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EFFECTS OF COLD PLASMA TREATMENT ON COLOR PIGMENTS AND BIOACTIVE COMPONENTS OF RED CHILLIES

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ABSTRACT

Cold plasma is an innovative and emerging technology in the food processing industry, recognized for its ability to modify surface properties while maintaining the quality of the product. The objective of this study was to evaluate the impact of different cold plasma exposure conditions on the color pigments and bioactive component of red chilli samples. The effects on color values, total carotenoids, beta-carotene, and anthocyanin content were analyzed under varying cold plasma voltages (20, 25, and 30 kV) and treatment durations (1, 5, 10, and 15 minutes). Brightness (L^*) and redness (a^*) values improved notably during CP treatment, contributing to enhanced visual appeal of red chilli. The total carotenoid content reached its highest value of $165,203 \pm 3,304 \mu\text{g}/100\text{g}$ at 30 kV for 10 minutes, while beta-carotene content showed a significant increase ($p < 0.05$) to $2,561 \pm 244 \mu\text{g}/100\text{g}$ under 30 kV for 5 minutes. Anthocyanin content reached its highest level at $32.02 \pm 8.48 \text{ mg}/100\text{g}$ after 5 minutes of CP treatment at 30 kV, but prolonged exposure resulted in a decline, demonstrating that CP exposure enhances bioactive components only within an optimal time frame. Based on the observations, the sample treated with 20 to 30 kV for 15 minutes was found to be the most effective in achieving optimal improvement in color pigment factors, while the 30 kV treatments for 5 minutes demonstrated the best preservation of bioactive components.

Keywords : Bioactive components, Cold plasma treatment, Color pigments, Red chillis

Introduction

Red chilli (*Capsicum* spp.) is one of the essential and important spice crops in many countries of the world. It serves as an excellent cash crop, catering to both domestic markets and international trade in several developing and developed nations. In India, it is considered one of the most valuable cash crops (Cui *et al.*, 2023). Red chillies are highly valued for their intense heat, vibrant flavour and natural colour making them a staple spice in various cuisines around the world. It is grown commercially as a spice-cum-

vegetable crop in India, China, Ethiopia, Hungary, Indonesia, Japan, Spain, Mexico and other countries. India is the world's largest producer, consumer and exporter of red chilli. It is used in almost every cuisine as spice for its pungency, colour and flavour (Sekhar and Karunakaran, 2018).

Color is as an important attribute for determining the commercial value of red chilli. Product appearance is a key factor that significantly impacts consumers' perception of quality, both directly and indirectly. Color plays a pivotal role in determining a product's

overall acceptability. For instance, if the color of a product is unappealing or undesirable, other important quality attributes like texture and flavor are often overlooked. The vibrant red color, often attributed to pigments like capsanthin, directly influences consumer perceptions of freshness and quality. It is also widely used for cosmetic purpose due to the colour attribute of capsanthin and capsorubin (Deng *et al.*, 2018, Das *et al.*, 2024).

Additionally, red chilli is a rich source of bioactive compounds with health-promoting properties, including natural pigments like total carotenoids, β -carotene and anthocyanins. These compounds not only offer physiological benefits but also serve as natural dyes and antioxidants, enhancing food formulations by delaying oxidation and improving product stability during storage (Amorim *et al.*, 2023, Das and Sahoo, 2023). However, fresh chili peppers, with their high moisture content and delicate texture, are highly perishable and challenging to preserve. Drying is a crucial processing method to extend their shelf life and maintain their quality (Deng *et al.*, 2018, Das and Das 2023). The aim of drying is to reduce the moisture content to a safe level, which can hinder the growth and reproduction of microorganisms and prevent many moisture-mediated deteriorative reactions (Wang *et al.*, 2018; Yang *et al.*, 2018).

Unfortunately, during conventional processing, the natural color pigments in red chilli products often degrade due to heat exposure, resulting in the development of undesirable off-colors and a reduction in their bioactive compounds. This heat-induced deterioration negatively impacts the vibrant color that is crucial for both the visual appeal and the quality of red chilli products. These color modifications deeply influence the consumer's preferences and thus cause a significant loss of product marketability (Wang *et al.*, 2018, Sahoo *et al.*, 2023). Researchers have tried to improve the processing fundamentals to abate their detrimental effects on natural food pigments. However, their complete obliteration is still a massive task for the emerging food processing industries. The trend of the global food market in the last decade affirms the utilization of non-thermal processing techniques amalgamated with surging concerns for natural, green, and minimally processed food preparations (Deng *et al.*, 2018, Singh and Thakur 2024).

Presently, some common approaches include cold plasma, ozone, high hydrostatic pressure, irradiation, ultraviolet technology, pulsed electric field, ultrasound, and supercritical technology are extensively explored by the domestic as well as the global food processing industries (Nowacka *et al.*, 2021). Among them, Cold

plasma treatment (CPT), could be a promising alternative, as it may ensure adequate safety of the products and guarantee proficient conservation of chemical and physical features, bio-availabilities of functional compounds, and maintaining natural food pigments (Singh and Thakur 2024). Due to their non-thermal nature, cold plasma treatments are known to have negligible effects on the pigments of food products (Pankaj *et al.*, 2014).

However, there is limited research on the effects of cold plasma processing on spices, particularly regarding the impact of different CP treatment conditions on their color pigments and bioactive component. Therefore, this study was designed to investigate the impact of cold plasma (CP) treatment conditions on the color pigments as well as bioactive compounds in red chillies, addressing the current research gap. Additionally, the study aims to enhance understanding of plasma's interaction with food components and encourage the use of non-thermal technology on an industrial scale. CP-treated red chillies could potentially be used to produce functional foods, fostering further research into developing more sustainable, eco-friendly, and energy-efficient technologies for red chillies processing.

Materials and Methods

Procurement of raw material, Chemicals and equipment

Red chillies (Teja variety) were procured from commercial sources. Healthy Red chillies with no signs of infection were used in the current study. All the chemicals used in the present study were of AR grade. The glassware and equipment utilized were from MFPI- Quality Control Laboratory, Post Graduate and Research Centre, College of Community Science and Central Instrumentation Cell, PJTAU, Rajendranagar, Hyderabad.

Cold plasma treatment

For each cold plasma treatment, approximately 100 g of Teja variety red chilli samples were evenly spread on a PET (polyethylene terephthalate) tray (15×15 diameter) for processing. The experiments were conducted using an open-air multipin-plane plasma reactor (Ingenium Naturae Pvt Ltd, India). This reactor features a multipin electrode consisting of 88 pins arranged in an 11 × 8 grid, with a 20 mm inter-pin spacing and an adjustable distance between the pins and the plane. The reactor was powered by a high-voltage step-up transformer capable of producing up to 40 kV R.M.S. voltage from a standard input supply of 220 to 250 V at 50 Hz. The transformer's output voltage was regulated via an electronic control panel.

Samples were exposed to plasma at various durations 1, 5, 10, and 15 minutes under three voltage settings: 20 kV, 25 kV, and 30 kV as shown in Fig. 1. Each treatment condition was replicated three times to ensure the accuracy of the results. An untreated red

chilli sample was used as the control to enable a comprehensive comparison of the effects of cold plasma treatment on the physical properties of the red chillies.

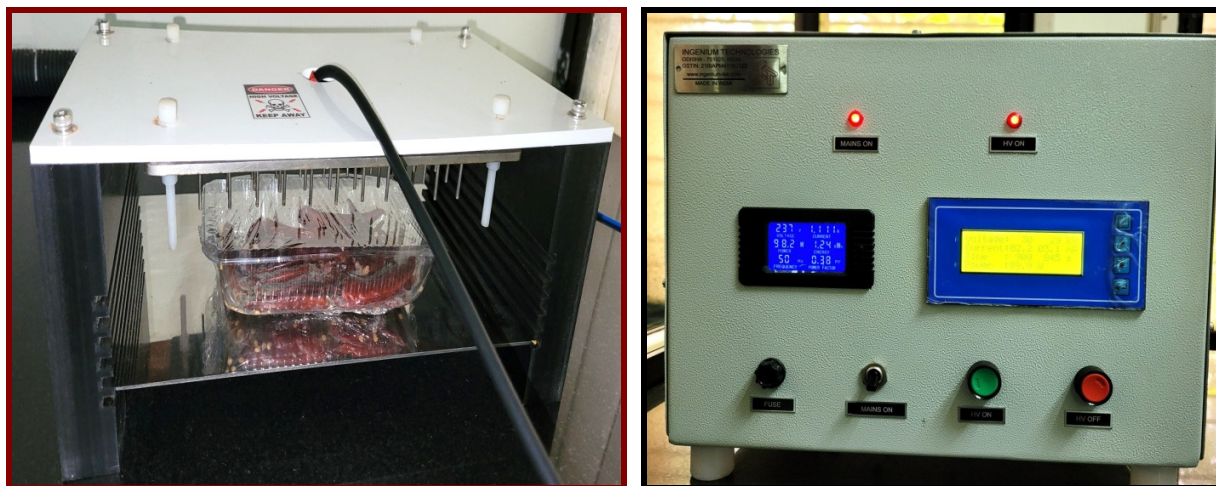


Fig. 1 : Cold plasma treatment

Estimation of color pigments in control and cold plasma treated red chillies

The color quality of red chilli was evaluated by Hunter Lab $L^*a^*b^*$ color parameters. The color of the red chilli samples were measured using a Hunter Lab spectrophotometer, with the parameters L^* , a^* , and b^* (Hunter Lab Color Flex, Firmware version 1.1, Reston, Virginia) and a measuring aperture of 36 mm. Calibration was performed before each trial using manufacturer-provided white, green, and black tiles to ensure accuracy. A circular glass cuvette was used for sample measurements, where the sample was placed directly on the reading lens for testing. Three readings were taken for each sample, and the average values were recorded for L^* (lightness), a^* (redness), and b^* (yellowness). The L^* values range from 0 (black) to 100 (perfect white), while the a^* values indicate red when positive and green when negative. Similarly, positive b^* values correspond to yellow, while negative b^* values represent blue hues (AOAC, 2005).

Determination of bioactive component

The bioactive compounds of control and cold plasma treated red chillies were analyzed for total carotenoids content (AOAC 2000); β -Carotene content (AOAC 2000) and anthocyanin content by method described by Hou *et al.* (2019).

Statistical analysis

The generated data was subjected to Analysis of Variance (ANOVA) using SPSS version 23 (SPSS,

IBM, Chicago USA) and means were separated using the Duncan multiple range test. The values obtained are presented as mean \pm standard deviation of three parallel measurements. Significant differences among different treatments were accepted at 95% confidence interval ($p < 0.05$).

Results and Discussion

Effects of cold plasma treatment on color indices of red chillies

Color is a crucial factor in food selection, serving as a sign of freshness and quality that significantly impacts consumers' purchasing decisions. The color parameters (L^* , a^* and b^*) of control and cold plasma treated red chilli are presented in Table 1.

Color parameters (L^* , a^* and b^*)

Cold plasma (CP) treatment significantly ($p < 0.05$) influenced the color parameters (L^* , a^* , and b^*) of red chilli samples, with notable changes observed in brightness, redness, and yellowness compared to the untreated red chilli. The L^* value of the untreated sample was 35.44 ± 0.14 , which was significantly ($p < 0.05$) lower than that of the cold plasma treated samples. The results showed no significant difference in the L^* value among samples treated with cold plasma for 5 minutes at 25 kV (38.12 ± 0.68) and 30 kV (38.58 ± 0.57), 10 minutes at 20 kV (38.54 ± 0.28), 25 kV (38.68 ± 0.02), and 30 kV (38.70 ± 0.31), as well as 15 minutes at 20 kV (38.77 ± 0.20) and 30 kV (38.79 ± 0.23). The highest L^* value in the red chilli

samples was observed after 15 minutes of cold plasma treatment at 30 kV (38.93 ± 0.05). This increase in L^* value indicates enhanced brightness in the red chilli samples, indicating a significant change ($p < 0.05$) compared to the untreated sample.

The study showed no significant difference in the a^* value between the control sample (34.35 ± 0.14) and the sample subjected to 1 minute of plasma treatment at 20 kV (34.37 ± 0.02). However, a slight increase in a^* value was observed with increasing voltage, specifically at 25 kV (34.82 ± 0.17) and at 30 kV (35.23 ± 0.19) after 1 minute of treatment. The highest a^* value was observed at 30 kV (37.19 ± 0.03) after 15 minutes. The results showed that the a^* values for plasma treated red chillies tended to move towards red, signifying a significant change ($p < 0.05$) compared to the control sample. The increased redness in red chillies suggests that cold plasma could serve as an optimal postharvest treatment for enhancing and retaining color in chilli pods.

The initial b^* value in the red chilli samples was 39.44 ± 0.31 , which significantly ($p < 0.05$) reduced to 36.69 ± 0.07 , 33.90 ± 0.41 and 33.35 ± 0.37 at 20, 25 and 30 kV, respectively, after 1 minute of CP treatment. It

was observed that after 5 minutes of CP treatment at 20 kV (37.16 ± 1.03) and 25 kV (36.88 ± 0.21), there was no significant change in the b^* value. However, when the CP treatment time was extended to 15 minutes, the b^* value of red chilli samples decreased to 37.53 ± 0.28 , 35.99 ± 0.18 , and 34.19 ± 0.59 for the same voltage levels. The decrease in the b^* value indicates a reduction in yellowness in the red chilli samples, representing a significant change ($p < 0.05$) compared to the untreated sample.

The present study's findings align with those of Misra *et al.* (2014), Sarangapani *et al.* (2017), and Gavahian *et al.* (2020), where no significant differences ($p < 0.05$) were observed in the color parameters (L^* and a^*) of cherry tomatoes, blueberries, and shiitake mushrooms after cold plasma treatment. In contrast, Yong *et al.* (2019) reported an increase in L^* and a^* values, while the b^* value significantly decreased ($p < 0.05$) with longer cold plasma treatment durations and higher voltage levels. The increase in the color parameters (L^* and a^*) is likely attributed to the breakdown of carotenoid pigments by plasma species, as reported by Zhang *et al.* (2022).

Table 1 : Effects of cold plasma treatment on color indices of red chillies

S. No	Cold Plasma Treatments		L^*	a^*	b^*
	Voltage (kV)	Time (min)			
1.	Control		35.44 ± 0.14^a	34.35 ± 0.14^a	39.44 ± 0.31^h
2.	20	1	35.93 ± 0.25^{ab}	34.37 ± 0.02^a	36.69 ± 0.07^{de}
3.	25		36.51 ± 0.33^{bc}	34.82 ± 0.17^b	33.90 ± 0.41^{ab}
4.	30		37.12 ± 0.44^c	35.23 ± 0.19^c	33.35 ± 0.37^a
5.	20		5	38.09 ± 0.79^d	35.94 ± 0.21^d
6.	25	38.12 ± 0.68^{de}		35.95 ± 0.04^d	36.88 ± 0.21^{ef}
7.	30	38.58 ± 0.57^{def}		36.10 ± 0.11^d	35.48 ± 0.23^c
8.	20	10	38.54 ± 0.28^{def}	36.56 ± 0.04^c	38.09 ± 0.43^g
9.	25		38.68 ± 0.02^{def}	36.68 ± 0.15^{ef}	35.39 ± 0.06^c
10.	30		38.70 ± 0.31^{def}	36.79 ± 0.03^f	33.65 ± 0.45^{ab}
11.	20	15	38.77 ± 0.20^{ef}	36.82 ± 0.02^f	37.53 ± 0.28^{fg}
12.	25		38.79 ± 0.23^{ef}	36.84 ± 0.12^f	35.99 ± 0.18^{cd}
13.	30		38.93 ± 0.05^f	37.19 ± 0.03^g	34.19 ± 0.59^b
Grand Mean			37.86	35.97	35.97
SE of Mean			0.19	0.15	0.29
CD			0.66	0.20	0.72
CV%			1.05	0.33	1.19

Note: Values are expressed as mean \pm standard deviation of three determinations. Values with similar superscripts within columns are statistically similar at 0.05% level.

Effects of cold plasma treatment on bioactive component of red chillies

Bioactive components like total carotene, β -carotene and anthocyanin are vital phytochemicals

known for their antioxidant properties and health benefits. Total carotene and β -carotene contribute significantly to the red-orange pigmentation in foods and play a crucial role in vitamin A synthesis,

supporting vision and immune function (Fernandrs *et al.*, 2019, Chaijan *et al.*, 2021, Sruthi *et al.*, 2022). Anthocyanins, water-soluble pigments responsible for red, blue, and purple hues in plants, exhibit potent anti-inflammatory and antioxidant activities, promoting cardiovascular health and reducing oxidative stress. Together, these components enhance the nutritional and functional value of foods (Sarangapani *et al.*, 2017).

Total carotenoids content

Cold plasma treatment significantly influenced the total carotene content of red chillies, with optimal enhancement observed at 10 minutes, particularly at 30 kV ($165203 \pm 3304 \mu\text{g}/100\text{g}$). However, at 15 minutes, the total carotenoids content declined, dropping to $150667 \pm 5774 \mu\text{g}/100\text{g}$ at 20 kV and drastically to $110267 \pm 4839 \mu\text{g}/100\text{g}$ at 25 kV and $109333 \pm 4619 \mu\text{g}/100\text{g}$ at 30 kV (Fig 2).

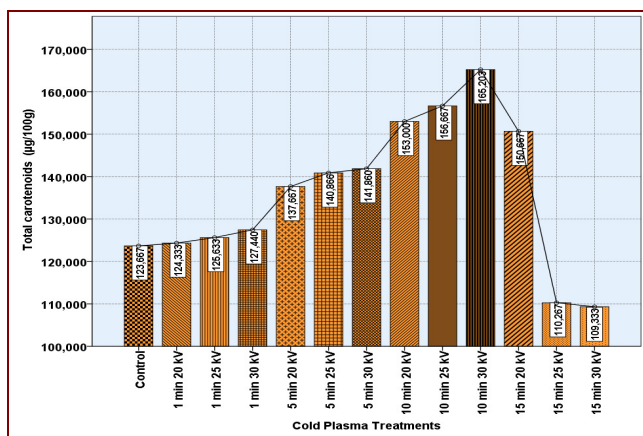


Fig. 2 : Effect of cold plasma treatment on total carotenoids content

Comparable findings were reported by Fernandrs *et al.* (2019) and Paixao *et al.* (2019), who observed significant increases ($p < 0.05$) in total carotenoids content in acerola juice and Siriguela Juice, respectively. This initial increase in total carotenoid content during cold plasma treatment is primarily due to the disruption of cell walls by reactive species, which enhances the release and extraction of carotenoids from their intracellular storage (Fernandrs *et al.*, 2019, Sruthi *et al.*, 2022). However, contrasting results were documented by Ramazzina *et al.* (2015) and Silveira *et al.* (2019), who observed a decrease in the total carotenoid content with prolonged cold plasma treatment and higher voltage levels. The increase in processing time reduced the retention of carotenoids due to the higher concentration of ionized and radical species that may have accumulated in the sample. Carotenoids have a radical scavenging

behavior, which contributes to their degradation when free radicals and ions are present (Jomova *et al.*, 2013).

β -Carotene content

The control red chilli sample contained $1707 \pm 225 \mu\text{g}/100\text{g}$ of β -Carotene, serving as the baseline as shown in figure 3. The most significant ($p < 0.05$) increase occurred at 5 minutes, with β -Carotene levels rising to $2311 \pm 163 \mu\text{g}/100\text{g}$ at 20 kV, $2480 \pm 202 \mu\text{g}/100\text{g}$ at 25 kV, and peaking at $2561 \pm 244 \mu\text{g}/100\text{g}$ at 30 kV. This enhancement aligns with the findings of Amorim *et al.* (2023), who reported that cold plasma treatment enhances β -carotene content by inactivating oxidative enzymes and inducing structural changes in cells. These changes stabilize and release more extractable β -carotene, enhancing its retention and bioavailability in treated red chilli samples. However, as the CP treatment time increased from 10 to 15 minutes, the β -Carotene content gradually decreased. The lowest β -Carotene level in the red chilli samples was recorded at 15 minutes with 30 kV ($2042 \pm 203 \mu\text{g}/100\text{g}$). The reduction in β -Carotene content during extended CP treatment may result from interactions between plasma species and carotenoids. Oxidative radicals transfer energy to carotenoids, forming unstable triplet states that undergo reactions such as epoxidation, hydrogen abstraction, and cyclization, leading to degradation products like apocarotenals and apocarotenes (Ramazzina *et al.*, 2015). These processes are likely the primary cause of the observed decline in β -Carotene in the present study. Similar findings were reported by Chaijan *et al.* (2021), highlighting oxidative degradation as a key factor in the decline of β -Carotene during prolonged exposure to reactive plasma species.

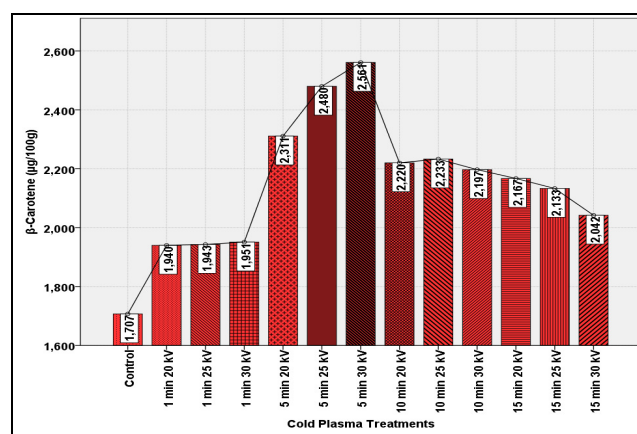


Fig. 3 : Effect of cold plasma treatment on β -Carotene content

Anthocyanin content

Figure 4 outlines the anthocyanin content in both untreated red chilli samples (25.01 ± 8.54) and those

treated with cold plasma (CP). At 20 kV, anthocyanin content in red chilli samples decreased from 26.21 ± 7.80 mg/100g after 1 minute to 16.99 ± 7.37 mg/100g after 15 minutes. Similarly, at 25 kV, the anthocyanin content reduced from 26.54 ± 11.92 mg/100 g at 1 minute to 14.20 ± 5.47 mg/100 g by the 15 minutes. The highest anthocyanin content was recorded at 30 kV for 5 minutes, with a value of 32.02 ± 8.48 mg/100g, while the lowest value was observed at 30 kV for 15 minutes, with a significant ($p < 0.05$) decrease to 13.88 ± 8.06 mg/100g. The data indicates that cold plasma treatment can alter the anthocyanin content with extended exposure times, particularly at higher voltages, leading to significant degradation. Similar findings were reported by Hou *et al.* (2019), where CP treatment for 4 and 6 minutes resulted in significantly higher ($p < 0.05$) anthocyanin levels in blueberry juice compared to the control.

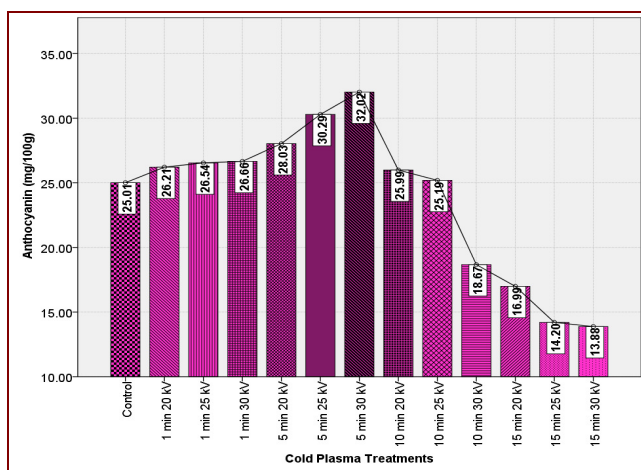


Fig. 4 : Effect of cold plasma treatment on anthocyanin content

However, a gradual reduction in anthocyanin content was observed with extended treatment durations and higher voltage levels. Active species especially ozone and hydroxyl radical are likely to cause oxidative cleavage of chromophores resulting in significant losses of anthocyanins (Saragapani *et al.*, 2017).

Conclusion

The present study highlights that cold plasma (CP) treatment significantly influences the bioactive components, color attributes, and pigment stability of red chilli samples. CP treatment effectively enhanced color quality, improving brightness and redness. It also increased levels of bioactive compounds such as total carotenoids, β -carotenes and anthocyanins within optimal treatment duration. However, extended CP exposure led to degradation of these components, likely due to oxidative reactions. These findings

underscore the potential of CP as a promising non-thermal technology for improving the nutritional and visual quality of red chilli, emphasizing the importance of optimizing CP treatment parameters for maximum benefit.

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