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Development of biodegradable smart packaging from chitosan, polyvinyl alcohol (PVA) and butterfly pea flower's (*Clitoria ternatea* L.) anthocyanin extract

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Abstract

Active compounds from natural sources such as butterfly pea flowers (*Clitoria ternatea* L.) such as anthocyanin was found able to be integrated with chitosan and Polyvinyl Alcohol (PVA) polymers in their application as smart packaging. Smart packaging that contains anthocyanin shows a colour response to changes in pH due to food spoilage. This study was aimed to evaluate the effects of chitosan (CH) and PVA proportion on the quality of biodegradable film incorporating butterfly pea flower's anthocyanin (BPFA) extract included film thickness, tensile strength (TS), elongation (E%), total anthocyanin content and the film colour response at different pH range were characterized. Concentrated anthocyanin extract was obtained from butterfly pea flower petals in ultrasonic-assisted leaching using ethanol as a solvent and followed by rotary vacuum evaporation. The film-forming solution was prepared from a binary mixture of CH:PVA in the ratio of 20:80, 40:60, 60:40, and 80:20. The results showed that films obtained from all combinations could exhibit different colour responses in various pH ranges, indicating the successful incorporation of anthocyanin. The film with CH:PVA ratio of 40:60 showed a more pronounced colour change, among other treatments. It was also characterized by a thickness of 0.15 mm, TS of 11.02 MPa, E% of 48.00%, and total anthocyanin of 25.08 mg/g. The film showed high potential to be used as biodegradable smart packaging for the food system which produces pH change upon deterioration.

1. Introduction

Efforts to maintain food availability are carried out by ensuring safety and reducing post-harvest loss. This system is applied to a variety of food commodities by providing packaging that has function as a protector, product container, has eased distribution, and communication. Based on these basic functions, a breakthrough is made in the form of smart packaging as an indicator of changes that lead to food spoilage (Kuswandi *et al.,* 2011). Spoilage of bacteria on food causes damage by impacting changes in food pH. Smart packaging with the addition of active compounds can detect changes in food pH through a colour response due to reactions with volatile amines produced by bacteria (Wei *et al.,* 2017).

The pH sensing natural dye that is commonly used is anthocyanin due to its colour producing response relating to pH changes, the presence of other colours, temperature, the concentration of other substances, certain chemical structures, oxygen, solvents, light exposure, the presence of enzymes and metal ions (Yoshida *et al.*, 2014). Anthocyanin can produce a variety of colours such as red, purple, dark blue, or dark red. It is responsible for colours in some fruits, vegetables and flowers including the butterfly pea flower (*Clitoria ternatea* L.). Anthocyanin extracts from butterfly pea flowers have characteristic similarities to anthocyanins, which are easily soluble in water, non-toxic, yet easily damage in high temperatures. Those characteristics are suitable for anthocyanin to be immobilized in making smart packaging or biodegradable films (Choi *et al.*, 2017).

Indicators film for food must be made from food-grade materials, including chitosan (CH), tapioca, agarose, PVA, and others. CH is a polymer that can produce a strong film, biodegradable, edible, and has good antibacterial properties. CH has good emulsifying activity and is thus often combined with other material/ polymer to improve its film properties, for instance, with

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hydrophobic substances like palm olein to improve its water vapour barrier (Pratama *et al.,* 2019). Whilst another polymer such as PVA can be used to improve the film mechanical features. Combining CH and PVA into a blended film has already been done in a previous study conducted by El-Hefian *et al.* (2010) and Bonilla *et al*. (2014). CH-PVA film that combined in various proportions, showed the difference in physical, structural, and antimicrobial properties. Moreover, CH-PVA film can be incorporated with a bioactive compound such as anthocyanin extract which functions as a natural pH sensing dye.

According to a previous study by Pereira *et al.* (2015), CH-PVA film can be immobilized with anthocyanin contain in red cabbage. However, based on that study, anthocyanin from red cabbage couldn't show pronounced colour changes in various pH ranges. Therefore, in this study, another source of anthocyanin, such as *Clitoria ternatea* L., contains a dense and high concentration of anthocyanin. *Clitoria ternatea* L. produced more stable anthocyanin compare to other sources (Chu *et al*., 2016). *Clitoria ternatea* L. is a native plant from Asia and can be found easily in Indonesia. The colour of anthocyanin extracted from *Clitoria ternatea* L. tends to have higher stability as long as it is kept away from direct contact with light at low room temperature, allowing it to be applied as a pH change indicator (Saptarini *et al*., 2015).

This study discussed the proportion of CH and PVA effect on the quality of indicators film containing anthocyanin from butterfly pea flower (*Clitoria ternatea* L.) including film thickness, TS, E%, the colour response of film and total anthocyanin content of film indicator. This study was aimed to determine the right proportion of CH and PVA to produce an indicator film for food pH changes.

2. Materials and methods

2.1 Materials

Chitosan (CH), *Polyvinyl Alcohol* (PVA), butterfly pea flower (*Clitoria ternatea* L.), acetic acid, ethanol 96%, glycerol, HCl, and aquadest.

2.2 Extraction of anthocyanins from butterfly pea flower

Butterfly Pea Flower Anthocyanin (BPFA) was extracted according to Damodaran *et al.* (2018) and was based on previous work (unpublished data). Fresh butterfly pea flower petals were washed and crushed. The material was dissolved in solvent 96% ethanol in an Erlenmeyer flask with a solid to solvent ratio of 1:10. The sample was extracted sonically (Branson 2510, United States) for 15 mins with a depth of 2 cm. The extract was filtered on filter paper. The filtrate was concentrated in a vacuum rotary evaporator (Biobase RE -2000A and SHZ-D (III) Water Aspirator, China) until equilibrium was reached. The resulting concentrate was stored in an opaque glass bottle coated with aluminium foil at 6^{o}C.

2.3 Film preparation

The films were made according to El-Hefian *et al*. (2010) with slight modification. Chitosan solution of 2% (w/v) was prepared by dissolving 2 g of CH in 100 mL 1% (v/v) acetic acid under 4 speed stirring at 130^{o}C for 30 mins with a magnetic stirrer (Ika T25 Digital Ultra-Turrax, China). PVA solution of 2.3% (w/v) was prepared by dissolving 2.3 g of PVA in 100 mL distilled water (v/v) under 4 speed stirring at 130^{o}C for 90 mins. The two solutions were mixed according to the proportion of CH:PVA (20:80, 40:60, 60:40 and 80:20) for 5 mins, then the glycerol 1% (v/v) was added as the plasticizer under constant stirring for 10 mins. The film hydrogel was spread (100 mL) in a Teflon pan and was then was placed in an oven (Memmert, Germany) at 80^{o}C for 5 hrs.

2.4 Immobilization of anthocyanin extract from butterfly pea flower

Immobilization of Butterfly Pea Flower Anthocyanin (BPFA), according to Warsiki *et al.* (2013) with slight modification. BPFA of 1 mL natural pH sensing dye was smeared on a 40 cm^2 film surface using a cake brush. The BPFA application was done gradually to make sure the colour was applied evenly.

2.5 Film thickness

The film thickness was measured according to Pereira *et al.* (2015), by using a micrometre screw (Herma, Germany) on five different spots. The reported value is the average of the thickness taken randomly for each sample.

2.6 Tensile strength and elongation at break

The tensile strength (TS) of the film according to Saxena *et al.* (2009), films were cut into 1 × 5 cm pieces, respectively. The elongation at break (E%) of the film was carried out according to Jirukkakul (2013) with slight modification. The films were cut into 3 × 3 cm, respectively. The pieces were fixed into tensile grips on a texture analyser (Brookfield CT-3, United States). The following equation was used in calculating the TS and E% :

$$\text{TS } \sigma \text{ (MPa)} = \frac{\text{F (N)}}{\text{A (m}^2\text{)}} \quad (1)$$

$$E\% = \frac{\text{final length - initial length}}{\text{initial length}} \times 100\% \quad (2)$$

2.7 Total anthocyanin content

Total anthocyanin content in the film was measured using the pH differential method. Indicator films were cut into 4 cm^2 for each solvent. The cut film was submerged in the blank solvent 1 mL KCl buffer pH 1 and 1 mL sodium acetate buffer 4.5 pH, respectively. Both solvents were settled for 30 mins of operating time. The total anthocyanin absorbance was measured using a UV-Vis spectrophotometer (Shimadzu, Japan) at 520 nm and 700 nm wavelengths. The absorbance of the samples was determined by the following equation:

$$A = (A_{520} - A_{700})_{pH\ 1} - (A_{520} - A_{700})_{pH\ 4.5} \quad (3)$$

Total anthocyanin content was determined by the following equation according to Luchese *et al.* (2017):

$$\text{Anthocyanin (mg/g)} = \frac{A \times MW \times FP \times V}{\varepsilon \times L} \times 100 \quad (4)$$

Whereby, A represents the absorbance value, MW is the molecular weight of predominant anthocyanin cyanidin 3-glucoside = 449.2 (g /mol), FD is the dilution factor, "ε" is the molar absorbance of cyanidin 3-glucoside = 26,900 and L is the width of the cuvette.

2.8 Colour change of indicator film in various pH range

Indicator films response to various pH ranges were performed in Koosha and Hamedi (2019) method with modification. The indicator films were cut into 4 cm^2 pieces for each seven pH buffer solutions. The samples were placed in a petri dish, and then 1 mL of buffer solution pH 1, pH 3, pH 5, pH 7, pH 9, pH 11 and pH 13 were dropped, respectively. The film images were captured in a light-controlled environment with a digital camera. The colour intensities were expressed in Red, Green, and Blue (RGB) values using Colour Grab version 3.6.1. Grayscale Index (I_G), where the lower value indicates more pronounced colour intensity.

2.9 Data analysis

The film colour response was analysed descriptively. While, film thickness, tensile strength, elongation at break, and total anthocyanin content were statistically analysed using the Analysis of Variance (ANOVA) test with a significance level of 5%. Then proceed with the Duncan test to find out the differences between each treatment.

3. Results and discussion

3.1 Film thickness

The average thickness of the CH-PVA film ranged between 0.12 to 0.16 mm (Table 1). Based on ANOVA with a significance level of $p<0.05$, there was no influence of CH and PVA proportion on film thickness. Based on the Duncan test it was found that there were no significant differences among the film treatments. Based on Table 1, it can be seen that the variation of CH and PVA proportion does not affect film thickness. The increase of PVA addition was affecting the increase of the film thickness. This is due to PVA in the form of a polymer solution that has a high viscosity. When the CH and PVA were combined into one polymer solution, PVA played a role in influencing the viscosity of the mixture (Bonilla *et al.*, 2014).

The film thickness was related to the film degradation rate. The degradation rate of the film was influenced by several factors, including the characteristics of the material used and the thickness of the film (Gomes *et al.*, 2011). PVA increases the film thickness due to its hydrophilic properties. PVA as the hydrophilic polymer increases the film water binding capacity and the film thickness. The film thickness also improves the mechanical properties of the film, light transmission, and WVP. Based on a previous study by Wang *et al*. (2019), black soybean seed coat extract as a pH sensing dye does not affect the thickness of the chitosan film.

3.2 Tensile strength

The average calculation of the TS of CH-PVA films ranged from 3.1-13.18 MPa (Table 1). Based on ANOVA with the significance level of $p<0.05$ it was shown that there was an effect of the proportion of CH and PVA on the tensile strength of the film. Based on Duncan's test it was found that there were significant differences in each treatment. As shown in Table 1 it is known that the increase in the PVA ratio was affecting the increase in tensile strength. Otherwise, a higher CH

Table 1. Solubility, tensile strength and total anthocyanin content of indicator film with various proportion of CH and PVA with the addition of BPFA Extract

Proportion	Thickness (mm)	Tensile Strength	Elongation	Total Anthocyanin
T1 (20:80)	0.16±0.06^{a}	13.18±1.17^{a}	50.00±0.00^{a}	13.87±1.13^{d}
T2 (40:60)	0.15±0.04^{a}	11.02±1.06^{b}	48.00±2.55^{a}	25.08±2.63^{b}
T3 (60:40)	0.13±0.05^{a}	5.0±0.73^{c}	15.80±4.66^{b}	17.45±1.62^{c}
T4 (80:20)	0.12±0.03^{a}	3.1±0.25^{d}	10.80±3.11^{c}	26.81±2.10^{a}

Value with different superscript in the same column indicates the significant difference ($P<0.05$).

ratio added affected the decrease in TS (Hajji *et al.*, 2016). The TS was influenced by the formation of intramolecular and intermolecular bonds that occurred between CH and PVA. Crosslinking that occurred between OH groups in PVA and NH_2 groups in CH would increase the tensile strength of films (Abraham *et al.*, 2016; Mittal *et al*., 2016).

The film CH:PVA (20:80) showed the highest TS of 13.18±1.17 MPa. This showed a similar result with research conducted by Bonilla *et al.* (2014) whereby the CH-PVA film with a ratio of 20:80 has the highest TS value of 43 MPa, compared to the TS of pure chitosan and pure PVA films. TS was needed to determine the mechanical properties of the film. This test could show the strength of the film in resisting the force given. Based on the test of film CH:PVA (40:60), (60:40), and (80:20), it was shown that the decrease in TS was due to the higher ratio of CH added. CH was built by the structure of O-H and N-H which are not flexible and difficult to stretch. Therefore, a higher CH ratio will decrease the TS of the film (Kurek *et al.*, 2012).

At a certain ratio, CH and PVA would form a cross-link that could increase the TS of the film. However, the excessive addition of PVA as a plasticizer would cause a decrease in the TS. Film CH:PVA (40:60) and (60:40) showed a decrease in TS compared to film CH:PVA (20:80), whereas film CH:PVA (80:20) had the lowest TS of 3.1±0.25 MPa. The dominant PVA ratio could prevent the formation of crosslinking with CH that could decrease the film's TS (Munthoub and Rahman, 2011).

3.3 Elongation at break

The average of film CH-PVA elongation ranged from 10.8 to 50.0% (Table 1). Results of ANOVA with the significance of $p<0.05$ shows that the CH and PVA proportions were affecting the film E%. Based on the Duncan test it was found that there were significant differences in each treatment. Based on Table 1, it can be seen that the E% was decreasing along with the decrease in the ratio of PVA added.

The film with the highest percentage of E% was filmed CH:PVA (20:80) with the E% of 50.00±0.00%, followed by film CH:PVA (40:60) with the E% of 48.00±2.55%. Based on the Duncan test there were no significant differences between the two treatments. This is due to the amount of PVA that was more dominant compared to chitosan in both film proportions. PVA could increase E% as well as increase the tensile strength of the film. PVA was able to form intermolecular reactions with chitosan through hydrogen bonds. In this reaction, the positively charged polysaccharide compound in chitosan moved toward the negatively charged hydroxyl group in PVA (Abraham *et al.*, 2016).

The film CH:PVA (60:40) E% was 15.80±4.66% and film CH:PVA (80:20) E% was 10.80±3.11%. Based on the Duncan test it is known that there were significant differences in the two treatments. The proposition of CH and PVA in both films were dominated by CH. The higher ratio of CH in a polymer film was causing a decrease of E%. Lower E% of a film would weaken the film mechanical properties and made the film more brittle with low elasticity (El-Hefian *et al.*, 2010). However, the film CH:PVA (60:40) E% was higher than the film CH:PVA (80:20). This result was consistent with the research by Ma *et al.* (2010) about hydroxyethyacryl-chitosan/PVA film with a ratio of 60:40 had an E% of 35%, which was higher than films with a ratio of 80:20 which has an E% of 25%.

3.4 Total anthocyanin content

The average total anthocyanin content in the indicator films was in the range of 13.87-26.81 mg/g (Table 1). Results of ANOVA showed that there was a significant effect of the proportion of CH and PVA on the total anthocyanin content a significance level of $p<0.05$. Based on the results of Duncan's, it was known that there were significant differences in each film composition. As seen on Table 2 it was known that the highest total anthocyanin content was owned by film CH:PVA (80:20) which is 26.81±2.10 mg/g, followed by film CH:PVA (40:60) as the second place which is 25.08±2.63 mg/g.

The total anthocyanin content in the film is influenced by the amount of BPFA that was absorbed during the immobilization process. CH ratio could affect the total BPFA that can be immobilized by the film. The effect of CH was seen in the total anthocyanin content in the film CH:PVA (80:20) and (60:40) which the CH ratio was higher than the PVA. Immobilization of BPFA could form cross bonds with CH. The phenol group contains in BPFA interacts with the hydroxyl group and the amine group in CH. The phenol group could also react with CH through electrostatic forces and ester chains (Halász and Csóka, 2018). Based on research conducted by Phrueksanan *et al.* (2014), butterfly pea flower extract contains a total phenol of 53.00±0.34 mg gallic acid/g dry extract and flavonoid content of 11.20±0.33 mg catechin/g dry extract. These results indicated that the phenol content of BPFA had a high probability to conduct interaction with CH.

Based on Table 1 it is known that film CH:PVA (20:80) has the lowest total anthocyanin content in the amount of 13.87±1.13 mg/g, while the film CH:PVA (40:60) was 25.08±2.63 mg/g. The proportion of PVA in

Table 2. Sensitivity test of indicator film with various proportion of CH and PVA with the addition of BPFA Extract

CH: PVA Ratio	Buffer pH						
	pH 1	pH 3	pH 5	pH 7	pH 9	pH 11	pH 13
20:80							
40:60							
60:40							
80:20							

those films was higher than CH. This affects the physical properties of the final film polymer becoming stiff and compact. Therefore, BPFA was not perfectly bound to the film when immobilizing by the smearing method on the film's surface. This is due to the PVA hydrophilic properties, while BPFA was extracted in ethanol. Some parts of BPFA were bound due to the interaction of phenol groups with CH and a small amount of water contained in the BPFA was bonded with PVA. The previous study by Pourjavaher *et al.* (2017) showed a similar result, in which the red cabbage anthocyanin extract was immobilized in the chitosan-corn starch film polymer. The amount of anthocyanin absorbed was influenced by the morphology of the polymer film. The film's pores were a potential space to be filled by anthocyanin extract.

The film CH:PVA (40:60) has a higher total anthocyanin content than film CH:PVA (20:80) even though both film's composition is dominated by PVA. This is due to the film's CH:PVA (40:60) hydrophilic properties that were stronger. Total anthocyanin content analysis with UV-Vis was influenced by the film water-binding ability, which in this case is the anthocyanin extract (Halász and Csóka, 2018). The film's CH:PVA (40:60) high total anthocyanin content was induced by the balanced ratio of CH and PVA that portrayed good BPFA binding properties. In a previous study conducted by Maciel *et al.* (2015), the pectin-chitosan indicator film (4.3:1) was the best proportion in binding anthocyanin extract powder from grapes due to the optimal reaction of the two polymers.

Total anthocyanin analysis using pH difference method which includes acidic buffer pH. In a low pH environment, anthocyanin will occur in the form of flavilium cation which is more stable in giving a clearer colour (Betz *et al*., 2012). In the buffer pH 1 solvent, the aglycone nucleus of anthocyanin forms a positive oxonium ion called a flavilium cation. Positive oxonium ions will form a double bond chain and turn into pink that can be analysed through visible wavelength. The buffer pH 1 solvent will turn red, which can be analysed in the 520 nm wavelength (Pereira *et al.,* 2015). In the buffer pH 4.5 solvent, the carbinol form was more dominant than the flavilium cation. The carbinol in anthocyanin does not generate any colour or remain the same as the initial colour. In this case, the colour of the film remained blue or even transparent and was analysed at 700 nm wavelength. The usage of 700 nm wavelength was for detecting residues in the solvent (Paes *et al.*, 2014).

The indicator films were showing different colour changes after various pH buffer was dropped (Table 2). At pH 1 or strong acid buffer, the colour of the film turned pink. At pH 3 and pH 5, the colour of the film became purple or purplish-blue. At pH 7 the film showed the original colour of BPFA which is blue. At pH 9 and pH 11, the colour of the film turned green. At pH 13, the colour of the film turned yellow. These colour changes were similar to the previous study by Kang *et al.* (2018) that the indicator films with anthocyanin mulberries addition turned into purplish-pink to purple at pH 2-7, blue at pH 8-10, greenish-blue at pH 11 and greenish-yellow at pH 12 or strong alkali.

The pH could influence anthocyanin colour changes. Anthocyanins extracted from butterfly pea flower consists of glycosides. Glycosides contain aglycone nuclei, which contain cations of flavilium that produces the red colour in acidic pH. At acidic pH (pH<2) the flavilium cation becomes dominant and forms a double bond to produce the colour red (Kungsuwan *et al.,* 2014). A higher pH will inhibit the production of the red colour and will eventually fade due to the hydrolysis of flavilium cation (Choi *et al*., 2017). At pH 3-5 a purplish colour was still visible due to the dominant of carbinol. Carbinol does not provide any colour or even prefers to maintain the initial colour of BPFA, especially at neutral pH. At the base pH, the colour changes appeared to be green to yellow.

As shown in Figure 1, it is known that the highest I_G average was film CH:PVA (80:20) with I_G 155.81. The lowest I_G average was film CH:PVA (40:60) with I_G 135.67. The I_G value was obtained from the average RGB value. The higher I_G value indicates that the level of white colour was high, which means the colour was fading or tended to be transparent. The lower I_G value

indicates the level of black colour was high, which means the colour appearance was stronger (Hidayah *et al.*, 2017).

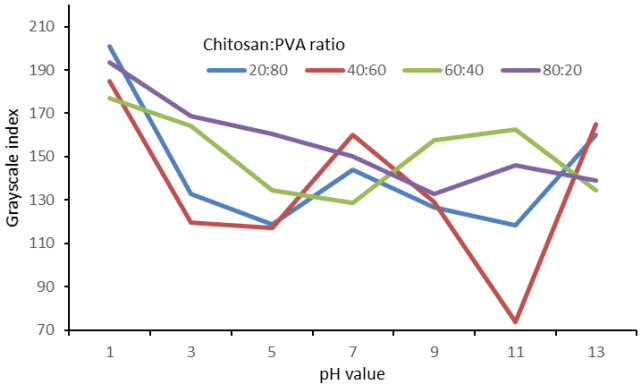

Figure 1. Grayscale index of film indicator with the various proportion of CH and PVA with the addition of BPFA extract at different pH values

The film CH:PVA (80:20) colour changes in various buffer pH were not very obvious due to the opaque yellowish film colour. The colour of the film CH:PVA (80:20) was influenced by the high CH ratio. The higher CH ratio affected the colour of the film becoming opaque and decreases the intensity of the colour changes (Ningrum *et al.,* 2019).

The film CH:PVA (40:60) could show a significant colour change at any pH level. A low I_G value of film CH:PVA (40:60) indicates a more intense colour than the others. It was influenced by the amount of BPFA that were trapped inside the PVA chains. On the other hand, the film CH:PVA (40:60) colour change was also related to the high total anthocyanin content. According to previous research by Zhai *et al.* (2017), the starch-PVA film with the addition of plasticizer was able to form a hydrogen bond with anthocyanin from rosella. Subsequently, the anthocyanins from rosella were trapped inside the polymer. The higher total anthocyanin content in a film could show clearer colour changes in a film in which the PVA ratio was more dominant. The PVA is a clear and transparent polymer which it does not display any colour that could sway the BPFA colour changes. Based on the previous study by Bonilla *et al.* (2014) that the film CH:PVA (30:70) displayed a transparent or white coloured film under the UV light transmission test compare to any other film.

A good indicator film can show a variety of vivid colour change when placed at different pH. This function is the most important aspect of making the indicator film. The indicator film is required to be able to provide colour changes that can be seen by normal eyes. The main purpose of the application of film indicators in food packaging is to monitor the food quality through changes in pH without opening its packaging or giving any change to the product (Pourjavaher *et al.,* 2017).

Despite the indicator film's ability to indicate food spoilage, the CH-PVA film has limitations in the application for dry products. The film needs direct contact with the food surface with enough water content to be absorbed into the polymer film. Moreover, the application of this film is preferable for a product that is kept in a chiller or freezer to avoid the damage of anthocyanin extract due to high temperature.

4. Conclusion

All films with various CH and PVA ratio can show colour changes in various pH ranges. However, the indicator film with the proportion of CH:PVA (40:60) can provide the clearest colour changes following its main purpose as an indicator film of pH change. The best result was found in the ratio of CH:PVA (40:60) with a thickness of 0.15 mm, TS of 11.02 MPa, E% of 48.00%, and total anthocyanin of 25.08 mg/g.

Conflict of interest

The authors declare no conflict of interest.

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