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Optimization of manufacturing conditions of the new purple leafed Kenyan teas (TRFK 306) – maceration style and withering duration

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Abstract

Purple tea in Kenya, TRFK 306, has received great attention due to its perceived health benefits though how it should be optimally manufactured has not been researched on exhaustively. The current study investigated on the two possible methods of manufacture – Cut Tear and Curl (CTC) and orthodox manufacture - with varying withering time of 0, 5, 10, 15 and 20 hours. Catechins, gallic acid, caffeine, total polyphenols and antioxidant activity were assayed as quality indicators. If gallic acid and caffeine are the main chemicals targeted, then orthodox type with longer withering, 15 – 20 hours, is usually recommended. When targeting catechins especially epigallocatechin gallate and high total polyphenols, orthodox type of manufacture whichever the withering time is proposed. CTC with withering hours of 15-20 produces teas with high antioxidant activity. It was concluded that TRFK 306 is best processed by orthodox or CTC but with longer withering time.

Introduction

Tea was first introduced in Kenya from India by a colonial settler G.W. Caine in 1903 and in the 1930's commercial planting began (Watts, 1999). Planted tea area in Kenya has grown from 21,448 hectares in 1963 to over 180,000 hectares in 2011 and 203, 000 hectares by 2014 (AFFA, 2014). Kenya is mostly known for its Cut Tear Curl (CTC) tea production, but the country also produces orthodox (ORTHO) tea. Kenya is among the few Orthodox tea producers worldwide accounting for 1.3 percent of total production. In 2012, Kenyan production of orthodox tea accounted for approximately 3.5% of total Kenyan production. Production of Kenyan orthodox tea showed an average annual increase of 4.3% in the period 2008-2012 (International Tea Committee, 2013). Kenya also produces low amount of green tea and with the introduction of purple tea, TRFK 306, Kenya is destined for mass production of green tea. World green tea production is expected to grow at a faster rate than black tea, 8.2 percent, reflecting the growth in China where production of green tea is expected to reach 2.97 million tonnes by 2023. (Chang, 2015).

Green tea is a non-aerated tea which is produced by steaming the plucked leaves to inactivate the enzyme polyphenol oxidase, which can oxidize tea polyphenols resulting in the formation of a brown colour. Green tea can be processed in various methods like CTC and orthodox. In CTC type of manufacture, there is full maceration of the tea leaves such that we have smaller sized teas whereas in orthodox manufacture, the tea leaves are rolled either by hand or by rollers to get relatively bigger sized teas. Green tea has naturally occurring catechins, including (-) epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC) and (-) epicatechin gallate (ECG), all in higher concentrations than other types of teas (Hung *et al*., 2010). The quality of processed tea is basically determined by the chemical constituents in tea leaves (Cabrera *et al*., 2006; Chaturvedula and Prakash, 2011). The compounds determining tea flavour and astringency include catechins (flavan-3-ols), including (-)-epigallocatechin gallate (EGCG), (+)-catechin (C), (+)-gallocatechin (GC) and their oxidation products (Liang *et al*., 2006; Narukawa et al., 2010; Chaturvedula and Prakash, 2011).

Polyphenolic compounds are known to be responsible for the antioxidant properties in many plants (Wen *et al*., 2013; Chiu *et al*., 2013). Epigallocatechin-3-gallate among other catechins play a key role as antioxidants in prevention and treatment of many diseases (Khan and Mukhtar, 2007; Sinija

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and Mishra, 2008; Kanwar *et al*., 2012). Green tea also contains flavonols (e.g., quercetin, kaempferol, myricetin), phenolic acids (e.g., gallic and chlorogenic acids), proanthocyanidins (prodelphinidin), xanthic bases (caffeine, theophylline), polysaccharides, essential amino acids (e.g., glycine, serine, valine, leucine, threonine, and characteristic amino acid theanine). Tea also has vitamins (B, C, E), and minerals and trace elements (calcium, magnesium, manganese, copper, zinc, selenium, potassium) (Sinija *et al*., 2008; Chacko *et al*., 2010). Although the mechanism is not completely clear, there is no doubt that ECs have beneficial effect on oxidative stress. Green tea polyphenols can be direct antioxidants by scavenging reactive oxygen species or chelating transition metals (Lambert and Elias 2010; Kanwar *et al.*, 2012). Alternatively, they may act indirectly by up regulating phase II antioxidant enzymes (Forester and Lambert, 2011). Spadiene *et al.* (2014) in their study found the catechins having antioxidant properties in this increasing order: catechin < epicatechin < epigallocatechin < epicatechin-3-gallate < epigallocatechin-3-gallate. The current study also investigated the antioxidant activity of the newly developed purple tea variety, TRFK 306.

Withering is an important process in tea manufacture. Suzuki *et al.* (2003) in their study found out that, as the moisture content of tea flushes decreases, withering increases the permeability of the membranes, thus enabling the catechins stored in the leaf cell vacuoles to flow out of the cytoplasm and come into contact with the oxidase in the cell cytoplasm. This study varied withering durations to investigate its effect on the quality.

Kenyan teas have high total polyphenol content (Kilel *et al*., 2013). This is because the tea breeding programme in Kenya has indirectly and consistently selected tea germplasm for high total phenol content to produce good teas with high levels of theaflavins and thearubigins. A study by Wachira and Kamunya, (2005), confirmed the superiority of Kenyan tea germplasm in total polyphenol content and that is why Kenyan teas are used for blending in other countries. This study also investigated the total polyphenols in the new purple tea variety.

TRFK 306 is a tea clone which has purple red leaf pigmentation which is due to anthocyanins. Presence of anthocyanin is a genetical characteristic. The purple red anthocyanin pigment tends to mask the normal green colour of chlorophyll. The clone was commercialised in Kenya in the year 2011 and little research on its manufacturing method has been done. Previously these authors studied various purple leafed teas including some green clones (Kilel *et al*., 2013) processed as CTC teas with uniform withering time.

The current study investigated the effect of two processing methods (CTC and Orthodox) at varying withering durations on the quality of the new released purple leafed Kenyan clone, TRFK 306, so as to inform potential processors on the best manufacturing method. Total polyphenols, catechins, gallic acid and caffeine were investigated as major quality parameters.

Materials and Methods

Tea manufacture

Tea samples for the analysis were plucked from Timbilil estate of Tea Research Institute, Kericho (0˚22' South, 35˚21' East, elevation 2180 m above mean sea level). Young tender shoots of the youngest two leaves and a bud were plucked and withered under ambient conditions. The plucked leaf were thinly spread in withering troughs and allowed to wither at different duration as is necessary. The freshly plucked tea leaves were divided into two where some were processed as CTC teas others as Orthodox teas at withering intervals of 0 hours, 5 hours, 10 hours, 15 hours and 20 hours. All samples were steamed for one minute using an electric steamer after each withering time before manufacture. For CTC teas, the withered leaves were put through the mini CTC machine and macerated three times before drying. Drying was done using fluid bed driers (Tea Craft, UK) for both CTC and orthodox teas. For orthodox teas, they were hand rolled for 20 minutes by experienced persons before drying. Samples were packed in well labeled aluminum lined sachets for further analyses.

Determination of dry matter

Approximately 2 g of milled samples were weighed and heated in an oven at a temperature of 103 ± 2^{O}C for at least 8 hours to constant weight. The percentage dry matter was then calculated when all the moisture has been removed. The dry matter content was then computed and expressed as a percent as follows; $(\Delta W/1W)^{*}100$ % = % DM. Where; ΔW is the change in weight, IW is the initial weight whereas % DM is the percent dry matter. Dry matter determination was necessary since all the parameters were expressed on dry weight basis.

Reagents

Double distilled water was used and all the solvents were of HPLC grade. 1,1 – diphenyl-2-picryhydrazyl radical DPPH, Folin-Ciocalteu phenol reagent, sodium carbonate, caffeine standard, acetonitrile and acetic acid were obtained from Sigma Aldrich, through Kobian Kenya Limited in Nairobi.

Sample preparation for total polyphenol and catechins analysis

Finely milled samples of 0.2 g were weighed into extraction tubes. Five millilitres of hot 70% v/v methanol/distilled water was then dispensed into the sample as an extraction mixture and vortexed. Heating of the extraction tube was continued in the water bath maintained at 70^{O}C for 10 minutes with mixing in the vortex mixer after every 5 minutes (the sample was vortexed immediately, after 5 minutes and after 10 minutes). The samples were then centrifuged at 3500 revolution per minute (rpm) for 10 minutes. The supernatant was decanted into a graduated tube and the extraction procedure repeated. The extracts were then combined and made up to 10 ml with cold 70% methanol/water mixture.

Determination of total polyphenols

The Folin-Ciocalteu phenol reagent method was used to determine total polyphenols as described by Pourmorad *et al.* (2006).

Analysis of catechins, gallic acid and caffeine

A modified HPLC method of Zuo *et al.* (2002) was used to assay for the tea catechins, gallic acid and caffeine.

Antioxidant activity

The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of free radical scavenging of the tea extracts using a modified method of Brand-Williams *et al.* (1995). The soluble solid extract was standardized to give stock solutions of 50mg soluble solids per 100ml of 50% methanol. A methanolic solution (50 µl) of the antioxidant was placed in a cuvette and 2ml solution of freshly prepared DPPH added (DPPH solution was made using 80% methanol).The decrease in absorbance at 517 nm was determined using a CE 393 digital grating spectrophotometer after 15 minutes. The DPPH solution was always prepared afresh and kept in the dark to minimize the loss of free radical stock solution. All determinations were performed in duplicate. The (%) inhibition of DPPH radical was calculated from the absorbance data according to (Yen and Duh, 1994).

% Inhibition against DPPH = [(AB -AA)/AB] *100

Where AB is the absorbance of the blank sample (50 µl double distilled water and 2 ml DPPH) and AA is the absorbance of the tested sample after 15 minutes.

Statistical analysis

Completely randomized design, with three replications, was adopted for this experiment. Data analysis was done using General Linear Model of Statistical Analysis System (SAS, version 9.1). Least Significant Difference was used for means' separation. The significance level of $p < 0.05$ was considered significantly different.

Results and Discussion

The quality of green teas is affected by the inherent biochemical compounds which can vary from variety to variety. Choice of manufacture and withering duration also affects the residual levels of these biochemicals. The current study analysed the catechins which included (ΔW/1W)*100 % = ffeine and total polyphenol were also investigated. All these parameters were done on the purple leafed tea, TRFK 306, processed into CTC and Orthodox teas and withered at varying durations. The results are presented in figures accordingly.

Gallic acid and caffeine

Gallic acid and caffeine results are presented in Figure 1. Gallic acid alongside catechins and caffeine influences green tea quality. In the current study, the results showed there were significant differences ($p < 0.05$) in the type of manufacture and in withering time. The highest mean values of gallic acid in this study was noticed in orthodox type of manufacture at 5 hours wither and 10 hours wither with 1.28% and 1.24% respectively. The lowest mean value was at 10 hours CTC type of manufacture with 0.74%. In this study, TRFK 306 was found to have a mean value of 1.08% to 1.28% gallic acid. It is worth noting here that, the average gallic acid in the non withered (0 wither hour) purple tea both CTC and orthodox and that subjected to 20 hours of withering for both types of manufacturing processes, though not varying significant ($p<0.05$), those withered for 20 hours had slightly higher average values. This could be because of oxidative degallation of catechin especially EGCG where free gallic acid is released. Gallic acid exists in plant material in the form of free acids, esters, catechins derivatives and hydrolysable tannins. Some studies have shown that gallic acid and its derivatives have antioxidant activity (Karamaæ *et al*., 2005, Gramza *et al*., 2005).

The caffeine levels as seen in Figure 1 were significantly different ($p<0.05$) with regard to the type

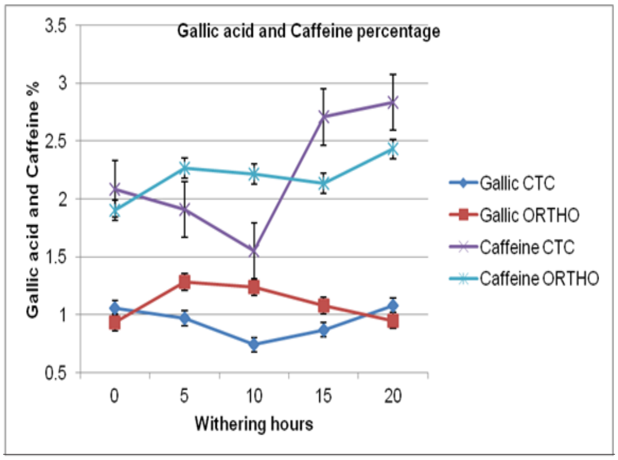

Figure 1. Effect of manufacturing method and withering duration on percent gallic acid and caffeine. Each value is a mean of three replicates. Bar means ± standard error. Key CTC, Cut Tear Curl; ORTHO, Orthodox

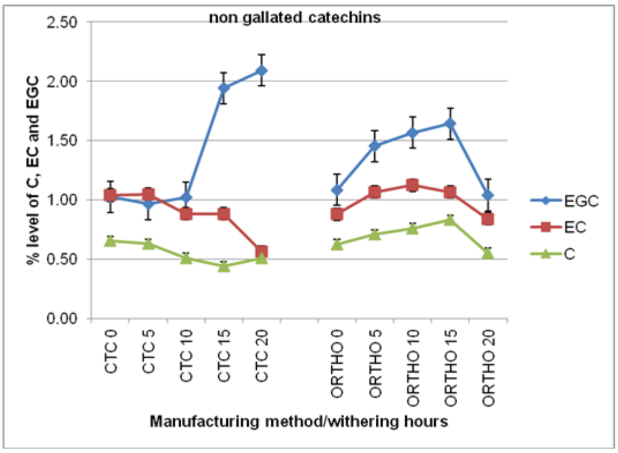

Figure 2. Effect of manufacturing method and withering duration on non gallated catechins percent levels. Each value is a mean of three replicates. Bar means ± standard error. Key CTC, Cut Tear Curl; ORTHO, Orthodox; C, Catechin; EC, Epicatechin; EGC, Epigallo catechin.

of manufacture and withering time. The highest mean value of caffeine was observed in CTC manufactured teas which had been withered for 20 hours (CTC 20 hours) with 2.83% and the lowest mean value observed in teas processed via CTC and withered for 10 hours wither with 1.55% caffeine. From Figure 1 it can be observed that the caffeine content percent increased with withering time. Previous studies had also found the same trend (Bhatia, 1962; Sanderson and Graham, 1973; Dev Choudhug and Bajaj, 1980a; Owour *et al*., 1986, Owour *et al*., 1989; Bhuyan and Mahanta, 1989). The literature explaining the increase in caffeine with longer time of withering is scarce and this warrants a research to demystify this. Caffeine is important in quality of tea since it is responsible for the briskness of the tea liquor and also good for human health as it prevents tumorgenesis (Lin and Liang, 2000).

Non gallated catechins (Epigallo catechin, Epicatechin and catechin)

The results for non gallated catechins are presented in Figure 2. There were significant differences ($p<0.05$) in the type of manufacture processes and in the time of withering. CTC teas which had been withered for 20 hours had relatively higher mean value of EGC with 2.09% while CTC processed teas that had been withered for 5 hours showed the lowest mean value with 0.97% EGC. On the (+) C, significant difference ($p<0.05$) was observed both on the type of manufacturing process and withering time. Orthodox processed teas that had been withered for 15 hours had the highest mean value of 0.83% of C whereas CTC processed teas which had been withered for 15 hours had the lowest mean value of 0.44%. There was a significant difference ($p<0.05$) in the mean values of EC with orthodox processed teas which had been withered for 10, 15 and 5 hours ,and non withered CTC teas having relatively higher mean values. The order of the non gallated catechins in percent was EGC>EC>C in both manufacturing methods as observed in figure 2. EGC is noted to be increasing with withering time and this can be attributed to degradation of EGCG which is decreasing with withering time. EGCG might degrade with time after plucking resulting in formation of EGC and the liberation of free gallic acid. It can also be noted in figure 2 that, the C and EC are decreasing with increase in withering time in CTC manufacture while it seems to be increasing in orthodox manufacture with withering time. This can be explained by the fact that in CTC, because of full maceration and hence higher surface area for the enzyme polyphenol oxidase, there is more oxidation of the catechins and hence their decrease in CTC type of manufacture. Whereas in orthodox manufacture the leaves are not macerated and hence there is less oxidation of the catechins.

Gallated catechins

The results of effect of processing and withering duration on gallated catechins levels are presented in Figure 3. The gallated catechins are the predominant catechins among the catechins. EGCG levels varied significantly ($p < 0.05$) influenced by the type of manufacture and withering duration. The highest mean value of EGCG content was noted in orthodox processed teas withered for 5 hours which had 5.05% EGCG and CTC processed teas withered for 15 hours recorded the lowest mean with 1.65%. There were significant differences ($p< 0.05$) in ECG percentage with regard to the type of manufacture and the

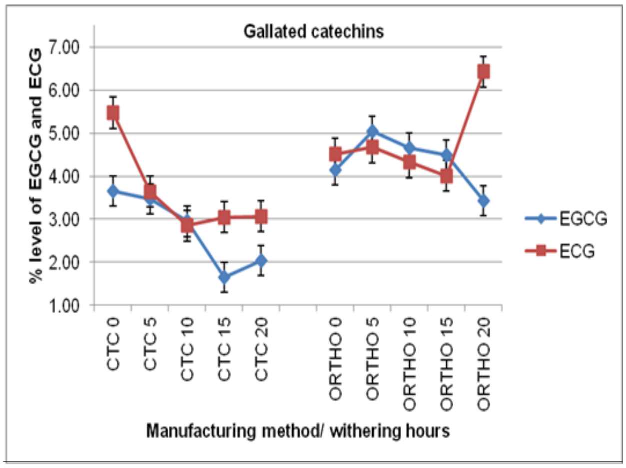

Figure 3. Effect of manufacturing method and withering duration on gallated catechins percent levels. Each value is a mean of three replicates. Bar means ± standard error. Key CTC, Cut Tear Curl; ORTHO, Orthodox; ECG, Epicatechin gallate; EGCG, Epigallo catechin gallate.

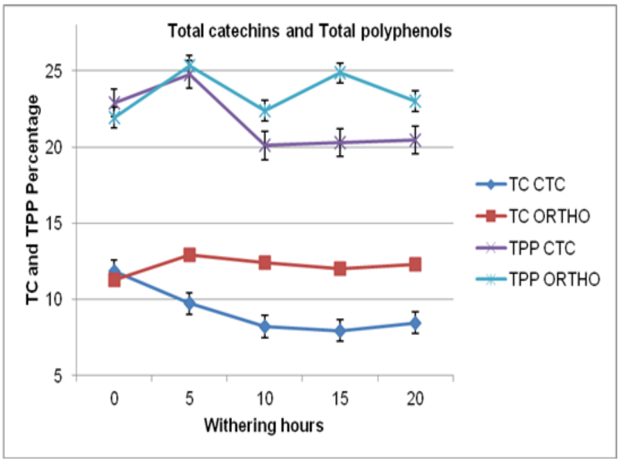

Figure 4. Effect of manufacturing method and withering duration on percent total catechins and total polyphenols. Each value is a mean of three replicates. Bar means ± standard error. Key CTC, Cut Tear Curl; ORTHO, Orthodox; TC, total catechins; TPP, total polyphenols.

withering duration. The highest mean value was observed in orthodox processed teas whose leaves had been withered for 20 hours which had 6.43% ECG and the lowest mean was noted in CTC processed teas withered for 10 hours which had 2.85% ECG.

From Figure 3, it can be observed that, withering for 5 hours onwards, there is a consistent trend where the orthodox type of manufacture displays a higher mean value of both EGCG and ECG. Moreover, ECG and EGCG are decreasing with withering time for both CTC and orthodox types of manufacture. This is because during withering stage, the content of catechins is mostly influenced by polyphenol oxidase and peroxidase. The oxidation of catechins under the action of the enzymes results in the decrease of catechins (Theppakorn, 2016).

Total catechins and total polyphenols

Total catechins results are presented in Figure 4. There were significant differences ($p<0.05$) in the type of manufacture and withering duration. Orthodox teas whose leaves had been withered for 5 hours had relatively higher mean of 12.95% with CTC teas whose leaves had been withered for 15 hours had the lowest mean of 7.96% total catechins. Except for non withered, all orthodox processed teas showed higher mean value than the corresponding CTC processed. The total catechins are observed to be decreasing with increasing withering time especially in CTC type of manufacture. Consequently there is a general decline in total polyphenols. This is because catechins forms most total polyphenols.

Total polyphenols results are presented in Figure 4. Significant differences were observed in the type of manufacture and the withering duration. High percentage of TPP was noted for leaves withered for 5 hours for both CTC and orthodox processed teas and for orthodox processed teas withered for 15 hours. Relatively lower TPP percentage was observed at CTC processed tea that had been withered for 10 and 15 hours. The Tea Research Institute of Kenya, through continuous research, has established that teas can be categorized as follows: high quality teas have 24.8–27.1% TPP; medium high quality have 22.5–24.4% TPP; medium quality have 19.6–22.3% TPP; low quality teas have 17.5–19.2% TPP. In this study the first category of high quality teas included; those withered for five hours and processed using CTC and orthodox methods and also orthodox processed teas that had been withered for 15 hours. The medium high quality teas included those non withered CTC processed and orthodox processed teas withered for duration of 20 hours. The rest which comprise those withered for 10 hours and processed and manufactured by either way, those CTC processed teas which were withered for 15 and 20 hours and non withered orthodox teas fall under medium quality teas.

Antioxidant activity

The results of antioxidant activity are presented in Figure 5. There is a significant difference ($p<0.05$) noted in the type of manufacture and withering duration. The highest antioxidant activity was observed in teas withered for 20 hours and CTC manufactured, whereas the lowest mean value was noted at 5 hours wither orthodox manufacture with 92.07% and 89.99%, respectively. There was an increase in the inhibition percentage with increase in withering time especially so in CTC type of

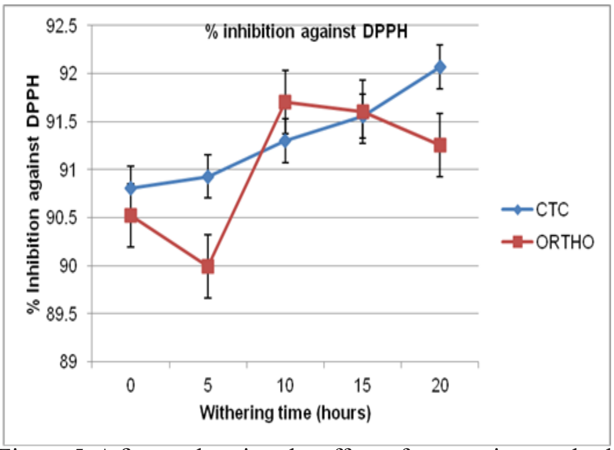

Figure 5. A figure showing the effect of processing method and withering duration on the percentage inhibition against DPPH. Each value is a mean of three replicates. Bar means ± standard error.

manufacture. The increase in the antioxidant activity with withering time could be because of products of enzymatic oxidation which starts immediately after plucking. During enzymatic oxidation of catechins which is faster in CTC type of manufacture than orthodox type because of larger surface area, products like theaflavins are produced. Luczaj and Skrzydlewska, (2005) in their study found out that the antioxidant activity of theaflavins is comparable to that of catechins. In fact, theaflavins were shown to be able to scavenge superoxide radicals more efficiently and ten times faster than EGCG (Luczaj and Skrzydlewska, 2005). The current findings however disagree with Carloni *et al.* (2013) who found the opposite results. They argued that CTC method creates a greater surface area for enzymatic oxidation and therefore the CTC teas have lower catechin content and antioxidant capacity compared to orthodox teas.

Conclusion

From the findings of this study, it is concluded that TRFK 306, can be processed either through orthodox or CTC method of manufacture with specific withering times. When gallic acid and caffeine are the main target compounds, then withering between 15-20 hours is suggested. Withering time of fifteen hours and processing through orthodox method, results in teas with relatively higher non gallated catechins. EGCG and total polyphenols are highest when the teas are processed through orthodox method irrespective of withering duration. TRFK 306 produces teas with high antioxidant activity especially when teas are withered for 15 -20 hours and processed via CTC method of manufacture. Long withering hours increases percent inhibition against DPPH.

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