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Research Article

Fortification of 'Zobo' (*Hibiscus sabdariffa*) Drink with Pineapple and Watermelon enhances its Nutritional Qualities and Phytochemical Compositions

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Abstract

This study sought to investigate the influence of pineapple and watermelon on the nutritional qualities and phytochemical compositions of zobo drink. Dry calyces of *H. sabdariffa* (zobo) leaves were obtained from Lafenwa market in Abeokuta, Nigeria. The leaves were boiled, cooled, filtered and stored in 3 different containers labelled A, B and C respectively. Pineapple and watermelon juice were extracted and added to the zobo drink labelled B and C respectively in ratio 1:4. The samples were immediately transferred to the laboratory for analysis. The proximate and phytochemical analyses were carried out using standard methods. The results showed that zobo drinks flavored with pineapple and watermelon was generally higher in proximate and phytochemical contents. It was observed that zobo drink flavored with watermelon has the highest concentrations of protein (0.91%) and ash (0.79%). Zobo drink flavored with pineapple was observed to contain the highest concentrations of crude fat (3.04%), crude fibre (0.16%) as well as energy value of 73.12 KJ/100mL. On the other hand, unflavored zobo drink was highest in moisture (87.33%) and carbohydrate (11.07%) concentrations. The results of the phytochemical analysis showed that concentrations of alkaloid (0.47 mg/100mL), flavonoid (1.71 mg/100mL) and phytate (2.83 mg/100mL) were highest in zobo drink flavored with pineapple while the concentrations of saponnin (7.95 mg/100mL), tannin (0.89) and phenol (2.76 mg/100mL) as well as pH (4.95) value were highest in zobo drink flavored with watermelon. This result showed that fortification of zobo drinks with pineapple and watermelon enhances its nutritional qualities and phytochemical compositions.

Keywords: Nutritional Qualities; Phytochemical Compositions; Pineapple; Watermelon; Zobo Drinks

Introduction

By boiling and filtration, zobo drink (Sorrel, zoborodo) is a non-alcoholic local beverage created from many varieties of dried petals, acid-succulent calyces of the flower *Hibiscus sabdariffa* [1,2]. Nigerians produce a vast range of cuisines using local technology and a variety of plant products. Beverages are food items that are unique and different from other foods. They are liquid in nature but have lower nutritional value than food products. Beverages can be liquid or thin gruel preparations with a high water content, which provides the body with the water it needs to be healthy.

Zobo is mostly consumed by low-income individuals, but it is gaining widespread acceptance, with millions of people from vari-

ous socioeconomic classes and backgrounds throughout the West African sub-region using it. Its nutritional value, as well as its organoleptic characteristics and keeping quality, must be improved to promote its acceptance. This will supply consumers with more nutrient-dense Zobo variations that will also last longer than traditional Zobo. Zobo has a sour (vinegar) flavor and is popular throughout the country. It is a hot water heater. *Hibiscus sabdariffa* extract, which is used to satisfy thirst in the tropics when it's hot [3].

It is a beverage manufactured on a small scale by traditional women at minimal cost because its ingredients are cheap and readily available. It is primarily consumed by low-income individuals of society. Given the expanding popularity, socio-economic potentials, and ready source of protein, Vitamin C, and other minerals, Zobo drink, if appropriately prepared and marketed, will compete favorably with most foreign non-alcoholic beverages available in Nigeria [4].

Figure 1: Dry Zobo (*Hibiscus sabdariffa*) Leaves.

The following components are used to make Zobo: dry Zobo leaves (Figure 1), artificial tastes, as well as water, can be employed. Water is the most abundant constituent in Zobo, and it serves as the solvent for all other constituents. Zobo is strong in vitamins, natural glucose, protein, Vitamin C, and other antioxidants, despite its inexpensive cost of manufacture [5].

Despite these clear benefits, the transition from locally marketed to commercial product is still a long shot due to its short shelf life, which would necessitate very little inventory and storage time. Several spices, such as garlic, ginger, and clove, have long been recognized and used for their scent and, to a lesser extent, preservation properties [6]. The goal of this study was to see how pineapple and watermelon affected the nutritional value and phytochemical makeup of zobo drink.

Materials and Methods

Preparation of zobo drinks

Dry calyces of *H. sabdariffa* (zobo) leaves were obtained from Lafenwa market in Abeokuta, Nigeria and were identified by a botanist. They were manually cleaned by handpicking stones and other unwanted debris. They were thoroughly washed using sterile de-ionized water. About 400g of the washed calyces zobo leaves were boiled in 2000 mL of water for 15 minutes as described by Oghier and Nwafor [7] and was left to cool for 15 minutes before removing the calyces using a white muslin cloth and left to stand in a vessel. The cooled zobo drink was stored in 3 different containers and were labelled A, B and C respectively.

The pineapple and watermelon were also purchased from the same market. They were separately washed, peeled and chopped into small bits with a clean stainless knife. The seeds of the watermelon were removed. The chopped pineapple and watermelon were separately blended with a blender with stainless steel blades until juice and pulp was obtained. The juice was filtered using a white muslin cloth and the resulting extract was stored in a clean bottle [8]. Pineapple juice was added to the zobo drink labelled B and watermelon was added to the zobo drink labelled C in ratio 1:4. Sample B therefore contained 80 mL of zobo drink and 20 mL of pineapple juice while sample C contained 80 mL of zobo drink and 20 mL of watermelon juice. The samples were immediately transferred to the laboratory for analysis.

Determination of proximate and energy composition of zobo drink samples

The samples' proximate compositions were analyzed using methods developed by the Association of Official Analytical Chemists (AOAC) [9]. The seed's moisture content was measured gravi-

metrically. The nitrogen conversion factor was 6.25, and the protein level was measured using the micro Kjeldahl method. Soxhlet extraction with petroleum ether was used to determine the fat content. The crude fibre content was determined using the acid-base technique. The ash composition of the samples was evaluated by incineration at 600°C in a muffle furnace. The carbohydrate concentration was calculated using the Airaodion., *et al*. [10] method. Using the approach of Airaodion., *et al*. [11], the energy value was estimated by multiplying the percentages of crude protein and carbohydrate by 4 and crude fat by 9. The calories per 100 grams of each sample were then calculated.

Energy = (carbohydrate 4) + (crude protein 4) + (crude fat 9) [11].

Quantitative determination of phytochemical composition of zobo drink samples

The phytochemical composition of zobo drink samples was evaluated quantitatively using the AOAC [9] techniques.

Determination of pH of zobo drink samples

The pH value was calculated using Airaodion., *et al.* [12] approach. Using a previously standardized pH meter, 10 mL of the sample was poured into a beaker and the pH was calculated (Jen-Way 3505). A phosphate buffer of pH 4.0 and 7.0 was used to calibrate the pH meter.

Statistical analysis

The results were calculated using Microsoft Excel 2013 program. The results of the triple analysis (n = 3) were provided as means standard deviation (SD). Graph Pad Prism was used to do analysis of variance on the data. The mean was compared using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. At p0.05, differences between means were judged significant.

Results

The results showed that zobo drinks flavored with pineapple and watermelon was generally higher in proximate (Table 1) and phytochemical (Table 2) contents. It was observed that zobo drink flavored with watermelon has the highest concentrations of protein (0.91%) and ash (0.79%) when compared with those in zobo drink flavoured with pineapple and the unflavored drink. Zobo drink flavored with pineapple was observed to contain the highest concentrations of crude fat (3.04%), crude fibre (0.16%) as well as energy value of 73.12 KJ/100mL when compared with those in zobo drink flavored with watermelon and the unflavored drink. On the other hand, unflavored zobo drink was highest in moisture (87.33%) and carbohydrate (11.07%) concentrations when compared with those in zobo drink flavored with both pineapple and watermelon (Table 1).

The results of the phytochemical analysis showed that concentrations of alkaloid (0.47 mg/100mL), flavonoid (1.71 mg/100mL) and phytate (2.83 mg/100mL) were highest in zobo drink flavored with pineapple when compared with those in zobo drink flavored with watermelon and the unflavored drink while the concentrations of saponnin (7.95 mg/100mL), tannin (0.89) and phenol (2.76 mg/100mL) as well as pH (4.95) were highest in zobo drink flavored with watermelon when compared with those in zobo drink flavored with pineapple and the unflavored drink.

Parameters	Zobo Only	Zobo + Pineapple	Zobo + Watermelon	p-Value
Protein (%)	0.73 ± 0.01	0.84 ± 0.02	0.91 ± 0.00	0.05
Crude Fat (%)	0.22 ± 0.11	3.04 ± 0.08	2.06 ± 0.12	0.01
Crude Fibre (%)	0.09 ± 0.00	0.16 ± 0.00	0.14 ± 0.01	0.03
Ash (%)	0.56 ± 0.01	0.76 ± 0.00	0.79 ± 0.01	0.04
Moisture (%)	87.33 ± 3.05	85.44 ± 4.22	85.05 ± 3.72	1.25
Carbohydrate (%)	11.07 ± 0.97	10.60 ± 1.31	11.05 ± 1.53	0.47
Energy (KJ/100mL)	49.18 ± 2.92	73.12 ± 3.36	67.98 ± 3.64	0.02

Table 1: Proximate and Energy Composition of Flavored Zobo Drinks.

Results are presented as means ± standard deviation (SD) of triplicate analysis (n = 3). Mean values are significantly different at p 0.05.

Phytochemical	Zobo Only	Zobo + Pineapple	Zobo + Watermelon	p-Value
Alkaloid	0.16 ± 0.00	0.47 ± 0.02	0.44 ± 0.06	0.02
Flavonoid	1.25 ± 0.06	1.71 ± 0.08	1.69 ± 0.03	0.04
Saponin	4.83 ± 0.21	7.07 ± 0.48	7.95 ± 1.11	0.00
Tannin	0.09 ± 0.00	0.77 ± 0.01	0.89 ± 0.02	0.00
Phytate	1.03 ± 0.00	2.83 ± 0.16	2.67 ± 0.06	0.01
Phenol	1.42 ± 0.02	2.69 ± 0.69	2.76 ± 0.39	0.01

Table 2: Phytochemical Composition (mg/100mL) of Flavored Zobo Drinks.

Results are presented as means ± standard deviation (SD) of triplicate analysis (n = 3). Mean values are significantly different at p 0.05.

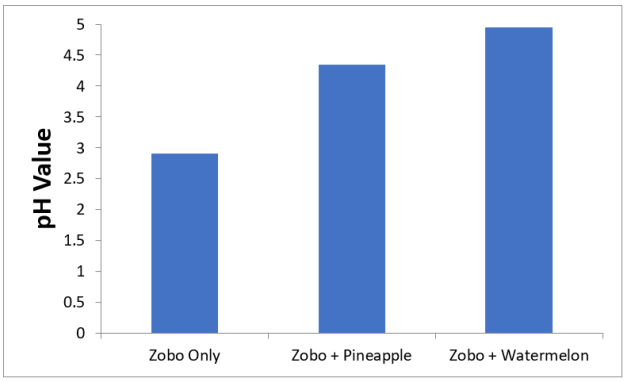

Figure 2: pH Values of Flavored Zobo Drinks.

Discussion

Proximate composition of flavored zobo drink

Rural residents rely on wild fruits to meet their daily nutritional demands as well as generate revenue [13]. The importance of determining the proximate composition of plants is that it predicts the profitability of a certain plant as a possible source of nutrients. This study's proximate analysis revealed that flavored Zobo drinks contain significantly more nutrients than unflavored Zobo drinks (Table 1). Unflavored zobo drink has a crude protein percentage of 0.73 percent. This is similar to the 0.76 percent protein found in unflavored zobo drink reported by Akujobi., *et al.* [14]. However, it is much lower than the 9.64 percent recorded by Nwankwo., *et al.* [15] and the 8.00 percent reported by Mohammed., *et al.* [16]. Furthermore, the crude protein concentration in unflavored zobo drink found in this study is higher than the 0.36 percent and 0.28 percent reported for unflavored zobo drink by Fasoyiro., *et al.* [17] and Gbadegesin and Gbadamosi [18]. The protein concentration of the non-alcoholic drink was boosted to 0.83 percent and 0.91 percent, respectively, by flavoring it with pineapple and watermelon. The protein level of flavored zobo drink is higher than unflavored zobo drink, possibly due to the protein content of pineapple and watermelon. The 0.83 percent crude protein found in pineapple flavored zobo drink in this study matches the 0.82 percent found in zobo drink flavored with pineapple by Akujobi., *et al.* [14]. In a study published by Koopman [19], flavoring zobo drink with turmeric resulted in increased protein content than unflavored zobo drink. Protein has been highlighted as a nutrient in short supply in poor countries; this means that ingesting zobo drinks, particularly those flavored with pineapple and watermelon, as well as other protein-rich foods, will enhance protein intake. Protein deficiency is a severe problem on the African continent, particularly in Nigeria [20].

The 0.22 percent crude fat concentration in unflavored zobo drink observed in this study is similar to the 0.21 percent and 0.26 percent crude fat concentrations in unflavored zobo drink reported by Gbadegesin and Gbadamosi [18] and Nwankwo., *et al.* [15] respectively. This figure is much lower than those of Fasoyiro., *et al.* [17] and Mohammed., *et al.* [16], who reported 1.14 percent and 3.00 percent, respectively. However, the result is much greater than Akujobi., *et al.* [14]'s finding of 0.05 percent crude fat in unflavored zobo drink. Low-fat substances will not go rancid during storage [10]. Because of the low fat content in this study, unflavored zobo drink could be stored for a long time without discoloring or de-

veloping an off-odor. This study also discovered that the crude fat concentrations in zobo beverages flavored with pineapple (3.04%) and watermelon (2.06%) were considerably greater than those in the unflavored drink. This could indicate that flavored zobo drinks cannot be stored as long as unflavored ones without discolouration or off-odor development.

Fibre has been shown to improve digestion [10] and reduce the risk of constipation in consumers [11]. Fibre included in plant materials is also important for lipid metabolism [22]. Both flavored and unflavored zobo beverages had low crude fiber contents. The flavored drinks' values were much greater than the unflavored drinks'. This could mean that flavored zobo drinks are better for digestion than unflavored ones. The low crude fibre levels found in this study are similar to those found in investigations by Fasoyiro., *et al*. [17], Gbadegesin and Gbadamosi [18], and Akujobi., *et al*. [14]. In fact, neither Adeniji [3] nor Ekanem [23] detected fibre in zobo beverages.

The ash value has been used as a criterion for evaluating food quality [11]. When Airaodion., *et al*. [10] reported the nutritional and anti-nutritional evaluation of garri prepared by conventional and instant mechanical processes, they explained that ash content is a measure of the total minerals present inside a product. The ash content of the unflavored zobo drink used in this study was 0.56 percent, which is similar to the 0.52 percent and 0.50 percent reported by Gbadegesin and Gbadamosi [18] and Koopman [19] respectively, but lower than the 1.30 percent and 2.31 percent reported by Adeniji [3] and Fasoyiro., *et al*. [17]. However, it is greater than the 0.24 percent found by Akujobi., *et al*. [14]. The ash content of zobo drink with pineapple flavor is 0.76 percent, whereas zobo drink with watermelon flavor is 0.79 percent. They are both much higher than the unflavored zobo drink. Adeniji [3] found that ash content in zobo drinks flavored with ginger was higher than in unflavored zobo drinks in a similar investigation.

Moisture content determines the sample's water content and, indirectly, its dry matter content. It's also a measure of the flour samples' storage stability [11]. Moisture level more than 14 percent makes substances susceptible to microbial growth and so has a lower storability [10]. Unfortunately, the moisture level of both flavored and unflavored zobo drinks found in this investigation is more than 500% greater than the 14 percent spoiling threshold. This means that flavored and unflavored zobo drinks are susceptible to microbial growth and have a shorter shelf life. This result is consistent with previous findings by Fasoyiro., *et al*. [17], Gbadegesin and Gbadamosi [18], Adeniji [3], Koopman [19], and Akujobi., *et al*. [14], who all reported moisture content in both flavored and unflavored zobo drinks to be greater than 80%.

For unflavored zobo drink, the carbohydrate and energy content were 11.07 percent and 49.18 KJ/100mL, respectively. The sample has low carbohydrate and calorific values. At rest, the human body requires a lot of energy. The amount needed is around 1 kcal per kg of body weight every hour, or 1,500 to 2,000 kcal per day. This is dependent on the metabolism of the individual. The majority of human energy consumption comes from food and is utilized to maintain vital life functions and body temperature [20]. The quantity of energy obtained by the body from food is less than that obtained by burning or totally oxidizing food in a bomb calorimeter [11]. This is due to calorie-producing foods such as protein, lipids, and carbs not being completely digested, absorbed, or oxidized in the body to produce energy [24]. The carbohydrate content of the unflavored zobo drink used in this study is identical to that of unflavored zobo drink reported by Gbadegesin and Gbadamosi [18]. The finding is much higher than Fasoyiro., *et al*. [17] and Akujobi., *et al*. [14]'s 6.31 percent and 5.34 percent, respectively. Furthermore, flavored zobo drinks were found to contain more energy than unflavored ones. Because both pineapple and watermelon contain calories, this is to be expected. This could indicate flavored zobo beverages. Based on the daily energy requirement (1,500-2,000 Kcal) [25], zobo drinks may only provide a percentage of the daily energy requirement when eaten, but they would considerably contribute to meeting the daily energy requirement, especially the flavored ones.

Differences in the analyzed values of proximate composition of zobo drink samples compared to certain earlier research could be related to differences in the soil, meteorological circumstances, harvest time, geographical condition, relative humidity of the surrounding atmosphere, and cultural practices. According to Airaodion., *et al*. [11], the aforementioned factors influence the composition of plant products.

Phytochemical composition of flavored zobo drink

Bioactive molecules present in vegetables, seeds, cereal grains, and plant-based beverages like tea and wine are known as phy-

tochemicals. Plants produce them as a result of their primary or secondary metabolism. They've been employed in traditional medicine and as a poison [26]. Table 2 shows the results of the phytochemical content of the zobo drink samples. Both flavored and unflavored zobo beverages were found to be low in alkaloid content. The alkaloid levels in zobo flavored with pineapple (0.47 mg/100mL) and watermelon (0.44 mg/100mL) are much greater than in the unflavored drink (0.16 mg/100mL). Antihypertensive effects (various indole alkaloids), antiarrhythmic effects (quinidine, spareien), antimalarial activity (quinine), and anticancer effects (dimeric indoles, vincristine, vinblastine) are just a few of the pharmacological properties of alkaloids [27]. Caffeine, nicotine, and morphine are examples of alkaloids that have stimulant qualities and are employed as analgesics [28]. For millennia, alkaloids have been connected with medical purposes, and cytotoxicity is one of their most prevalent biological features [29]. Alkaloids have been shown to have analgesic, antispasmodic, and antibacterial effects by several researchers [30-35]. Alkaloids have been linked to antihypertensive and detoxifying activities [36-40]. As a result, flavored zobo will be more therapeutic than plain zobo.

Flavonoids have been shown to have antibacterial, cytotoxic, anti-inflammatory, and anticancer characteristics, but their ability to act as antioxidants, which can protect the human body from free radicals, is the most well-known property of practically every group of flavonoids [41]. Flavonoid concentrations in the unflavored zobo drink utilized in this study are 1.25 mg/100mL, while flavonoid concentrations in pineapple and watermelon-flavored drinks are 1.71 mg/100mL and 1.69 mg/100mL, respectively. These results are higher than those reported by Akujobi., *et al*. [14] for unflavored zobo (0.05 mg/100mL) and orange-flavored zobo (0.06 mg/100mL). Flavonoids' ability to serve as antioxidants is determined by their chemical structure. Flavonoids' antioxidant and free radical scavenging capabilities are influenced by the location of hydroxyl groups and other factors in their chemical structure [11]. Anti-inflammatory activity, enzyme inhibition, antibacterial activity, oestrogenic activity, anti-allergic activity, antioxidant activity, vascular activity, and cytotoxic anticancer activity have all been reported for flavonoids [42]. Flavonoids are a type of secondary plant metabolite that have anti-inflammatory and antioxidant properties [11]. Flavonoids are a diverse group of compounds that protect biological systems from the detrimental effects of oxidative processes on macromolecules such as carbohydrates, proteins, lipids, and DNA [25]. Several studies have found that the presence of flavonoid in several plant extracts can help prevent peptic ulcers [43-45].

Saponin has been shown to have a variety of biological activities and potential health benefits, including hypocholesterolemic, anti-coagulant, anti-carcinogenic, hepatoprotective, hypoglycemic, immunomodulatory, neuroprotective, anti-inflammatory, anti-oxidant, and platelet aggregation inhibition [46]. In this investigation, saponin concentrations in zobo drink samples were found to be 4.83 mg/100mL, 7.07 mg/100mL, and 7.95 mg/100mL for unflavored zobo, zobo flavored with pineapple, and zobo flavored with watermelon, respectively. The amounts of saponin in flavored zobo beverages are much higher than in the unflavored drink, according to this result. Saponin has been discovered to have a favorable effect on growth, feed intake, and reproduction in animals and could be utilized to treat hypercalciuria. Saponins have also been found to kill protozoa and mollusks, as well as acting as antifungal and antiviral agents [11,47].

Tannin-containing plant extracts are employed as astringents, diuretics, anti-inflammatory, antibacterial, antioxidant, and hemostatic medicines, as well as against stomach and duodenal cancers [11,48]. Both flavored and unflavored zobo beverages have minimal tannin concentration, according to the findings of this study. Tannin levels in zobo flavored with pineapple (0.77 mg/100mL) and watermelon (0.89 mg/100mL) are much greater than in the unflavored drink (0.09 mg/100mL).

Tannins are known to react with proteins to create the usual tanning effect, which is useful for the treatment of inflamed or ulcerated tissues, according to Han., *et al*. [29], Parekh and Chanda [49], and Senchina., *et al*. [50]. Tannins attach to proline-rich proteins, preventing them from being synthesized. Astringent herbs with tannins as their primary constituents are used to treat intestinal problems such as diarrhea and dysentery [51].

It's important to know how much phytate is in your food because too much can affect your digestion [10,52]. Because of its negative consequences, phytate has been labeled as an anti-nutrient. Mineral bioavailability was lowered, and growth was inhibited. Phytate can chelate divalent cationic minerals such calcium, iron, magnesium, and zinc, causing deficit in the diet [53,54]. According to Wise [55], dietary calcium levels influence phytate solubility and

the fraction of minerals bound to the complex. The concentration of phytate in the unflavored zobo drink used in this study is 1.03 mg/100mL, whereas the concentrations of phytate in the pineapple and watermelon flavored zobo beverages are 2.83 mg/100mL and 2.67 mg/100mL, respectively.

Phenols have been shown to exhibit a variety of biological functions [56,57]. Biological properties of phenol include increased bile production, lower blood cholesterol and lipid levels, and antibacterial activity against bacteria such as staphylococcus aureus. Antiulcer, anti-inflammatory, antioxidant, cytotoxic and anticancer, antispasmodic, and antidepressant effects are among the many biological activities of phenolics [11]. In this investigation, phenol concentrations in unflavored zobo, zobo flavored with pineapple, and zobo flavored with watermelon were found to be 1.42 mg/100mL, 2.69 mg/100mL, and 2.76 mg/100mL, respectively. The amounts of phenol in flavored zobo beverages were substantially higher than in the unflavored drink, according to this finding.

Figure 2 depicts the results of the pH of zobo beverages. The pH of the unflavored zobo drink is 2.91, whereas the pH of zobo flavored with pineapple is 4.35, and zobo flavored with watermelon is 4.95, according to the findings. This demonstrates that zobo beverages, particularly the unflavored variety, are acidic. It is said to be a naturally acidic fruit with a high content of organic acids [58]. This means that zobo drink should not be eaten on an empty stomach or without a snack. Because of the low pH, those with peptic and stomach ulcers should avoid drinking unflavored zobo drink on a regular basis [18]. Instead, they should drink a zobo drink with pineapple and/or watermelon flavors.

Conclusion

The results from this study showed that fortification of zobo drinks with pineapple and watermelon enhances its nutritional qualities and phytochemical compositions.

Ethical Approval

Not Applicable

Consent for Publication

Not applicable.

Availability of Data and Material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Conflict of Interests

Authors declare that they have no conflict of interests in this research and publication.

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Authors' Contributions

The research was carried out by both authors. Both authors read and approved the final manuscript.

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