Bulgarian Chemical Communications, Volume 57, Special Issue A (pp. 63-69) 2025 DOI: 10.34049/bcc.57.A.MU14

Microwave-assisted isolation of inulin from shatavari roots - chemical characterization and functional properties

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Received: September 03, 2024; Revised: February 04, 2025

The aim of the current study was to isolate and characterize inulin from roots of shatavari (*Asparagus racemosus* Willd.) using microwave-assisted extraction. Physico-chemical properties of the isolated polysaccharide as yield, purity, degree of polymerization, molecular weight, polydispersity index and color characteristics were evaluated. The structural elucidation was performed by FTIR spectroscopy. The functional properties as swelling, water and oil holding capacities, tapped and bulk densities, flowability, cohesiveness, hygroscopicity and wettability were evaluated. The isolated inulin was characterized by low degree of polymerization 7-10 and molecular mass of 1.6 kDa. The structure of inulin-type fructan was confirmed by FT-IR spectroscopy, where 2-ketose and β (2→1) bonds were found. Inulin showed better oil-holding capacity than water-holding one, good swelling properties (6 g/cm^3), high cohesiveness and fair flowability. The conducted research was the first detailed study for the elucidation of structure and functional properties of inulin from shatavari (*Asparagus racemosus* Willd.) roots. The isolated inulin is characterized as low-molecular fructan with good swelling and oil-holding capacities and it can be used in food or pharmaceutical formulations as taste enhancer and potential prebiotic.

Keywords: shatavari *(Asparagus racemosus* Willd.), inulin, FTIR spectroscopy, functional properties

INTRODUCTION

Shatavari *(Asparagus racemosus*), known also as satawar, or shatamuli is a medicinal plant originated from India and the Himalayas that belongs to the Asparagus genus, *Liliaceae* family [1, 2]. The roots of *A. racemosus* are finger-shaped, bitter in taste and they are sweet, oleaginous, indigestible, cooling, and an appetizer [2]. This plant is considered as one of the most popular herbs in Ayurvedic system, the category of 'Ayurvedic Rasayana' which essentially comprises herbs possessing adaptogenic and immuostimulating properties [1]. *A. racemosus* possesses aphrodisiac, immune-modulatory, adaptogenic, anaphylactic, antiulcer, molluscicidal and radioprotective properties [1-4]. The extracts of shatavari roots contain many bioactive compounds, especially carbohydrates, phenolic compounds, steroidal glycosides. Tannins were detected in ethanolic and aqueous extracts, whereas steroids, terpenes, and saponins were found in ethanolic extracts. The major compounds that bring about its biological activities are steroidal saponins, especially Shatavarins I–IX [3]. Shatavarins (I–IV) are antimicrobial compounds that inhibit mold growth and possess insecticidal activity [2]. However, the information about carbohydrate composition is unsufficient. Some recent studies have demonstrated that fructooligoacchrides (FOSs) from the roots of *A. racemosus* showed *in vitro* immunomodulatory activity, higher stimulatory activity of 51.8 ± 1.2% and low cytotoxicity [1]. The aqueous extracts of the roots of *Asparagus racemosus* rich in 2→1 type FOS were evaluated for their efficacy against streptozotocin- and alloxan-induced diabetes leading to sexual dysfunction in rats [5]. Some authors reported that the content of inulin is 12 % with degree of polymerization 7-8 [1, 6, 7]. However, there is no detailed study so far of the characteristics of inulin from shatavari roots and evaluation of its functional properties. Revealing the polysacharide characteristics of shatavari roots will bring about its future application. Therefore, the aim of the current study was to isolate inulin from roots of shatavari (*Asparagus racemosus* Willd.) using microwave-assisted extraction and to characterize its physicochemical and functional properties.

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EXPERIMENTAL

All used solvents and chemicals were of analytical grade.

Plant material

The finely ground powder of shatavari (*Asparagus racemosus*) roots was purchased from on-line shop Zoya (Bulgaria) with LOT 2006030006, with declared origin from India. The roots were used as they were received without any pretreatment.

Carbohydrate analysis in aqueous extracts

The aqueous extracts of shatavari roots (2.5 g) were obtained using an ultrasonic bath (Isolab, Germany) as previously decribed [8]. The total fructan content in the extracts was determined by resorcinol-thiourea spectrophotometric method at 480 nm [9]. The individual sugars and inulin were analyzed by HPLC-RID method [9].

Microwave-assisted isolation of fructan from shatavari roots

The shatavari root sample (50 g) was extracted with 500 ml of boiling distilled water in duplicate using a microwave device Crown (2452 kW), with microwave power of 700 W for 5 min [10]. The hot water extract was filtered through a nylon cloth. The extract was cooled to room temperature and then was precipitated with acetone (1:4 v/v). The obtained sample was separated by centrifugation and then purified by recrystallization from boiling water and precipitated with acetone (1:5 v/v). The obtained polysaccharide was filtered and dried at 40 °C.

Characterization of fructan from shatavari roots

The melting point was measured with a Kofler apparatus. The reducing groups were determined by the PAHBAH method at 410 nm [10], while total fructose content - by resorcinol-thiourea reagent at 480 nm. The purity of inulin was analyzed by HPLC on an Elite Chrome - Hitachi instrument with a Shodex® Sugar SP0810 column (300 mm × 8.0 mm i.d.) at 85°C, flow rate of 1.0 ml/min and injection volume of 20 µl [10]. High-performance size-exclusion chromatography (HPLC-SEC) was used for the determination of number average molecular weight (Mn), and weight average molecular weight (Mw). The analysis of isolated inulin was performed on HPLC ELITE LaChrome (VWR Hitachi, Japan) chromatograph with a column Shodex OH-pack 806 M (ID 8 mm and length 300 mm), (Shodex Co., Tokyo, Japan) at 30°C and a RI detector (VWR Hitachi Chromaster, 5450, Japan) with 0.1M $NaNO_3$ [9]. Polydispersity index (X) of inulin was calculated as the ratio of the two molecular weights (Mw/Mn).

FTIR spectroscopy

The FTIR spectrum of isolated shatavari fructan (2 mg) was collected in KBr tablet on a Nicolet FTIR Avatar Nicolet (Thermo Scientific, USA) spectrometer in the wavelength range of 4000–400 cm^{-1} after 132 scans at a resolution of 2 cm^{-1}.

The color measurement of shatavari inulin was done using a portable colorimeter Model WR-10QC D 65 lighting, following the CIELAB (L*, a*, b*) system [11]. The swelling properties, water- and oil-holding capacities of shatawari inulin were evaluated according to Robertson *et al.* [12]. Other characteristics as true, bulk, and tapped densities, angle of repose, flowability and wettability were determined according to the methods described in [13].

Statistical analysis

All experimental measurements were carried out in triplicate and are expressed as average of three analyses ± standard deviation.

RESULTS AND DISCUSSION

Before isolation of polysaccharide, a screening of the aqueous extract for carbohydrate composition is needed. The results from the analysis of the aqueous extract from shatavari roots are summarized in Table 1. The carbohydrate profile is presented on Figure 1. The total fructan content in the analyzed sample was 24 g/100 g dw, as 21.22 g/100 g dw was inulin. In the aqueous extract of shatavari roots were found also 1-kestose (2.91 g/100 g), sucrose, glucose and fructose. Therefore, inulin is the dominating polysaccharide in shatavari roots (88 % from the total detected fructans), followed by prebiotic 1-kestose. The inulin level in *Asparagus racemosus* found [14] in some studies varied between 10 − 15 g/100 g fw [14, 15]. *A. falcatus, A. racemosus* and *A. officinalis* have shown inulin content in the roots of 17.74, 11.83 and 15.3% on a fresh weight basis, respectively [16]. According to some authors [16] asparagus roots contain around 28% fructans on a dry weight basis, which was near to our results (24% dw).

Table 1. Fructan and sugars content in shatavari roots, g/100 g dw

Characteristics	Values, g/100 g dw
Total fructans	24.07±2.68
Inulin	21.22±2.30
Nystose	-
1-Kestose	2.91±0.05
Sucrose	2.73±0.25
Glucose	0.70±0.21
Fructose	1.90±0.19

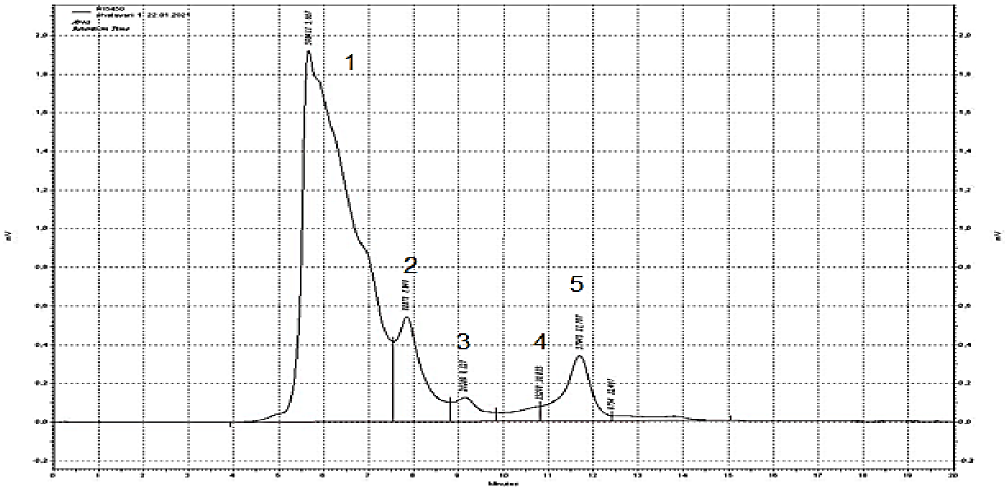

Fig. 1. HPLC-RID chromatogram of an aqueous extract of shatavari roots, where 1. inulin, 2. 1-kestose, 3-sucrose, 4-glucose and 5-fructose.

Isalation and characterization of inulin-type fructan from Asparagus racemosus roots

The yield was low (2.6 % dw), with 19 % fructose content. The purity of the obtained inulin was 58%. The degree of polymerization was evaluated to be 7-10, and the obtained inulin from *Asparagus racemosus* can be evaluated as low-molecular fructan, part of fructooligosacchrides with molecular mass 1655 Da. The polydispersity index is 1.03, which is near to the chicory inulin Frutafit HD with DP 8-12 used as a reference (Table 2). In some early study about phyto-chemical evaluation 26.7% yield of 2 → 1 linked fructo-oligosaccharides (FOS) with degree of polymerization (DP) of nearly 7–8 in *Asparagus racemosus* roots was reported [1]. Sun *et al*. [17] reported a new inulin-type fructan from *Asparagus cochinchinensis* with molecular weight of 2690 Da isolated using distilled water and it could be used as a dietary supplement to improve health. The average degrees of polymerization were 8.8 in raw inulin (*Asparagus racemosus*) according to another study [18]. Therefore, our data about inulin with low molecular mass and low degree of polymerization coincided with most of the reports in literature for *Asparagus racemosus* [1, 18]. HPLC-RID chromatogram of the polysaccharide from shatawari isolated by microwave–assisted extraction is presented in Fig. 2. The purity of the obtained inulin was 58% and from the chromatogram can be seen also presence of sucrose and fructose, which are degradation products of inulin-type fructan during the sample preparation. The low molecular weight of shatavari inulin led to the presence of a small amout of free fructose as degradation product during heating of the sample.

The HPLC-SEC chromatogram (Fig. 3) shows the molecular weight distribution pattern of the isolated polysaccharides from shatavari roots. Four peaks were observed, the first two being due to polysaccharides. The first peak with a retention time of 11.49 min was due to a polysaccharide fraction with Mw 43038 Da and Mn 38765 Da, while the second peak with the retention time of 13.2 min is inulin with Mw 1655 Da and Mn 1605 Da with degree of polymerization 10.

Some authors have described up to 14 different isomers of DP 4-8 from the roots of *A. officinalis*, belonging to both inulin and neo series [19-21]. Inulin with DP 7 and three neo series (F-β(2→6)-G-β(2→1)-F5, F2-β(2→6)-G-β(2→1)-F4 and F4-β(2→6)-G-β(2→1)-F2) were previously reported in the roots of *A. officinalis and A. racemosus*, as the greatest variety of oligomers of DP 3–10 reported in *A. officinalis* [19]. Therefore, the isolated short-chained inulin-type fructan in our study was in accordance of the data reported in the literature for short-chained fructans in Asparagus genus.

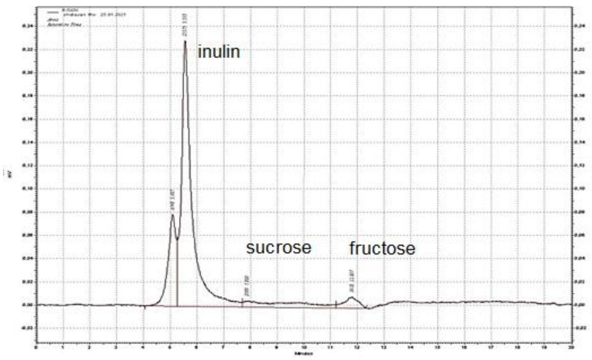

Fig. 2. The purity of inulin from shatavari isolated by microwave–assisted extraction

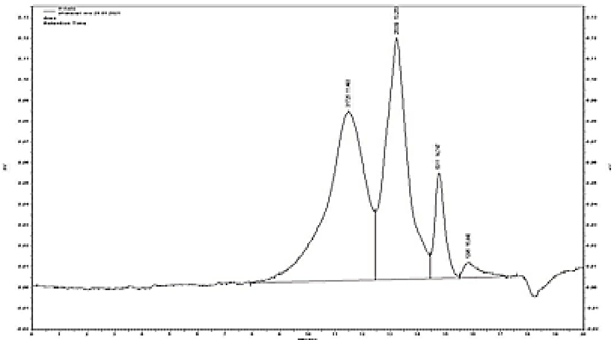

Fig. 3. HPLC-SEC of polysaccharide from shatavari isolated by microwave–assisted extraction

Table 2. Physicochemical characteristics of inulin isolated from shatavari roots by microwave-assisted extraction

Characteristics	Shatavari inulin MW	Inulin Frutafit HD DP 8-12
Yield , %	2.6±0.4	-
Purity, %	58.0±0.6	82.5±0.3
Fructose content, %	19.1±0.6	75.0±0.4
Reducing groups, %	3.0±0.3	5.4±0.5
DP by spectrophotometric method	7	15
DP by HPLC-SEC	10	12
Mw, Da	1655	2185
Mn, Da	1605	2105
Polydispersity index	1.03	1.04
Color characteristics		
L	63.72±1.64	100.00±0.00
a	5.04±0.99	0.08±0.04
b	5.17±1.29	6.79±0.10
C	7.22±1.61	6.79±0.10
h	45.34±1.84	92.29±5.16
Δ E	32.39±0.87	4.81±0.55

The color of shatavari inulin was darker than that of commercial low molecular inulin from chicory (Table 2). Shatawari inulin was pale-white and darker than that obtained from *Asparagus falcatus* [6]. The possible explanation for this dark color could be the presence of polyphenolic compounds, as previously described by some authors [6, 16]. The color of shatavari inulin was clearly different from that of chicory inulin because the asparagus extract was dark brown. The L* (lightness) value decreased and the b* value (yellowness) increased. The similar observation was made for other unpurified fructans from natural sources such as Jerusalem artichoke, *Asparagus falcatus* and *Taraxacum javanicum* plants [14, 16]. The reported by Hamdi *et al.* [16] data for color characteristic for asparagus inulin demonstrated even higher results for darker inulin (L=50.5203; a=3.9032; b=17.3581) in comparison to our study.

Functional properties of shatavari inulin

The degree of polymerization is one of the most important fructan characteristics for further application in food products. Oligofructose with DP ≤ 10 has some degree of sweetness (30–65% compared to sucrose). It is very soluble in water, not texturizing, but is easily and quickly fermented by colonic microbiota with high prebiotic activity [16]. Therefore, the evaluation of functional properties of the obtained short-chained inulin from shartavari will reveal its potential for futher application in food products.

The functional properties of inulin isolated from shatavari roots are summarized in Table 3.

Table 3. Functional properties of inulin isolated from shatavari roots

Functional properties	Shatavari iunulin MW	Inulin Raftiline HD
Swelling, g/cm^3	6.70	0.74
Water-holding capacity, g water/g	2.90	0.40
Oil-holding capacity, g oil/g	3.10	0.40
Bulk density, g/mL	1.01	24
Tapped density, g/mL	0.61	18.5
True density, g/mL	1.17	1.11
Carr's index	40	23
Hausner ratio	1.67	1.30
Angle of repose,°	5.14	25.64
Flowability	Very poor	Fair
Cohesiveness	Very poor	Fair
Hygroscopicity, %	7.9	3.7
Wettability, s	225	9

Shatavari inulin demonstrated good swelling properties (6.7 g/cm^3), better oil-holding and water-holding capacity in comparison with commercial chicory inulin Frutafit DP 8-12. In our case, inulin from *Asparagus racemosus* demonstrated 3.1 g/g sample oil-holding capacity, which was three times higher in comparision to chicory inulin used as reference with DP 8-12.

However, inulin from other asparagus sources demonstrated higher oil-holding capacity, more than 3 times higher than our results. For example, Hamdi *et al*. [16] reported oil-holding capacity for asparasus inulin about 94 g/100 g, and explained this fact with low purity of inulin (near 58% in fructans), while inulin from *Asparagus falcatus* demonstrated oil-holding capacity of- 101.62 g/100 g inulin [6]. Functional characteristics regarding swelling properties, oil-holding capacity of this polysaccharide (3.1 g oil/g sample), and water-holding capacity (2.90 g water/g sample) were similar to those of echinacea inulin obtained by microwave-assisted extraction [11, 22]. The values for water- and oil-holding capacities of shatawari inulin were close to previously reported data for long-chained chicory (1.59 g water/g sample and 3.4 g oil/g sample, respectively) [23]. However, our results for oil-holding capacity were higher in comparision to commercial chicory inulin and globe artichoke inulin (1.37 and 1.38 g oil/g sample, respectively) and Jerusalem artichoke 1.02 g oil/g sample [24].

Shatavari inulin with oil-holding properties can be used as a functional ingredient to improve taste. In addition, this is the first detailed study that evaluates functional properties of shatavari inulin. Based on Nandi's classification [25] shatavari inulin demostated high cohesiveness and fair flowability, based on Carr`s index (40) and Hausner ratio (1.67) (Table 3). Shatavari inulin is more hygroscopic (7.9 %) than chicory inulin (Table 3), that could be explained with low degree of polymerization DP 7-10 (Table 2). Moreover, the higher sharavari wettability could be explained with presence of other fractions of high-molecular polysaccarides (Fig. 3). The flow properties—the angle of repose, Carr's index, and Hausner ratio are very important for the packaging and transportation of powdered pharmaceutical and food additives. Based on these characteristics, the isolated shatavari inulin can be evaluated as hygroscopic with very poor cohesiveness and flowability and low wettability.

FTIR specroscopy

The structure of shatawari inulin isolated by microwave-assisted water extraction was confirmed by FT-IR spectroscopy (Fig. 4).

In general, the FTIR spectrum of shatavari inulin mainly shows all typical bands for inulin-type fructans [16, 26, 27]. A broad band at 3470 cm^{-1} was due to O–H stretching vibrations assossiated with inter- and intramolecular hydrogen bonds in the inulin structure. Smilar bands between 3600 and 3200 cm^{-1} due to the stretching of OH-groups from carbohydrate were detected earlier in asparagus inulin [6, 16]. The bands at 2967 cm^{-1} were due to C–H asymmetric stretching vibrations. The bands at 2894 cm^{-1} were characteristic for symmetric stretching vibrations of C–H from CH_2. The bands at 1458 cm^{-1} were due to symmetric stretching vibrations C–H in pyranosyl ring and $\beta_{O\text{–}H}$ (OH). The band at 1654 cm^{-1} was assigned to the absorption of water.

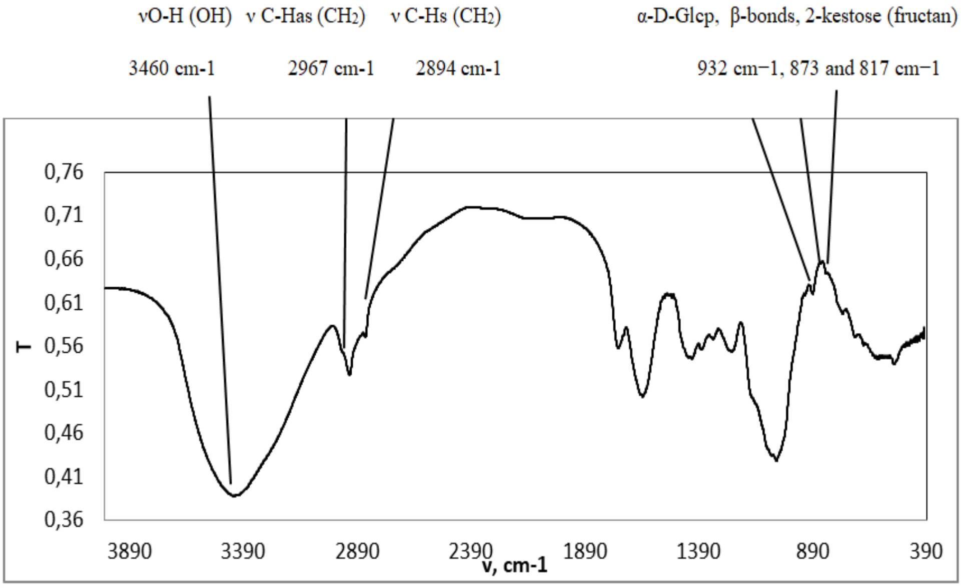

Fig. 4. FT-IR spectrum of shatavari inulin isolated by microwave-assisted extraction with DP 7-10

The bands at 1166 cm^{-1} were assigned to C–O–C ring stretching vibrations from glycoside linkage. The band at 1024 cm^{-1} was assigned to C–O stretching vibrations. The band at 932 cm^{-1} showed the presence of α-D-glucopyranosyl residue in the carbohydrate chain. A band for 2-ketofuranose and β-anomer bendings in C1–H was detected at 873 cm^{-1} and the occurence of a typical band at 817 cm^{-1} confirmed the presence of 2-ketose in pyranosyl or furanosyl ring. Moreover, the band at 783 cm^{-1} was a confirmation for the presence of ketopyranoses, as well as aldopyranoses with symmetrical ring breathing vibrations [29]. A band between 950 and 920 cm^{-1} was diagnostic for fructans, previously detected in other fructan-bearing species such as *A. potatorum* and *A. angustifolia* [27]. In our spectrum a small band at 940 cm^{-1} (Fig. 4) was detected that was due to 2→6 as in levan structure described previously [26]. Moreover, the bands at 932.62 and 822.22 cm^{-1} indicated the presence of fructose (Fru) with a *β*-configuration glycosidic bond in inulin-type fructan from *A. cochinchinensis* [17]. The observed bands were in accordance with previous reports [16, 17, 27]. The bands at fingerprint region in the FT-IR spectrum were reported earlier for inulin-type fructan. The bands at 932, 873, and 817 cm^{-1} were typical for inulin from different plant sources such as burdock, echinacea, dahlia, chicory [10, 11, 15, 22, 23, 28, 29]. In addition, asparagus plant is rich in fructooligosaccharides and contains neokestose type (2→1, 2→6) fructopyranosyl linked units of fructans [1, 16, 20, 21]. In the FTIR spectrum bands at 2938 cm^{-1} typical for lipids, and at 1080 cm^{-1} for carbohydrates were found [26]. However, the band at 1741 cm^{-1} was typical for C=O stretching vibration that could be due to the protein or phenolic compounds bound to the carbohydrate. A similar observation was reported earlier for the FTIR spectrum of asparagus inulin-type fructan in the zone 1700–1500 cm^{-1}, where bands were assigned to proteins (amide I and amide II vibrations) and to aromatic compounds (C-H and C=C-C aromatic bond stretching) [16]. In conclusion, inulin, isolated from shatavari in the present study, contained mainly bands typical for inulin-type fructan with *β* 2→1 bonds.

CONCLUSION

The conducted research elucidated the structure and functional properties of inulin from shatavari (*Asparagus racemosus* Willd.) roots. The obtained shatavari inulin was low-molecular with DP 7-10, good swelling properties and oil-holding capacity, high hygroscopicity, very poor cohesiveness and flowability and low wettability. Inulin molecular-weight properties revealed its potential to be used as taste modifier in food products with potential prebiotic effect, due to its low-molecular weight and degree of polymerization.

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