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## **Effect of the Addition of Okra Seed (*Abelmoschus esculentus*) Flour on the Antioxidant Properties of Plantain *Musa paradisiaca* Flour**

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### **ABSTRACT**

**Aim:** This study aims at determining the antioxidant properties of plantain flour fortified with okra seed flour (full fat and defatted).

**Methodology:** Plantain *Musa paradisiaca* and fibrous okra *Abelmoschus esculentus* that cannot be cut with kitchen knife were used for this work. The plantains were made into flours while the seeds were removed from the okra pod, sundried, milled and sieved. The Okra seed flour (full fat and defatted) was used to fortify the plantain flour separately in the following ratio 90:10, 80:20 and 70:30. The order of antioxidant activity was evaluated by measurement of total phenolic content, vitamin C content, ABTS scavenging ability and the ferric reducing antioxidant property (FRAP) of the fortified plantain flour.

**Results:** The process of defatting caused a significant reduction in the total phenolic content (2.85%), vitamin C content (2.63%), ABTS scavenging ability (17.2%) and the reducing power (13.75%) of the okra seed flour. The antioxidant properties of the okra seed flour were significantly higher ( $P < 0.05$ ) than that of the plantain flour except the vitamin C where there was no significant difference ( $P > 0.05$ ) (plantain flour: 6.30mg/100g, defatted okra seed flour: 6.66 mg/100g and full fat okra seed flour: 6.84 mg/100g). The fortification of the plantain flour with the okra seed flour resulted in significant increase ( $P < 0.05$ ) in the total phenolic content, ABTS scavenging ability and the ferric reducing power of the fortified plantain flour as the percentage of okra seed flour increased.

**Conclusion:** The addition of okra seed flour to plantain flour should be encouraged because it increased the antioxidant properties of the resultant fortified plantain flour.

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**Key word:** Plantain flour; full fat okra seed flour; defatted okra seed flour; total phenol; vitamin C; FRAP reducing power; ABTS scavenging ability.

## 1. INTRODUCTION

Free radicals are molecules with incomplete electron shells, which make them more chemically reactive than those with complete electron shells. Free radical can be formed as a result of exposure to various environmental factors like tobacco smoke and radiation (Gate et al., 1999). Free radicals produced as a result of normal biochemical reactions in the body are implicated in various human diseases; therefore interest in reactive oxygen species (ROS) has substantially increased in direct relation to cellular abnormalities. The antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) play an important role in scavenging oxidants and preventing injury to cellular macromolecules. However, quantities of ROS which overwhelm the capacity of the body's defence system may result in irreversible oxidative damage to DNA, proteins and lipids causing cellular and metabolic injury and accelerating aging, cancer, cardiovascular diseases, neurodegenerative diseases and inflammation (Zarena and Sankar, 2009). Antioxidants neutralize the electrical charge and prevent the free radical from taking electrons from other molecules (Oboh and Rocha, 2006). Antioxidants are substances that are capable of counteracting the damaging but normal effects of the physiological process of oxidation in animal tissue; they are also nutrients such as vitamins and minerals as well as enzymes such as protein in the body that assists in chemical reactions (Sun et al., 2002). Increased concern over the safety of synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has led to an increased interest in exploration of effective and economical natural antioxidants (Iqbal et al., 2005). Natural polyphenols exert their beneficial health effects by their antioxidant activity, these compounds are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce -tocopherol radicals and inhibit oxidases (Oboh, 2006). Okra (*Abelmoschus esculentus* L.) is widely consumed as a fresh vegetable in both temperate and tropical countries. Although the seed pods are most often used (Camciuc et al., 1998), the mature seed is known to have superior nutritional quality. Rubatzky and Yamaguchi (1997) reported that the seed is a rich source of protein and oil; contains cyclopropanoid fatty acids which cause some toxicity concerns and is used as a substitute for coffee in some countries. In an earlier study, Karakoltsides and Constantinides (1975) found that the Protein Efficiency Ratio (PER) of okra seed flour heated at 130°C for 3hr was not different from the non-heated flour, indicating the absence of anti-nutritional factors. According to these authors, the amino acid composition of okra seed protein is similar to that of soybean and the PER is higher than that of soybean. Okra seed is known to be rich in high quality protein especially with regards to its content of essential amino acids relative to other plant protein sources (Oyelade et al., 2003). It is also reported to be rich in minerals and vitamins. Plantains (*Musa paradisiaca* Linn) are major starch staple crops of considerable importance in the developing world. They are consumed as an energy yielding food and as a dessert. It has been estimated that plantain and other bananas provide nearly 60 million people in Africa with more than 200 calories (food energy) a day. Nearly 90% of the total banana and plantain produced worldwide are consumed locally in producing countries leaving only 10% for export (CGIAR 1992, 1993; Stover and Simmonds, 1987). In Nigeria plantain have been popular for many years and are an ingredient in many traditional recipes.

Another popular method of preparing and preserving the unripe plantain is the production of flour: the fruit is sliced, sundried and then ground into a fine powder 'elubo ogede'. The flour is used in gruel and eaten with vegetables or okra soup (Ohiokpehai, 1985). Instant plantain flours were prepared from ripe and unripe plantain (*M. paradisiaca*) fingers, by cooking and subsequent oven dehydration at 76°C and at 88-92°C, respectively (Ukhun and Ukpebor, 1991). These authors considered the products as having commercial potential on their own or as ingredients for other foods such as baby weaning foods, puddings, soups and gravies. The over matured okra pod that cannot be cut with kitchen knife are thrown away in Nigeria, this lead to postharvest loss of okra. The seed in this okra could be utilised by processing into okra seed flour for the fortification of plantain flour. Since the okra seed flour is rich in oil and the oil contains high proportion of cyclopropene fatty acids (CPFA) which cause some toxicity concerns therefore the aim of this work is to evaluate the antioxidant properties of plantain flour fortified with full fat and defatted okra seed flour.

## **2. MATERIALS AND METHODS**

### **2.1 Sample Collection**

The plantains were bought from 'Emure' market in 'Emure Uli' in Ondo State. The okra used was freshly harvested, matured and fibrous okra that cannot be cut with kitchen knife. The okra was harvested from a farm in Rufus Giwa Polytechnic, Owo.

### **2.2 Sample Preparation**

The plantain flour was produced according to the method of Mepba et al. (2007). The fingers were washed, peeled, cut into thin slices of 2 cm thick and blanched in 1.25% NaHSO<sub>3</sub> solution at 80°C for 5 min. Blanched plantain slices were drained and dehydrated in an air-recirculating oven at 60°C for 24 hours. Dried plantain slices were milled into flour using Hammer mill. Flour obtained were sifted through a 250µm aperture sieve and packed in a two-ply medium density polythene bag. The okra seed were removed from the pod, sundried, dry milled and sieved to obtain a particle size of less than 250µm, the sieved okra seed flour was divided into 2 parts, one part was defatted using petroleum ether in a soxhlet extractor, Samples were extracted for 16hr at a condensation rate of 2-3 drops/s, and the fat was dried at 100°C for 30min (Aminigo and Akingbala, 2004) while the other was termed full fat. The plantain flour was then fortified with the defatted and full fat okra seed flour separately in the following ratio 90:10, 80:20 and 70:30. All determinations were done in triplicates, vitamin C, total phenol, ABTS scavenging ability and ferric reducing antioxidant property (FRAP).

### **2.3 Antioxidant Analysis**

The total phenol content was determined according to the method of Singleton et al. (1999). Appropriate dilutions of the aqueous extracts were oxidized with 2.5ml of 10% Folin-Ciocalteou's reagent (v/v) and neutralized by 0.2ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45°C and the absorbance was measured at 765nm in the JENWAY UV – Visible spectrophotometer. The total phenol content was subsequently calculated as garlic acid equivalent.

The vitamin C content of the aqueous extract was determined using the method of Benderitter et al. (1998). 75µl DNPH (2gdinitrophenyl hydrazine, 230mg thiourea and 270mg

CuSO<sub>4</sub> · 5H<sub>2</sub>O in 100ml of 5M/L H<sub>2</sub>SO<sub>4</sub>) were added to 500 µl extracts mixture (300 µl of an appropriate dilution of the extract with 100 µl 13.3% trichloroacetic acid (TCA) and water). The reaction mixture was subsequently incubated for 3 hours at 37°C, then 0.5ml of 65% H<sub>2</sub>SO<sub>4</sub> (v/v) was added to the medium and the absorbance was measured at 520nm in the JENWAY UV-Visible spectrophotometer. The vitamin C content of the extracts was subsequently calculated using ascorbic acid as standard.

The ABTS scavenging ability of the extracts were determined according to the method describe by Re et al. (1999). The ABTS was generated by reacting an ABTS aqueous solution (7mM/L) with potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>) (2.45mM/L, final concentration) in the dark at room temperature for 16 hours and adjusting the Abs734nm to 0.700 with ethanol. 0.2ml of appropriate dilution of the extract was added to 2.0ml ABTS solution and the absorbance was measured at 734nm after 15 minutes in the JENWAY UV – Visible spectrophotometer. The trolox equivalent antioxidant capacity was subsequently calculated. The reducing property of the extract was determined by accessing the ability of the extract to reduce FeCl<sub>3</sub> solution as described by Oyaizu (1986). A 2.5ml aliquot was mixed with 2.5ml 200mM/L sodium phosphate buffer (pH6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20min then 2.5ml 10% trichloroacetic acid (TCA) was added. This mixture was centrifuged at 650rpm for 10min. 5ml of the supernatant was mixed with an equal volume of water and 1ml of 0.1% ferric chloride. The absorbance was measured at 700nm using the JENWAY UV–Visible spectrophotometer. The ferric reducing antioxidant property was subsequently calculated using ascorbic acid as a standard.

## 2.4 Statistical Analysis

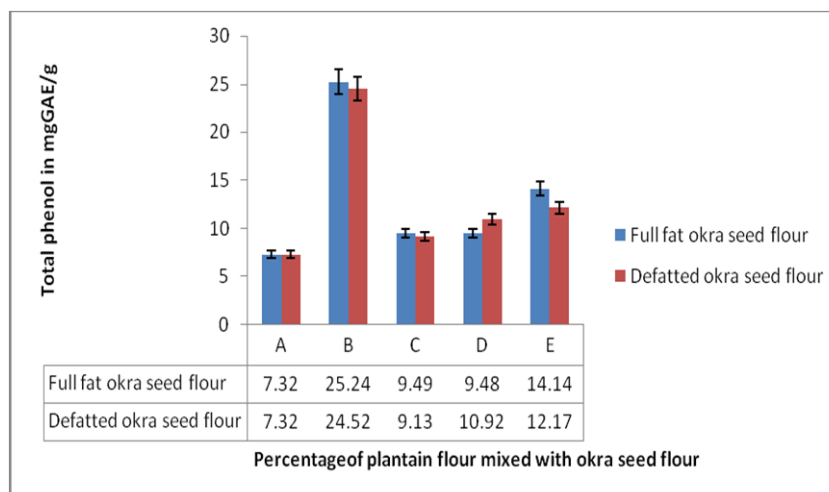
Analysis of variance (ANOVA) was performed on the results for each quality variable to determine the significance of the fortification (SAS, 2002). Mean separation was done where there is a significant difference using Duncan multiple range test procedure as described in the SAS software. Significance was accepted at P = 0.05.

## 3. RESULTS AND DISCUSSION

### 3.1 Total Phenol

The total phenol of the fortified flour is presented in Figure 1. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Rice-Evans et al., 1995). Phenolic phytochemicals inhibit autoxidation of unsaturated lipids thus preventing the formation of oxidized low-density lipoprotein (LDL) which is considered to induce cardiovascular disease (Amic et al., 2003). The process of defatting resulted in 2.85% loss in the total phenol content of the okra seed flour. The total phenol content of the full fat okra seed flour was higher than that of the defatted; this could be attributed to the removal of the fat from the okra seed flour using a non-polar solvent. The total phenol content of the plantain flour 7.32mgGAE/g was higher than the value reported for yam by Marie et al. (2005) and Kouakou et al. (2010). The total phenol content of the okra seed flour both defatted (24.25mgGAE/g) and full fat (25.24mgGAE/g) were significantly higher (P = 0.05) than the total phenol content of the plantain flour (7.32mgGAE/g). The total phenol content of the resultant fortified plantain flour increased as the percentage of okra seed flour (defatted and full fat) increased. This could be attributed to the high content of phenols in the okra seed flour which was significantly higher (P = 0.05) than that of the plantain flour. It is also shown in Figure 1 that at 70:30 ratio of plantain flour to okra seed flour, the value of the full fat okra

seed flour fortification 14.14mgGAE/g was significantly higher ( $P = 0.05$ ) than the defatted okra seed flour fortification 12.17mgGAE/g. Plant phenolics have multiple biological effects as they constitute one of the major groups of compounds acting as primary antioxidant or free radical terminator (Zarena and Sankar, 2009).



**Fig. 1: Total phenol content of plantain flour fortified with okra seed flour**

Value represents mean of triplicate. A = 100PF:0OSF, B = 0PF:100OSF, C = 90PF:10OSF, D = 80PF:20OSF, E = 70PF:30OSF. PF = Plantain flour, OSF = Okra seed flour (Full fat & defatted)

### 3.2 Vitamin C

The antioxidant properties of vitamin C is exhibited by protecting the membrane erythrocytes, protecting the body against cancer of the oesophagus, oral cavity and stomach, also by maintaining the blood vessel flexibility and improving blood circulation in the arteries of smokers as well as flexibility in the absorption of iron in the body (Obboh, 2005; Block et al., 1992). The vitamin C content of the fortified plantain flour is presented in Table 1. The vitamin C content of the plantain flour is 6.30mg/100g. This was not significantly different ( $P = 0.05$ ) from the vitamin C content of the okra seed flour both defatted 6.66 mg/100g and full fat 6.84 mg/100g. The vitamin C reported in this work was higher than the value reported for underutilised legumes 0.5 – 0.9mg/100g (Obboh, 2006) and “Gbodo” yam flour and “Elubo ogede” plantain flour (Jonathan et al., 2011), it was lower to the value reported for vitamin C of different water yam (*Dioscorea alata*) flour 16.72 – 35.20mg/100g (Udensi et al., 2008). However, the vitamin C content of the fortified flour was very low when compared with some commonly consumed green leafy vegetables and fruits which are established dietary sources of vitamin C (Obboh, 2006). The percentage loss of vitamin C in the okra seed flour as a result of defatting is 2.63%. The addition of okra seed flour to plantain flour caused a decrease in the vitamin C content of the fortified plantain flour, the vitamin C content of the plantain flour reduced from 6.30mg/100g to 2.59mg/100g when fortified with defatted okra seed flour while it reduced to 4.87mg/100g when fortified with full fat okra seed flour. The basis for this reduction in vitamin C content of the fortified plantain could not be categorically stated. It is revealed in Table 1 that, though there was decrease in the vitamin C content of plantain flour with the addition of okra seed flour but the vitamin C content of the fortified plantain flour increased with increase in the percentage of okra seed flour, for defatted, the vitamin C content increased from 1.85mg/100g at 90:10 to

2.59mg/100g at 70:30 of plantain flour and okra seed flour while for full fat, it increased from 1.85mg/100g at 90:10 to 4.87mg/100g at 70:30. The increase in the vitamin C content of the fortified plantain flour as the percentage of okra seed flour added increased agreed with the report of Otunola et al. (2006 and 2007) in the supplementation of "ogi" with okra seed flour and pawpaw slurry. This observation may not be unexpected since the vitamin C content of plantain flour and okra seed flour are not significantly different.

**Table 1: Vitamin C content of plantain flour fortified with okra seed flour in mg/100g**

% PF/OSF	Full fat okra seed flour	Defatted okra seed flour
100:0	6.30± 0.52a(a)	6.30± 0.52a(a)
0:100	6.84± 1.07a(a)	6.66±0.01a(a)
90:10	1.85± 1.57d(a)	1.85± 1.57d(a)
80:20	3.47± 0.01c(a)	2.22± 0.01c(b)
70:30	4.87± 0.44b(a)	2.59± 1.57c(b)

Value represents mean of triplicate±SD. Values with the same letter along the same column are not significantly different ( $P > 0.05$ ) while value with the same letter inside bracket along the row are not significantly different ( $P > 0.05$ ). PF: Plantain flour, OSF: Okra seed flour.

### 3.3 ABTS Scavenging Ability

The ABTS radical based model of free radical scavenging ability has the advantage of been more versatile as the polar and non-polar samples can be assessed and spectral interference is minimised as the absorption maximum used is 760nm, a wavelength not normally encountered with natural products (Re et al., 1999). Awika et al. (2003) also has reported the superiority of the ABTS assay because it is operable over a wide range of pH, inexpensive and more rapid. ABTS scavenging ability reported as trolox equivalent antioxidant capacity (TEAC) of the fortified plantain flour is presented in Table 2.

**Table 2: ABTS scavenging ability of plantain flour fortified with okra seed flour in mmol.TEAC/100g**

% PF/OSF	Full fat okra seed flour	Defatted okra seed flour
100:0	3.3± 0.05e(a)	3.3± 0.05e(a)
0:100	25.0± 0.01a(a)	20.7± 0.04a(b)
90:10	9.75± 0.01d(a)	4.5± 0.05d(b)
80:20	14.5± 0.01c(a)	5.7± 0.07c(b)
70:30	18.95± 0.07b(a)	6.5± 0.01b(b)

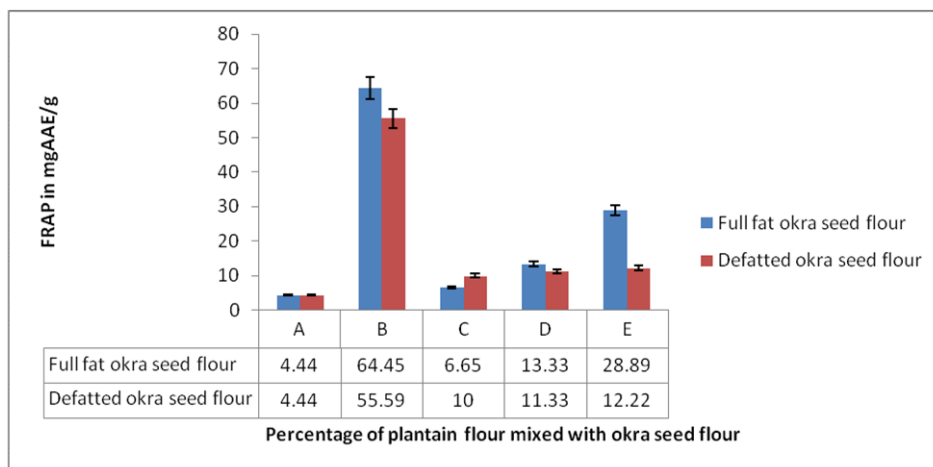
Value represents mean of triplicate±SD. Values with the same letter along the same column are not significantly different ( $P > 0.05$ ) while value with the same letter inside bracket along the row are not significantly different ( $P > 0.05$ ). PF: Plantain flour, OSF: Okra seed flour.

The result revealed that the ABTS scavenging ability of the full fat okra seed flour 25.00mmol.TEAC/100g was significantly higher ( $P > 0.05$ ) than the defatted okra seed flour 20.70mmol.TEAC/100g. This could also be attributed to the defatting of the okra seed flour. The ABTS scavenging ability of the okra seed flour (defatted and full fat) was significantly higher ( $P > 0.05$ ) than that of the plantain flour 3.30mmol.TEAC/100g. This suggested that okra seed flour has a better antioxidant activity than the plantain flour. It was observed that

the higher the TEAC value of the sample, the stronger was the antioxidant activity (Zarena and Sankar, 2009). It is worth noting that as the percentage of okra seed flour increased the ABTS scavenging ability of the fortified plantain flour increased, at 70:30 plantain flour and defatted okra seed flour, the ABTS scavenging ability of the plantain flour increased from 3.30mmol.TEAC/100g to 6.5mmol.TEAC/100g while at the same ratio for plantain flour and full fat okra seed flour the ABTS scavenging ability increased from 3.30mmol.TEAC/100g to 18.95mmol.TEAC/100g. The basis for this increase in the ABTS scavenging ability of the resultant fortified plantain flour could be because the ABTS scavenging ability of the okra seed flour was significantly higher ( $P < 0.05$ ) than that of the plantain flour. ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain breaking antioxidants (scavenger of lipid peroxy radicals) (Leong and Shui, 2002).

### 3.4 Reducing Power

Reducing power is a novel antioxidation defense mechanism and the mechanisms that affect this property are electron transfer and hydrogen atom transfer. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Dastmalchi et al., 2007; Lee et al., 2007). The reducing power of the flour extract were determined based their ability to reduce  $Fe^{3+}$  to  $Fe^{2+}$ . A Higher absorbance indicated higher activity (Zarena and Sankar, 2009). FRAP assay is quick and simple to perform; the reaction is reproducible and linearly related to the molar concentration of the antioxidants (Hodzic et al. 2009). Therefore in this work FRAP assay was used in the determination of the reducing power of the flour. The Ferric Reducing Antioxidant Property (FRAP) of the fortified plantain flour reported as Ascorbic Acid Equivalent (AAE) is presented in Figure 2.



**Fig. 2: Ferric reducing antioxidant property (FRAP) of plantain flour fortified with okra seed flour.**

Value represents mean of triplicate. A = 100PF:0OSF, B = 0PF:100OSF, C = 90PF:10OSF, D = 80PF:20OSF, E = 70PF:30OSF. PF = Plantain flour, OSF = Okra seed flour (Full fat & defatted)

The reducing power of the okra seed flour was 55.59mgAAE/g when defatted and 64.45mgAAE/g when full fat, this suggest 13.75% loss of reducing power as a result of defatting. The reducing power of the okra seed flour (defatted and full fat) was significantly higher ( $P < 0.05$ ) than that of plantain flour 4.44mgAAE/g. The addition of okra seed flour to

the plantain flour caused increase in the reducing power of the plantain flour from 4.44mgAAE/g to 12.22mgAAE/g when fortified with defatted okra seed flour while it increase to 28.89mgAAE/g when fortified with full fat okra seed flour. The reducing power of the fortified flour increased as the percentage of okra seed flour increase. This increase in the reducing power of the fortified plantain flour could be attributed to reducing power of the okra seed flour which was significantly higher ( $P < 0.05$ ) than that of the plantain flour.

The results of the reducing power of the flour (okra seed, plantain and fortified plantain) agreed in its entirety with the total phenol content of the flour because reducing power is directly proportional to the total phenol content of the sample. This observation is similar to the report of Oboh et al. (2008), where *Ocimum gratissimum* with the highest total phenol (polar and non-polar) had the highest reducing power. Lee et al. (2007) observed that higher reducing power activities can be attributed to higher amounts of polyphenolics, and the reducing capacity of a compound may reflect its antioxidant potential. The reducing power property indicated that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process (Tachakittirungrod et al., 2007).

#### 4. CONCLUSION

It is evident from the present work that defatting caused a reduction in the antioxidant properties of okra seed flour. Antioxidant potential differs as the percentage of okra seed flour added to plantain flour increased. The higher the percentage of okra seed flour the higher the antioxidant properties of the plantain flour. The addition of okra seed flour to plantain flour should be encouraged because it increased the antioxidant properties of the resultant fortified plantain flour. Moreover a detailed *in vivo* study is recommended to ascertain the effectiveness of the antioxidant properties.

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#### COMPETING INTERESTS

Author has declared that no competing interests exist.

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