

EVALUATION OF POTATO PEEL EXTRACT AS A SOURCE OF ANTIOXIDANT AND ANTIMICROBIAL SUBSTANCES

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ABSTRACT: Antioxidant and antimicrobial substances were extracted from potato peels by using different solvents (water and both methanol and ethanol at a concentration of 80 and 100 %). The potato peel extracts were evaluated for yield extract, antioxidants activity, total phenols and total flavonoids. The best extracts were selected for their efficacy as an antioxidant and antimicrobial. The proximate composition of dried potato peels indicated that total carbohydrates (65.47 %) were the major compound, while total lipids (2.60 %) were the minor compound. Potato peel methanol extract at concentration 80 % (PPME) had higher yield extract (10.42 %), total phenols (3.78 mg GAE g⁻¹ DW), total flavonoids (0.13 mg Rutine g⁻¹ DW) and antioxidants activity (80.86 %) than other solvent extracts. The vanillic (176.64 µg/l) and hesperdin (6058.52 µg/l) were the most abundant phenolic and flavonoid compounds, respectively in PPME. Induction period (using Rancimat instrument) was increased from 5.95 hr for sunflower oil free from PPME to 10.9 and 11 hr for sunflower oil containing 200 and 400 ppm of PPME, respectively, comparing with 14 hr for sunflower oil containing 200 ppm of butyl hydroxyl toluene (BHT). PPME at 400 ppm was more effective in inhibiting of both gram-positive and gram-negative bacteria and *C. albicans* than ampicillin.

Key words: Potato peels waste, proximate composition, antioxidants, antimicrobials.

INTRODUCTION

Potatoes (*Solanum tuberosum*) are one of the most widely consumed vegetables worldwide (Amado *et al.*, 2014). The world production of potatoes in 2017 was 4325478 tons (FAO STAT, 2017). Most of the liquid and solid wastes from potato arise from peeling, trimming, slicing, cleaning, and rinsing operations which creates a pollution problem. This has resulted in environmental problems associated with waste generated by such manufacturing processes. Potato peel waste is the major waste from the potato processing industry and a potential source of functional and bioactive compounds, including not only antioxidants but also pigments, dietary fibre, vitamins and minerals (Mohdaly *et*

al., 2010). Potato peel also has acquired attention as a natural antioxidant in food system due to its high content of polyphenols, which was reported to be 10 times higher than their levels in the flesh accounting for approximately 50 % of all polyphenols in potato tuber (Al-Weshahy and Rao, 2012). Therefore, the effective utilization of potato peel as an antioxidant in food has been investigated extensively. Phenolic compounds in potatoes and by-products also exhibit beneficial antioxidative, antibacterial and health-promoting properties (Friedman, 1997, Vinson *et al.*, 2012 and Akyol *et al.*, 2016). Mohdali (2010) reported that methanolic extract of potato peels exhibited the highest extraction ability for phenolic compound and also showed the strongest antioxidant capacity. Generally, polar

organic solvents are the most effective in bioactive substances solubilizing from plant tissues (Khalifa *et al.*, 2016). Therefore, the objectives of the present study were to evaluate the potato peel extracts as a source of antioxidant and antimicrobial substances.

MATERIALS AND METHODS

1. Materials:

1.1. Plant material:

Potato peels (20 Kg) waste (*Solanum tuberosum L.*) was collected from peeling carborundum machine unit, chips production factory (6th of October city, Giza Governorate, Egypt). The collected peels were hand-sorted to remove foreign particles, filtered to remove accumulated water generated from collection zone, then stored in polyethylene bags in the freezer (Ideal, Delta Home Appliance, Egypt) at $-18^{\circ}\text{C}\pm 1$ until used.

1.2. Microbial strains:

Three different microbial groups, two Gram-positive bacteria, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 29213, two Gram-negative bacteria, *Echerichia coli* ATCC 25922 and *Salmonella typhumurium* (ATCC 14028) as well as, *Candida albicans* ATCC 10231 were obtained from National Research Center, Giza Governorate, Egypt.

1.3. Refined sunflower oil:

Refined sunflower oil was obtained from Afia Oil Industries Company, Al-Adabia, Suez Governorate, Egypt.

2. Methods:

2.1. Preparation of plant material:

Frozen potato peels waste was dried in a ventilated oven (Memmert, GmbH+CoKG, UF 55, Germany), at $40^{\circ}\text{C}\pm 1$ until dryness. The sample was ground by using a laboratory disc mill (Perten, Model LM 3100, Sweden), sieved in 25 mm sieve

and kept in a tightly closed polyethylene terephthalate bottle at $-18^{\circ}\text{C}\pm 1$ for further analysis

2.2. Extraction of antioxidants:

Dried ground potato peels were extracted with different solvents (1: 10, w/v) such as distilled water and different concentrations (80 and 100%) of both methanol and ethanol for overnight (16 hr) at room temperature ($22^{\circ}\text{C} \pm 2$). The samples were sonicated for 20 min in ultra-sonication instrument (Elmasonic 15 Hans Schmidbauer GmbH), and then filtrated through Whatman No.1 filter paper. The precipitates were re-extracted under the same conditions twice and the filtrates were combined. Evaporation of solvents performed by using a rotary evaporator (Buchi 011, Buchi, Switzerland) below (40°C) under vacuum and then performed in a vacuum oven (Type SPT-200, vacuum dryer, Poland) at 40°C for 2 hr. The residues were weighted after drying to determine the yield percentage then stored in dark tight glass at $-18^{\circ}\text{C}\pm 1$ until further use.

2.3. Proximate composition of dried potato peels:

Proximate composition of dried potato peels was determined using the following AOAC (2005) methods: moisture, ash, ether extract, crude fibers and total nitrogen (micro-Kjeldahl). Protein was calculated as $\text{N} \times 6.25$. A total carbohydrate was calculated by difference.

2.4. Determination of total phenolics:

Total phenolic content of potato peel extracts was determined by using the Folin-Ciocalteu micro-method as described by Singleton and Rossi (1965). Gallic acid was used as a standard for calibration curve. Total phenolic content expressed as gallic acid equivalent.

2.5. Determination of total flavonoids:

Total flavonoids content was determined by the method of Zhishen *et al.* (1999). Total flavonoid content expressed as rutin equivalent.

2.6. Separation and identification of phenolic and flavonoid compounds:

Phenolic and flavonoid compounds were determined according to the methods of Goupy *et al.* (1999) and Mattila *et al.* (2000), respectively. The potato peels methanol extract was dissolved in a mixture of methanol and water (6: 4, respectively). Phenolic and flavonoid compounds of potato peels extract were separated and identified by HPLC apparatus (Type: Shimadzu LC-6A model) under the following conditions: Column: Water-Bondapak C₁₈ column (250 × 4.6 mm) and as SCL-6A system controller, the solvent system used was a gradient of A (CH₃COOH 2.5%), B (CH₃COOH 8%) and C (acetonitrile). The solvent flow rate was 0.7 ml/min and separation was performed at 35°C, injection volume: 20 µl, detector: UV-visible spectrophotometer SPD- 6 AV (Leicestershire LE17 5BH, UK), phenolic compounds were assayed by external standard calibration at 280 nm and expressed in µg L⁻¹ equivalent (+)-catechin, and equivalent quercetin-3-rutinoside for flavonoids at 330 nm, sensitivity of detector: 0.04 AUFS.

2.7. Evaluation of antioxidant activity:

Potato peel extracts were tested for the scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the method of Brand-Williams *et al.* (1995). The stock reagent solution (6 × 10⁵ M) was prepared by dissolving 0.0024 gm of DPPH in 100 ml of methanol and stored at -20°C ± 1 until use. The working solution (6 × 10⁻⁵ M) was measured at 515 nm using

a spectrophotometer (SCHOOT instrument, UV line 9400, EU). One hundred microliters of potato peel extracts were mixed with 3.9 ml of DPPH solution and left to react in dark for 30 min, after which the absorbance at 517 nm was recorded. A control with no added extract was also analyzed. Scavenging activity was calculated as follows:

DPPH radical-scavenging activity (%) = [(A_{control} – A_{sample})] / (A_{control}) × 100, Where: A is the absorbance at 517 nm.

2.8. Rancimat test:

Potato peels methanol extract was added to refined sunflower oil at concentrations of 100, 200, 400 and 800 ppm comparing to sunflower oil without any additives (control) and sunflower oil contained 200 ppm of BHT. Oxidative stability (induction period, protection factor, antioxidant activity and increasing index) of previous sunflower oil samples was evaluated by the Rancimat method using 679 Rancimat (Metrohm, Herisan, Switzerland) as described by Läubli and Bruttel (1986). Samples were loaded into the reaction vessel cylinder and the air supply was maintained at 20 ml/min and 100°C ± 2.0 throughout the experiment. The induction period (IP) was recorded automatically during determination by hours, while protection factor (PF), antioxidant activities (AA) and increasing index (II) were calculated using the following equations:

$$PF = \frac{IP_s}{IP_c}, \quad II = \frac{IP_s}{IP_c} \times 100 \quad \& \quad AA = \frac{IP_s - IP_c}{IP_{BHT} - IP_c}$$

Where; PF: Protection factor, IP_s: Induction period of sample containing antioxidant, IP_c: Induction period of control without antioxidant, II: Increasing index, AA: Antioxidant activities and IP_{BHT}: induction period of sample with BHT.

2.9. Antimicrobial activity of potato peels methanol extract:

Gram-positive bacteria, *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 29213, Gram-negative bacteria, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028 were used. The strains were cultivated on nutrient broth at 35°C for 22 hr as described by Bayder *et al.* (2004). Suspensions (250 µl) of the bacteria were added to flasks containing 25 ml sterile nutrient agar at 43- 45°C and poured into petri dishes (8 cm diameter), then the agar was allowed to solidify at 4°C for 1 hr. Wells (8 mm in diameter) were made in media using a sterilized stainless-steel borer. Each well was filled with 70 µl of potato peel methanolic extracts (200 and 400 ppm) and ampicillin as control. The plates were left at room temperature for 30 min to allow diffusion of materials in media. Plates were incubated at 37°C for 18- 24 hr and then inhibition zones in mm around wells were measured. The antibacterial activity was expressed as the diameter of inhibition zones produced by the extracts against tested bacteria. The *Candida albicans* ATCC 10231 cultured in yeast and mold extract broth (YM, Difco) for 48 hr at 25°C (Shin and Lim 2004). The antifungal activity was expressed as the diameter of inhibition zones produced by the potato peels methanol extracts (200 and 400 ppm) and ampicillin as control.

2.10. Statistical analysis:

Results are expressed as the mean value \pm SD of three separate determinations (proximate composition of dried potato peels, yield extract, total phenolics, total flavonoids and antioxidant activity). All results were analyzed using one-way analysis of variance. Least significant difference (LSD) was used for comparison among means, considering significance at 0.05% level, using Costat version 6.311 (Montgomery, 1984).

RESULTS AND DISCUSSION

1. Proximate composition of dried potato peels:

The proximate composition of dried potato peels is given in Table (1). Total carbohydrates (65.46%) was the major compounds followed by total protein (10.21%), both total ash and crude fiber (7.37 %), moisture (6.99 %) and total lipids (2.60 %) on dry weight basis. The protein content of potato peels was similar that the value (10.73%) reported by Hijosa-Valseiro *et al.* (2018). Total ash and total lipid values were nearly from the results reported by Liang *et al.* (2015). Crude fiber and total carbohydrates values were agree well with those reported by Badr and El-Waseif (2018). The moisture content value of potato peel was nearly to the value reported by Zhu *et al.* (2016).

2. Properties of potato peel extracts:

The properties of potato peel extracts are presented in Table (2). Methanol extract (80%) had higher ($p \leq 0.05$) yield, total phenolics and antioxidant activity than other extracts. However, the water extract had the lowest ($p \leq 0.05$) properties. Non-significant differences ($p \geq 0.05$) were observed between ethanol 80% extract and methanol 100% extract. Total flavonoid was similar ($p \geq 0.05$) in methanol 80% extract and ethanol 80% extract and both extracts had higher ($p \leq 0.05$) total flavonoids than other extracts. Mohdaly *et al.* (2010) and Barchan *et al.* (2014) reported that methanol and ethanol were the most efficient solvents resulting in high amount yield owing to their higher polarity and good solubility from plant materials. Chang (2011) reported that total phenolics extract were generally lowest in water and greater in methanol followed by ethanol. The results of total phenolics are in compliance with those reported by Silva-Beltrán *et al.* (2017), who mentioned that total phenolics of potato peel ethanol

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and water extracts were 14.031 and 4.160 mg GAE/g, respectively. The results of total flavonoids are higher than those reported by Friedman *et al.* (2017), who found that total flavonoids extracted by methanol 80% for six cultivars were ranged between 0.007 to 0.023 mg/gm⁻¹ DW. Silva-Beltrán *et al.* (2017) reported that potato peel ethanol extract had high level of total flavonoids (3.310 ± 0.331 mg QE/g), while potato peels water extract had lower level (1.016 ± 0.116 mg QE/g).

Results of antioxidant activity are greater than those reported by Zhu *et al.* (2016). They reported that antioxidant activity percentage of potato peels extract are ranged between 36.38 to 58.62%.

From previous results, it can conclude that methanol (80 %) extract was the best solvent to extract the antioxidants from dried potato peels comparing with the other solvents. Therefore, methanol extract 80% was selected based on their antioxidant properties for further studies.

Table (1): Proximate composition of dried potato peels waste*.

Components	Wet weight (%)	Dry weight (%)
Moisture	6.99 ± 0.62	--
Crude protein	10.21 ± 0.00	10.98
Crude lipids	2.60 ± 0.00	2.80
Total ash	7.37 ± 0.00	7.92
Crude fiber	7.37 ± 1.00	7.92
Total carbohydrates**	65.46 ± 0.00	70.38

* Means ± standard deviation of means of three determinations.

** Total carbohydrates= 100- (moisture+ crude protein+ crude lipids+ total ash+ crude fiber)..

Table (2): Yields, total phenolics, total flavonoids and antioxidant activity of the extract solvents*.

Solvents	Yield (%)	Total phenolics (mg GAE g ⁻¹ DW)	Total flavonoids (mg Rutine g ⁻¹ DW)	DPPH• radical scavenging activity (%)
Ethanol 100%	7.22 ^c ±0.01	2.61 ^c ±0.00	0.09 ^b ± 0.00	62.75 ^c ±1.72
Methanol 100%	9.01 ^b ±0.00	3.26 ^b ±0.00	0.09 ^b ± 0.00	71.15 ^b ±0.01
Ethanol 80%	9.00 ^b ±0.00	3.26 ^b ±0.00	0.12 ^a ± 0.00	72.18 ^b ±0.04
Methanol 80%	10.42 ^a ±0.00	3.78 ^a ±0.00	0.13 ^a ± 0.00	80.68 ^a ±1.21
Water	5.28 ^d ±0.15	1.91 ^d ±0.01	0.05 ^c ± 0.00	58.22 ^d ±0.00
LSD**	0.52	0.22	0.01	2.39

Means in the same column with different letters are significantly difference (p≤ 0.05).

* Means ± standard deviation of means of three determinations.

** Least significant difference.

3. Separation and identification of phenolic compounds of potato peels methanol extract (PPME):

Data in Table (3) showed that vanillic was the major phenolic compound, contributing about 19.87% of total phenolics. Iso-ferulic and benzoic acids were also predominant in methanol extract of potato peels, contributing 17.63% and 15.58%, respectively. *p*-cumaric revealed the minimum

concentration contributing about 0.62 % of total amount of phenolic compounds. Present results are close to those reported by Lewis *et al.* (1998). Mohadli (2010) found that potato peels methanol extract contains different phenolic compounds such as caffeic, gallic, pyrogallol, benzoic and chlorogenic. Sello (2011) demonstrated that major phenolic compounds in potato peels extract were gallic acid, caffeic acid, chlorogenic acid and protochatechuic acid.

Table (3): Fractionation of total phenolic compounds of potato peels methanol extract.

Phenolic compounds	Concentration (µg/ L)	Percentage (%)
Pyrogallol	108.81	12.28
Gallic	8.81	0.99
Protocatechuic	10.04	1.13
Catechol	9.09	1.02
4-Aminobenzoic	3.22	0.36
Chatchein	104.07	11.74
Chlorogenic	12.47	1.40
<i>p</i> -OH-benzoic	55.67	6.28
Benzoic	138.06	15.58
Caffeic	25.59	2.88
Vanillic	176.64	19.94
<i>p</i> -Cumaric	5.54	0.62
Caffeine	28.30	3.19
Ferulic	15.19	1.71
Iso-ferulic	156.21	17.63
α -Cuumaric	8.01	0.90
Coumarin	20.00	2.25
Total phenolic compounds	885.72	100 %

Average of two determinations.

4. Separation and identification of flavonoid compounds of potato peels methanol extract (PPME):

Data in Table (4) showed that Hesperidin followed by naringin were the most abundant flavonoid compounds in the methanol extract of potato peels which represented 93.58% and 20.18%, respectively of the total flavonoid compounds. However, kampferol and hespirtin (0.49 % of the total flavonoid compounds) were the lowest flavonoid compounds in the methanol extract of potato peels. Hsieh *et al.* (2016) reported that flavonoid compounds in water extract were quercetin (4.2 ± 0.3 mg/ 100 gm), hesperidin (0.3 ± 0.2 mg/ 100 gm), naringin (0.2 ± 0.1 mg/100 gm) and rutin (0.2 ± 0.1 mg/ 100 gm). Chu *et al.* (2000) found that potato peels flavonoids consist of myricetin, quercetin and kaempferol.

5. Oxidative stability of sunflower oil as affected by addition of potato peels methanol extract (PPME):

Data in Table (5) indicated that the sunflower oil oxidative stability parameters (IP, PF, AA and II) were increased with increasing the concentration of PPME up to 400 ppm then decreased. No distinct difference between 200 and 400 ppm PPME, where IP were 10.90 and 11.0 hr, respectively. The BHT at 200 ppm gave the highest values of IP, PF, AA and II comparing with PPME at all concentrations. The data indicated that all PPME concentrations produced an antioxidant power to retarding the oil oxidation. These results are in accordance with those mentioned by Franco *et al.* (2016), who demonstrate that antioxidant effect of potato peels ethanol extract in achieving long induction period was effective. However, even the highest potato peels extract concentration was

less effective than BHT. Samarin *et al.* (2001) revealed that potato peel extract retarded the oxidation of soybean oil and increased its induction period and protection factor. Samarian *et al.* (2012) and Amado *et al.* (2014) reported that synthetic antioxidants remained the most effective and gave the highest induction period than natural extracts, due to synthetic antioxidants are pure constituents whereas the natural extract was in complex mixtures with active compounds being present at low concentrations but considered safe for human consumption more than synthetic antioxidants that have potential health hazards.

6. Antimicrobial activity of potato peels methanol extracts (PPME):

Data in Table (6) illustrated that PPME was more effective in inhibiting of gram-positive, gram-negative and *C. albicans* than ampicillin (control). This is may be due to the antioxidant activity of PPME. The flavonoid compounds had antimicrobial and antifungal properties. These results are agree well with those found by Amnpour *et al.* (2015), who reported that potato peel extract has antibacterial activity and its activity on gram-positive bacteria was more pronounced than gram-negative bacteria. Khalifa *et al.* (2016) reported that potato peels methanol extract has antibacterial activity against *E. coli* and *Staphylococcus aureus*. Naz *et al.* (2017) reported that phenolic compounds extracted from potato peels and pulp has significant effect on *Bacillus subtilis* growth. Rauha *et al.* (2000) found that plant phenolics, especially dietary flavonoids have antimicrobial activity against *Candida albicans* ATCC 10231 as pathogenic fungal strain.

Table (4): Fractionation of total flavonoid compounds of potato peels methanol extract.

Flavonoid compounds	Concentration (µg/ L)	Percentage (%)
Hesperdin	6058.52	73.40
Naringin	1666.08	20.18
Rutin	123.93	1.50
Quercetrin	86.91	1.05
Quercetin	100.58	1.21
Naringenin	136.31	1.65
Hespirtin	40.68	0.49
Kampferol	40.95	0.49
Total flavonoid compounds	8253.96	100

Average of two determinations

Table (5): Oxidative stability of sunflower oil as affected by potato peels methanol extract (PPME)*.

PPME concentrations	Oxidative stability*			
	IP (hr)	PF	AA	II (%)
Sunflower oil (control)	05.96	1.00	0.00	100
Sunflower oil +100 ppm of PPME	10.70	1.80	0.59	180
Sunflower oil + 200 ppm of PPME	10.90	1.83	0.62	183
Sunflower oil + 400 ppm of PPME	11.00	1.85	0.63	185
Sunflower oil + 800 ppm of PPME	10.70	1.80	0.59	180
Sunflower oil + 200 ppm of BHT	14.00	2.34	1.00	234

*IP: Induction period; PF: Protection factor; AA: Antioxidant activity; II. Increasing index.

Table (6): Antimicrobial activity of 400 ppm potato peels methanol extract (PPME) on the growth of some pathogenic bacteria strains and *C. albicans*.

Antimicrobial substances	Strains / Inhibition zones (cm)				
	Gram-positive		Gram-negative		<i>C. albicans</i> (ATCC 10231)
	<i>B. subtilis</i> (ATCC 6633)	<i>S. aureus</i> (ATCC 29213)	<i>E. coli</i> (ATCC 25922)	<i>S. typhimurium</i> (ATCC 14028)	
PPME	5	7	2.5	4.5	5.9
Ampicillin (control)	3.5	5	2.4	2.2	3.5

CONCLUSION

Potato peel can be utilized as a source of antioxidant and antimicrobial substances in foods, as well as generating additional income and reducing waste disposal problems.

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تقييم مستخلص قشر البطاطس كمصدر لمضادات الأكسدة والمواد المضادة للميكروبات

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الملخص العربي

تم استخلاص المركبات المضادة للأكسدة، المضادة لنشاط الميكروبات من قشور البطاطس باستخدام مذيبات مختلفة (الماء وكل من الميثانول والإيثانول بتركيز 80 و 100%). تم تقدير إنتاجية المستخلص، والنشاط المضاد للأكسدة، الفينولات الكلية والفلافونيدات الكلية المستخلصة، وتم تقييم فاعلية المستخلصات كمضاد للأكسدة ومضاد للميكروبات. أوضحت نتائج التركيب الكيميائي لقشور البطاطس المجففة أن الكربوهيدرات الكلية (65,47%) كانت المكون الرئيسي، بينما كانت الليبيدات الكلية (2,60%) أقل مكون. مستخلص الميثانول لقشور البطاطس بتركيز 80% كان الأعلى في محتواة من الإنتاجية المتحصل عليها (10,42%)، الفينولات الكلية (3,78) مجم حمض جاليك/ جم⁻¹ وزن جاف، الفلافونويدات الكلية (0,13) مجم روتين/ جم⁻¹ وزن جاف والنشاط المضاد للأكسدة (80,86%) عما هو في مستخلصات المذيبات الأخرى. يشير فصل مستخلص الميثانول لقشور البطاطس (باستخدام جهاز HPLC) إلى أن حامض الفانيليك (176,64 ميكروجرام/ لتر) و الهسبيردين (6058,52 ميكروجرام / لتر) كانا أكثر المركبات الفينولية والفلافونيدية وفرة، على التوالي. تمت زيادة الفترة التمهيديّة (باستخدام جهاز Rancimat) من 5,95 ساعة لزيت دوار الشمس الخالي من مستخلص الميثانول لقشور البطاطس إلى 10,9 و 11 ساعة لزيت دوار الشمس الذي يحتوي على 200 و 400 جزء في المليون من مستخلص الميثانول لقشور البطاطس على التوالي، مقارنة بـ 14 ساعة لزيت دوار الشمس الذي يحتوي على 200 جزء في المليون من مضاد الأكسدة الصناعي الـ BHT. كان مستخلص الميثانول لقشور البطاطس عند تركيز 400 جزء في المليون أكثر فاعلية في تثبط كل من البكتيريا الموجبة والسالبة لجرام وفطر *C. albicans* من الأمبسيلين.

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