tropical climate. Sweet basil is 30-130 cm tall with silky leaves which are 3 cm long and 1 cm broad. Its flowers are small, white in colour and arranged in a terminal spikes [18]. The plant has a distinct scent and an essential oil called eugenol. The distinct scent is produced as a result of some of the chemical composition present in the plant such as citronellol, mycenae, tarpileol, octanes, methyl eugenol, eugenol etc. [19]. The sweet basil leaves have been found to contain carbohydrate, dietary fibre, fat, protein, water, vitamin A, B1, B2, B3, B6, C, E and K as well as minerals [20]. The leaves are traditionally used as supplementary treatment for stress, asthma, diabetes and also used in medicine for treating pimples [21]. Consequently, this suggests that the plant contains phytochemicals that have antioxidant properties.

Therefore the focus of this research is to obtain extracts from sweet basil leaves using methanol; investigating the antioxidative potential of the extract at varying concentrations (200-1000 ppm) on refined soybean oil; determining the effect of the extract on refractive index of the oil as well as comparing the antioxidant activities of the extracts with that of butylatedhydroxytoluene (400 ppm BHT) by monthly monitoring their free fatty acid (FFA), acid value (AV) and peroxide value (PV) during five months storage of the oil in white transparent plastic bottles. .

## Materials and Methods

### Sources of materials

Sweet basil leaves were obtained from a backyard garden at Oka in Ondo-City, Ondo-State, Nigeria. The refined soybean oil was obtained before being fortified with vitamin A at JOF Ideal Family Farms Limited, Owo, Ondo-State, Nigeria.

### Preparation and extraction of sweet basil leaves

The sweet basil leaves were removed from their stems, rinsed with water and cut into smaller pieces for easy air-drying. The dried leaves were ground using electric blending machine and it was sieved with 40 mm mesh size. The powdery samples were packed into a black polyethene bags labelled appropriately prior to extraction. Twenty gram of the powdery sample was weighed into a cleaned and dried reagent bottle; and 200 mL of methanol was added to the bottle and left for 72 hours during which it was intermittently shaken on a shaking orbit machine. The mixture was filtered through a 0.45 µm Nylon membrane filter. The extract was evaporated to dryness under reduced pressure at 40°C by a rotary evaporator [15,22].

### Addition of additives to refined soybean oil (RSBO)

Methanol extract of sweet basil leave at concentration of 200 ppm (0.02 g per 100 mL oil) to 1000 ppm (0.10 g per 100 mL oil)

were separately added to refined soybean oil (RSBO) contained in white transparent plastic bottles of equal capacity and they were thoroughly shaken for proper mixing. RSBO containing 400 ppm BHT (0.04 g per 100 mL oil) and that which contained no additive (0 ppm (control)) were also set-up. Each container was appropriately labelled and stored in an open place at room temperature ranging from 29°C to 35°C.

### Physical and chemical analysis

As soon as the set up was done, the refractive index (RI) was determined using Abbe's Refractometer at 40°C. Thereafter, the refractive index (RI), free fatty acid (FFA), acid value (AV), and peroxide value (PV) of each oil sample were monitored monthly using standard method of analysis [23] for a period of five months.

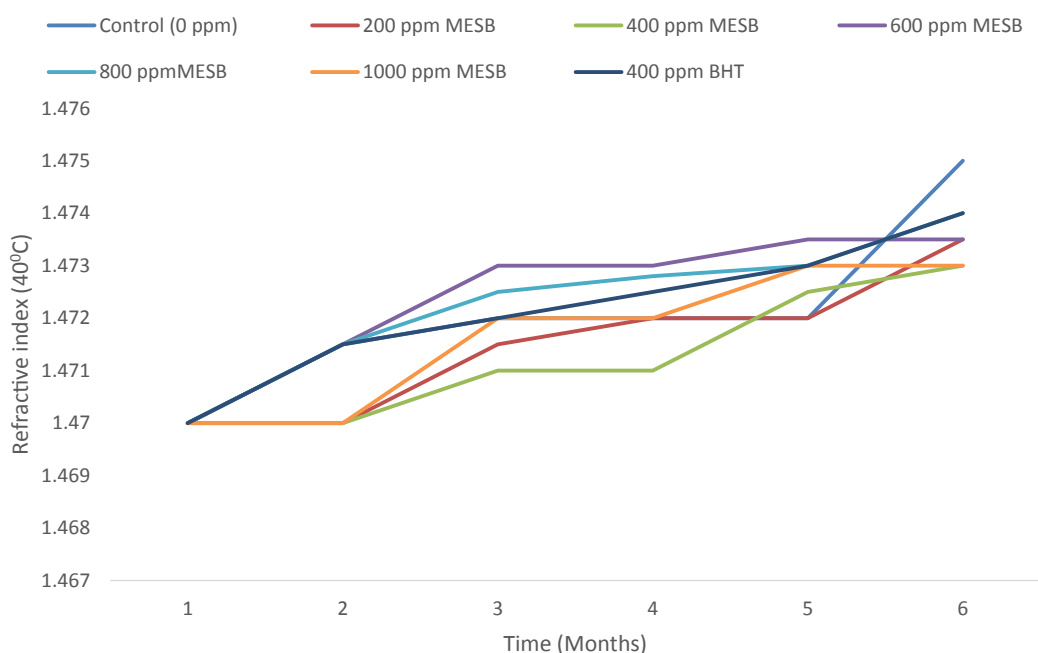
### Statistical analysis

The results were compared by one-way analysis of variance (one-way ANOVA) to test for significant difference. Means of the group were compared using Duncan Multiple Range Test (DMRT) [24].

## Results and Discussion

Figure 1 presents the refractive index of refined soybean oil stored with varying concentrations (200-1000 ppm) of methanol extract of sweet basil (MESB) and 400 ppm butylatedhydroxytoluene (BHT) for a period of five months. There was gradual increase in the refractive index of the oil samples stored for five months but the values were still within the accepted standard of 1.470-1.476 specified by Standards Organization of Nigeria [25]. Detailed discussion on the refractive index of refined soybean oil stored with the additives is made in Table 1 where the overall average for the five months can be conspicuously observed.

Figure 2 depicts the free fatty acid (FFA) of refined soybean oil (RSBO) stored with varying concentrations (200-1000 ppm) of methanol extract of sweet basil and 400 ppm

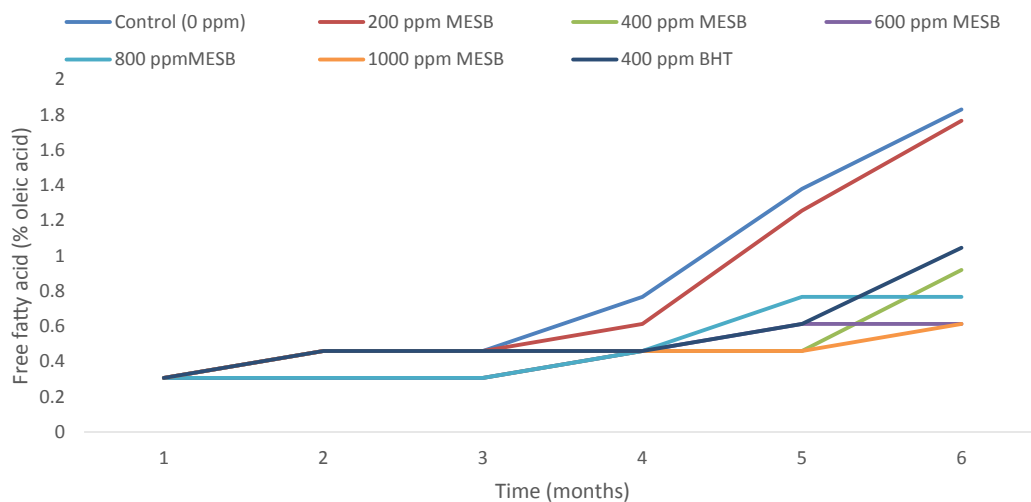


**Figure 1.** Refractive index of refined soybean oil stored with methanol extract of sweet basil and butylatedhydroxytoluene.

**Table 1.** Mean values of selected identity and quality properties of refined soybean oil stored with varying concentration of methanol extract of sweet basil (MESB) and 400 ppm butylatedhydroxytoluene (BHT) over a period of five months.

Concentration of Additive	*Refractive Index (RI) (at 40°C)	*Free Fatty Acid (FFA) (% Oleic acid)	*Acid Value (AV) (mgKOH/g oil)	*Peroxide Value (PV) (meqO <sub>2</sub> /Kg oil)
Control (0 ppm)	1.475 <sup>a</sup> ± 0.002	0.867 <sup>e</sup> ± 0.608	1.739 <sup>e</sup> ± 1.215	10.460 <sup>d</sup> ± 2.898
200 ppm MESB	1.473 <sup>a</sup> ± 0.001	0.810 <sup>d</sup> ± 0.575	1.618 <sup>d</sup> ± 1.151	9.510 <sup>bc</sup> ± 1.967
400 ppm MESB	1.473 <sup>a</sup> ± 0.001	0.460 <sup>ab</sup> ± 0.237	0.915 <sup>ab</sup> ± 0.472	8.786 <sup>a</sup> ± 1.663
600 ppm MESB	1.474 <sup>a</sup> ± 0.001	0.434 <sup>a</sup> ± 0.151	0.866 <sup>a</sup> ± 0.302	9.149 <sup>ab</sup> ± 1.475
800 ppm MESB	1.474 <sup>a</sup> ± 0.001	0.485 <sup>b</sup> ± 0.226	0.970 <sup>b</sup> ± 0.400	9.330 <sup>b</sup> ± 0.936
1000 ppm MESB	1.473 <sup>a</sup> ± 0.001	0.460 <sup>ab</sup> ± 0.097	0.916 <sup>ab</sup> ± 0.194	9.557 <sup>c</sup> ± 1.494
400 ppm BHT	1.474 <sup>a</sup> ± 0.001	0.557 <sup>c</sup> ± 0.258	1.113 <sup>c</sup> ± 1.086	9.572 <sup>c</sup> ± 1.859

NOTE: Within each column, mean values followed by the same superscript are not significantly different at P<0.05 level according to Duncan Multiple Range Test (DMRT). \*Mean Value ± Standard Deviation. MESB: Methanol Extract of Sweet Basil; BHT: Butylatedhydroxytoluene.



**Figure 2.** Free fatty acid of refined soybean oil stored with methanol extract of sweet basil and butylatedhydroxytoluene.

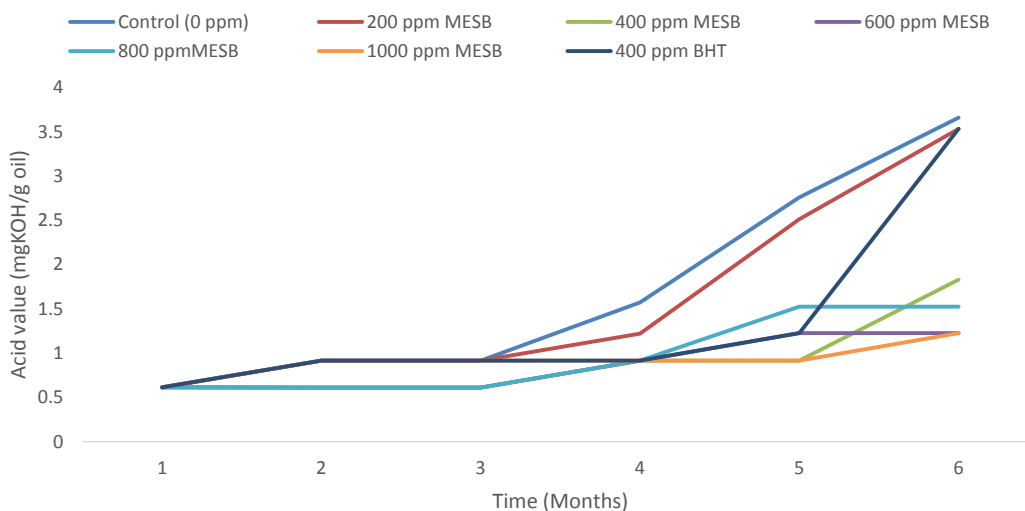
butylatedhydroxytoluene for a period of five months. The oil sample containing additives has lower free fatty acid than the oil sample without any additive (control) (0 ppm). Refined soybean oil containing 400 ppm to 1000 ppm methanol extract of sweet basil (MESB) has lower free fatty acid than refined soybean oil containing 400 ppm butylatedhydroxytoluene (BHT). There were gradually decrease in the free fatty acid of RSBO as the concentration of MESB increased from 200 ppm to 1000 ppm. The FFA plot resembles the plot obtained for FFA of palm kernel oil stored with varying concentration of sweet orange peel extract as reported by Arawande et al. [26]. Further discussion on the average effect of the additives on free fatty acid of RSBO is contained in Table 1 discussion.

Acid value (AV) of refined soybean oil stored with varying concentration (200-1000 ppm) of methanol extract of sweet basil and 400 ppm butylatedhydroxytoluene for a period of five months is shown in Figure 3. The AV plot resembles the plot obtained for AV of palm kernel oil stored with varying concentration of sweet orange peel extract as reported by Arawande et al. [26]. RSBO containing additives have lower acid value than the RSBO which contained no additive (0 ppm). It was observed that the acid value of RSBO containing methanol extract of sweet basil (MESB) decreased as the concentration of the extract increased in the oil sample. However, further discussion on the average effect of the additives on acid value of RSBO is contained in Table 1 discussion.

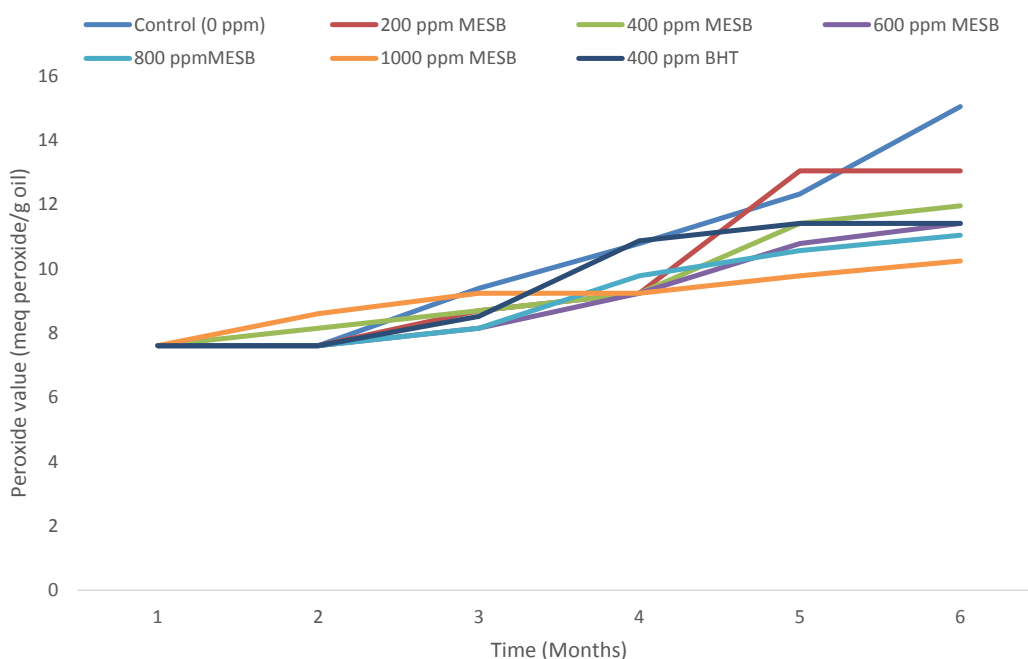
Figure 4 presents the peroxide value of refined soybean oil

stored with varying concentration (200-1000 ppm) methanol extract of sweet basil and 400 ppm butylatedhydroxytoluene for five months. The trend of the plot is very similar to the plot obtained by Amir et al. [15] for the plot of peroxide value of soybean oil mixed with pistachio hull extract. The oil sample containing additives have relatively lower peroxide values than the oil sample without any additive (control (0 ppm)). The RSBO containing 200 ppm-1000 ppm of methanol extract of sweet basil (MESB) had slightly lower peroxide value than RSBO containing 400 ppm BHT.

Table 1 shows the mean values of selected identity and quality properties of refined soybean oil stored with varying concentrations of methanol extract of sweet basil (MESB) and 400 ppm butylatedhydroxytoluene (BHT) over a period of five months. The identity property examined is refractive index (RI) while the quality properties considered are free fatty acid (FFA), acid value (AV) and peroxide value (PV). Refractive index of edible oil is an important physical identity parameter used to determine the level of purity of oil [27,28]. The addition of varying concentration (200-1000 ppm) of MESB to RSBO did not have much significant difference on the refractive index of RSBO. The average RI of RSBO containing 200-1000 ppm MESB was 1.473 ± 0.001 and 1.474 ± 0.001 while average RI of RSBO that contained no additive was 1.475 ± 0.002. The average RI of RSBO containing 400 ppm BHT was 1.474 ± 0.001. There was no significance difference (P<0.05) in average RI of RSBO containing additives (MESB and BHT) and RSBO



**Figure 3.** Acid value of refined soybean oil stored with methanol extract of sweet basil and butylatedhydroxyltoluene.



**Figure 4.** Peroxide value of refined soybean oil stored with methanol extract of sweet basil and butylatedhydroxyltoluene.

which contained no additive (control (0 ppm)). Therefore the presence of the additives in RSBO did not adulterate the oil.

The free fatty acid and acid value of edible oil is a measure of hydrolytic rancidity. The lower the values of both FFA and AV the better the quality of the oil and more stable is the oil against hydrolytic rancidity [28,29]. The mean FFA of RSBO containing MESB ranged between  $0.434 \pm 0.151$  and  $0.810 \pm 0.575\%$  oleic acid while the mean FFA of RSBO containing 400 ppm BHT had FFA of  $0.557 \pm 0.258\%$  oleic acid. The mean FFA of RSBO which contained no additive (0 ppm) was  $0.867 \pm 0.608\%$  oleic acid. The mean FFA of RSBO containing 400 ppm to 1000 ppm MESB was lower than mean FFA of RSBO stored with 400 ppm BHT. Apart from RSBO which contained 400 ppm and 1000 ppm MESB, the mean FFA of RSBO stored for five months was significantly different ( $P < 0.05$ ). The average AV of RSBO containing MESB ranged between  $0.915 \pm 0.472$  and  $1.618 \pm 1.151$  mg KOH/g oil and the average AV of

RSBO without additive (control (0 ppm)) was  $1.739 \pm 1.215$  mg KOH/g oil while the average AV of RSBO containing 400 ppm BHT was  $1.113 \pm 1.086$  mg KOH/g oil. The mean AV of RSBO containing 400 ppm to 1000 ppm of MESB were lower than the mean AV of RSBO without additive and RSBO containing 400 ppm BHT. There was no significant difference ( $P < 0.05$ ) in mean AV of RSBO containing 400 ppm and 1000 ppm MESB but there exist significant difference ( $P < 0.05$ ) in mean AV in other RSBO investigated. The optimal concentration of MESB against hydrolytic rancidity of RSBO is 600 ppm.

Peroxide value (PV) is one of the quality parameters of edible oils and it is an index of the extent of oxidative stability of edible oil. The lower the PV of edible oil the more stable is the oil against oxidative rancidity and the better the quality and safer of the oil for human consumption [26-30]. The mean PV of RSBO containing 200 ppm to 1000 ppm MESB ranged between  $8.786 \pm 1.663$  and  $9.557 \pm 1.494$  meq  $O_2$ /Kg oil while the mean PV of

RSBO without additive was  $10.460 \pm 2.898$  meq  $O_2$ /Kg oil. The mean PV of RSBO containing 400 ppm BHT was  $9.572 \pm 1.859$  meq  $O_2$ /Kg oil. The mean PV of RSBO containing varying concentrations (200-1000 ppm) of MEBS was lower than the mean PV of RSBO containing 400 ppm BHT. This shows that the methanol extract of sweet basil serves as a better antioxidant than BHT against oxidative rancidity of refined soybean oil. There was significance difference ( $P < 0.05$ ) in average PV of RSBO containing varying concentration of MESB but there was no significant difference ( $P < 0.05$ ) in average PV of RSBO containing 1000 ppm MESB and 400 ppm BHT. The optimal concentration of MESB in combating oxidative rancidity of RSBO is 400 ppm.

## Conclusion

Addition of varying concentration (200-1000 ppm) of methanol extract of sweet basil to refined soybean oil did not have significant difference on the refractive index of the oil but it lowered the free fatty acid, acid value and peroxide value of the oil than 400 ppm butylatedhydroxyltoluene did during the five months period of storage. The methanol extract of sweet basil served as a superior antioxidant than butylatedhydroxyltoluene in preventing the on-set of both hydrolytic and oxidative rancidity of refined soybean oil. It is therefore suggested that further investigation should be conducted by using other solvents in obtaining extract from sweet basil and examining their antioxidant activities on crude and refined edible oils such as groundnut oil, palm kernel oil, sesame oil, sunflower oil, palm oil etc. Higher concentrations of the extract above 1000 ppm can further be examined on these oils as well as extending storage period to twelve months.

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