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COOKING, A FACTOR INFLUENCING HEAVY METAL AND HERBICIDE CONCENTRATIONS IN FOOD: CASE OF THE SNAIL CEPHALOPOD *ACHATINA ACHATINA*

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ABSTRACT

Meat, the main source of protein, is used to improve health and well-being through the reform and construction of body tissues as well as the production of antibodies. The purpose of this study is to determine the effect of temperature on certain heavy metals such as Pb, Cd, Cu, Zn as well as the glyphosate-based herbicide in the cephalopod in order to evaluate the effect of cooking time on pollutants as well as the effect of variation in cooking temperature on these pollutants. Analytic methods used for the determination of heavy metals and glyphosate absorption spectrophotometry and HPLC. The results show that temperature significantly degrades glyphosate concentrations (0,037 mg/kg (raw) to 0,02 mg/kg (60°C); 0,012 mg/kg (90°C) and 0.0017 mg/kg (120°C)) and copper (0,0023 mg/kg (raw) to 0,0009 (60°C), 0,0008 mg/kg (90°C) and 0,0004 mg/kg (120°C)). But cadmium increases enormously when the temperature exceeds 120°C. As for time, it greatly deprives the concentrations of copper (0.2 mg/kg (raw) to 0,09 (30 min) and 0,02 (60 min) and that of glyphosate (0,85 mg/kg (raw), 0.69 (30 min) and 0,06) (60 min)). These results could help consumers to take greater care when cooking food.

KEYWORDS: food, cooking, *Achatina achatina*.

INTRODUCTION

It is undeniable nowadays that the food resources available in their natural state are dwindling day by day. Meat, the main source of protein, is used to improve health and well-being through the reform and construction of body tissues as well as the production of antibodies.[1,2] The giant snail is part of this very rich and comfortable source of nutrients. Its meat has been consumed by humans around the world since prehistoric times and remains one of the most prized meats by European, African and American populations.[3] Indeed, the cephalopod (the consumed part of the snail) represents, according to a study carried out in Côte d'Ivoire by[4] 30% of the live weight of the snail. It is very rich in water (80%) and contains 62 to 75% crude protein in dry matter. Although low in fat, it has a very interesting calorific value (341 to 358 Kcal/100g), a high content of calcium (1,3g/100g DM) and iron (491 mg/Kg DM). Naturally, iron is one of the effective remedies in the treatment of anemia, cephalopod is also recommended to fight ulcers and asthma.[5] As a result, the cephalopod is a likely source of iron intake for the body. At the time of the Roman Imperial Court, snail flesh, believed to have aphrodisiac virtues, was often offered in the late evening to visiting dignitaries.[5] Almost all the essential amino acids that humans need are present in snails as well as vitamins A, D3 and E.[4] In addition, snails are macro invertebrates that colonize dense and poorly anthropized forests. They are found on soils rich in calcium.[6] The soil is a food source for snails in which they ingest the resources necessary for their growth, a habitat and a support to which they are strongly subject. This soil provides them with mineral salts including calcium as well as various substances present in the organic matter.[7] However, the environment in which these critters thrive receives all kinds of pollutants: heavy metals and active substances. These toxic chemicals are constantly prevalent in the environment; they are bioaccumulative and non-biodegradable in food series.[8] As a result, most people come into contact with them through contaminated food.[9] To exceed their flavor, taste, palatability and to make them tender; various cooking processes are used to treat these meats and these methods consist of barbecuing, boiling, grilling, frying, stir-frying, and roasting.[10,11] However, with the different cooking methods and their effects on palatability, there is a paucity of information on the impact of cooking time and/or temperature on the concentration of heavy metals and active substances in snail meat. Consequently, the aim of this study is to assess the contamination of the snail cephalopod *Achatina achatina* by metallic elements and herbicides. More specifically, it is a matter of evaluating the effect of the duration and the variation of the cooking temperature on the Pb, Cd, Cu, Zn and the glyphosate in the snail cephalopod.

Study site

The present study took place in the Nawa region of south-western Ivory Coast. Located between 5°60' and 9°50' north latitude and 6°0' and 8°20' west longitude, this region covers an area of 9193 km2 with an estimated population of 314,192 inhabitants (INS, 2014). The sampling site is located in the department of Méagui, precisely in the locality of Raphaelkro. Geographically, it is located between latitude 5°21'38,7534 N and longitude 6°28'38,7534 W. The choice of this region is justified not only by its high production of cocoa beans but also of snails.

Biological material

This study focused on a species of land snail belonging to the family Achatinidae. It is *Achatina achatina* (Fig. 1). It is the most prized species in Ivory Coast in general and by the populations of the study area in particular.

Figure 1: ***Achatina achatina.***

Preparation of snail samples

Once in the laboratory, snails were fasted for two days (48 hours) to remove unabsorbed food and feces from their digestive tract. The live weight, shell length and shell diameter of each snail were determined before dissection. This was done according to the method described by.[12]

Mineralization and determination of heavy metals

Mineralization for the determination of lead, copper, zinc and cadmium was performed according to AOAC method 999.10 (2003). For mercury, the official AOAC method No.

977.13 was adopted (AOAC, 1978). For the digestion of each sample, a 0,5 g test sample of shredded material is placed in a Teflon tube under a fume hood. Then 5 mL of 65% concentrated nitric acid (HNO3) and 15 mL of 37% hydrochloric acid (HCL) are successively added to the shredded material in the ratio (HNO3/HCL1/3). Another Teflon tube containing bidistilled water is used as a blank (neutral control of the elements to be investigated or treated). To this blank tube, the same reagents that were used for the matrix digestion are added in the same order. The Teflon tubes are put in a water bath for 1 hour (1H) at a temperature of 90°C for the digestion of the content. The Teflon tubes are removed and placed on the bench for cooling. Thirty minutes (30) later, a decompression of 5 minutes is done under the hood by opening the Teflon tubes to let the gas escape. After cooling, the mixture is filtered into a 100 mL volumetric flask and supplemented with bidistilled water up to the mark and then homogenized by manual stirring. Then, the solutions obtained after the mineralization are transferred into a clean tube bearing the references of the samples and kept at 4°C until the analysis.

Quantification of metallic trace elements

The detection of the metallic trace elements was done with the help of the Atomic Absorption Spectrophotometer with graphite furnace, controlled by the LC solution software equipped with a non-specific background corrector (Deuterium lamp), an automatic sample changer. A calibration was done before the preparation of the standard solutions for each metal with increasing concentrations of 10ug/l, 800ug/l, and 1500ug/l. The coefficient of determination of each element chosen is between 0,995 and 1. The metal contents in mg/kg (ppm) were obtained from the concentrations read directly on the spectrometer.

Determination of glyphosate content

Twenty-five (25) g of snail meat samples are taken and weighed into a 50ml conical tube. Then, demineralized water is added up to the 50ml gauge, the whole is vortexed for 3 min. The resulting solution V_2 is filtered into another 50ml conical tube. At the end, a corresponding volume of 10% TCA (trichloroacetic acid) is added to V_2 up to the 50 ml gauge and then vortexed for 3 min. The whole is filtered into another 50 ml graduated conical tube to obtain the volume V_3.

Preparation of samples for quantification

To 1 ml of the diluted V3 solution, 1 ml of the tetraborate solution (5%) and 1 ml of the FMOC solution are added in order. The resulting solution is stirred for 1 hour in the dark at room temperature, then centrifuged at 4500 rpm for 5 min. The supernatant is collected in a vial and ready for HPLC detection and quantification.

Effect of temperature and cooking time on pollutants in the cephalopod of snails *Achatina achatina*

On the one hand, to know the effect of temperature on pollutants (lead, copper, zinc, cadmium, glyphosate) snail cephalopods were brought to different temperatures including 60°C, 90°C and 120°C for thirty (30) minutes. As for the evaluation of cooking time on pollutants in cephalopods, it was carried out at different cooking times. The concentrations were determined in the cool state then thirty (30) minutes and sixty (60) minutes at 100°C.

Statistical analysis

The concentrations obtained were first subjected to the Shapiro-Wilk normality and homogeneity tests to verify their distribution and equality of variances. Next, ANOVA 1 followed by the post hoc test were used to compare the different means for parametric tests. For non-parametric tests, the Kruskal Wallis test followed by the Mann-withtney test were applied to compare the different means. Differences were considered significant at the = 0,05 threshold ($p < 0,05$). Finally, these tests were performed using PAST4.11 software.

RESULTS

Comparison of pollutant concentrations in cephalopods as a function of temperature

The variation pollutants concentration in cephalopods as a function of cooking temperature is shown in Fig. 2, 3, 4, 5 and 6. These figures show that temperature acts differently on pollutants. It reduces the levels of some pollutants, such as lead, copper and glyphosate. On the other hand, the levels of other pollutants, such as zinc and cadmium, rise with temperature. For example, lead levels dropped from 0,034 mg/kg fresh cephalopod to 0,01 mg/kg at a cooking temperature of 120°C. Statistical analysis shows a significant difference ($p>0,05$) between lead levels measured in fresh cephalopods and those obtained in cephalopods subjected to the different cooking temperatures. Copper levels drop with temperature from 0,023 mg/kg (fresh) to 0,004 mg/kg at 120°C. There was also a highly significant difference ($p<0,001$) between copper levels recorded at different cooking temperatures. Zinc levels rise with increasing temperature from 0,013 (fresh) to 0,07 mg/kg at (90°C), then fall to 0,03 at 120°C. Cephalopod cadmium concentration decreases from 0.01 mg/kg (fresh cephalopod) to 0,003 mg/kg at 60°C, then increases to 0,005 mg/kg at 90°C, before rising again to 0,02 mg/kg at 120°C. Glyphosate levels in cephalopods fell significantly (p<0,01) with increasing temperature.

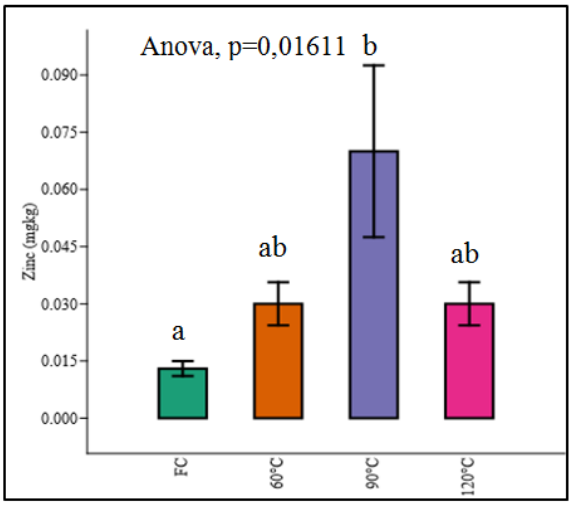

Figure 2: Effect of temperature on zinc content in *A. achatina* snail meat

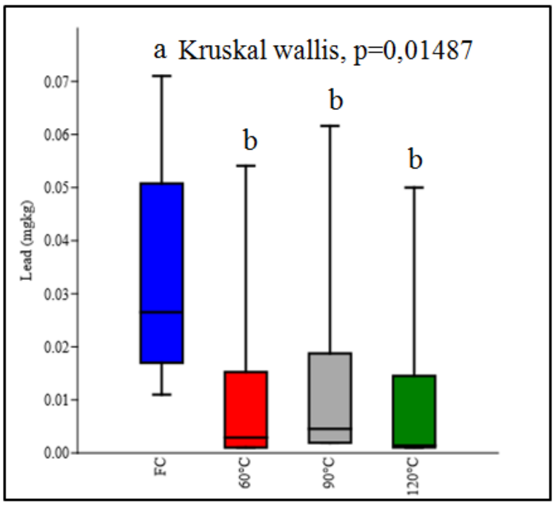

Figure 3: Effect of temperature on lead content in *A. achatina* snail meat

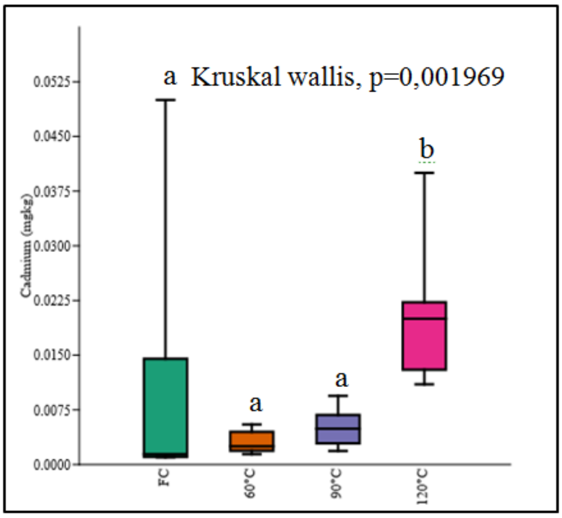

Figure 4: Effect of temperature on cadmium content in *A. achatina* snail meat.

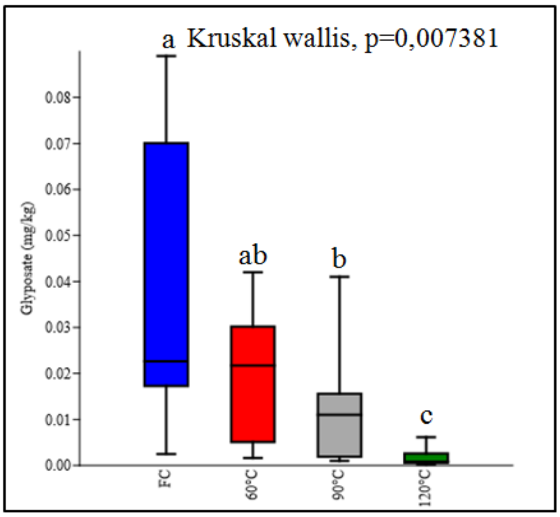

Figure 5: Effect of temperature on glyphosate content in *A. achatina* snail meat

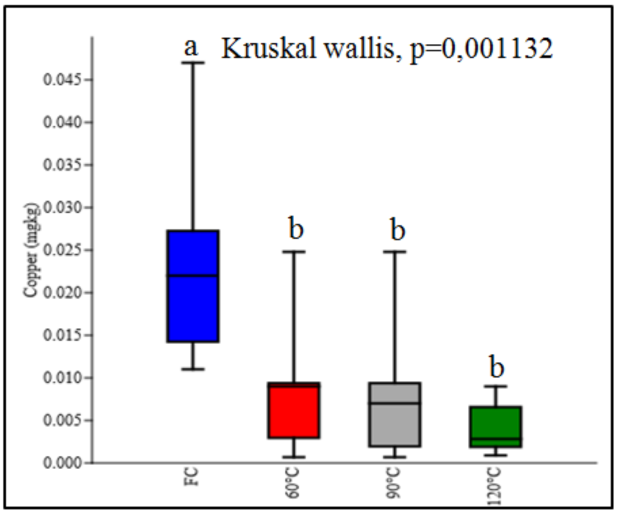

Figure 6: Effect of temperature on copper content in *A. achatina* snail meat.

Comparison of pollutant concentrations in cephalopods as a function of cooking time

The concentration of various pollutants in cephalopods decreases with cooking time at a temperature of 100°C (Fig. 7, 8, 9, 10 and 11). For example, lead levels in cephalopods vary according to cooking time, from 0.29 mg/kg (fresh) to 0,1 mg/kg after 60 minutes of cooking, via 0,14 mg/kg after 30 minutes of cooking, i.e. a 100% reduction. The Kruskal wallis test revealed no difference between the lead content of raw snails and those subjected to different cooking times. Copper concentration fell from 0,2 mg/kg (fresh) through 0,09 mg/kg (30 min) to 0,02 mg/kg (60 min). The Kruskal wallis test reveals a significant difference (p<0,05) between copper levels in cephalopods cooked for 30 min and 60 min. In contrast, copper levels in fresh cephalopods were statistically identical to those in cephalopods cooked for 30 min. Cadmium and zinc levels decreased with increasing cooking time. Levels of these pollutants in cephalopods varied respectively from 0,08 mg/kg (fresh) to 0,07 mg/kg (60 min) and from 0,64 mg/kg (fresh) to 0,45 mg/kg (60 min). However, according to the One-way ANOVA test, this decrease in cadmium (p=0,917) and zinc (p=0,939) was not statistically significant. Glyphosate levels fell from 0,85 mg/kg in fresh cephalopods to 0,06 mg/kg in cephalopods cooked for 60 min. The one-way ANOVA test revealed a difference between raw snail samples and those subjected to different cooking times. In fact, the glyphosate content in raw snail is different from those treated for 30 and 60 minutes of cooking. NB, FC: Fresh Cephalopod.

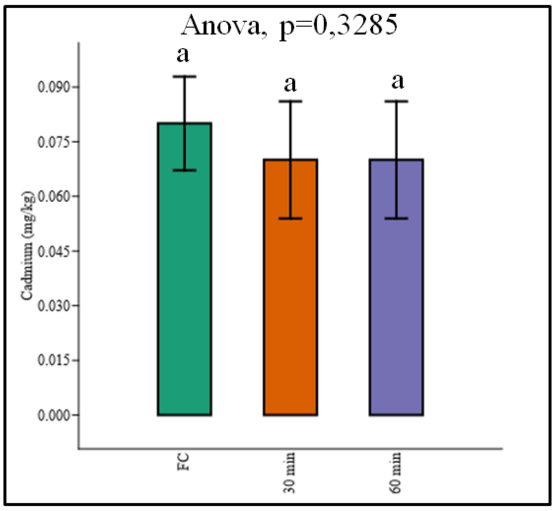

Figure 7: Effect of cooking time on cadmium content in *A. achatina* snail meat.

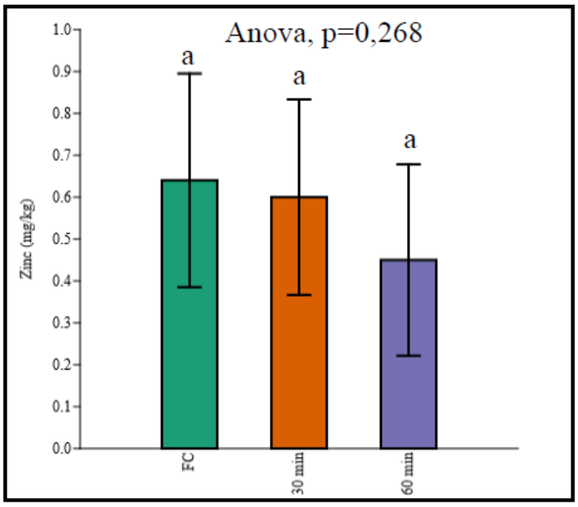

Figure 8: Effect of cooking time on zinc content in *A. achatina* snail meat.

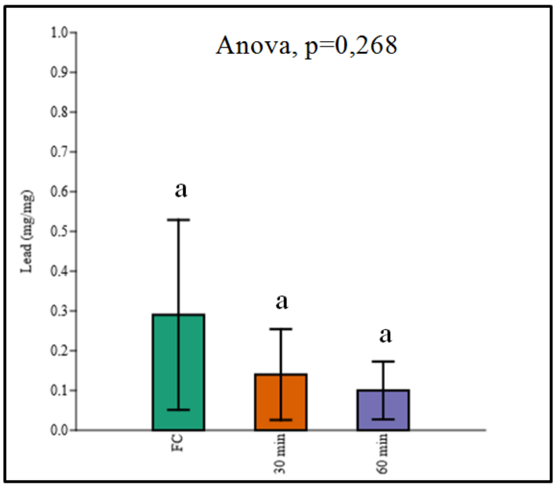

Figure 9: Effect of cooking time on lead content in *A. achatina* snail meat.

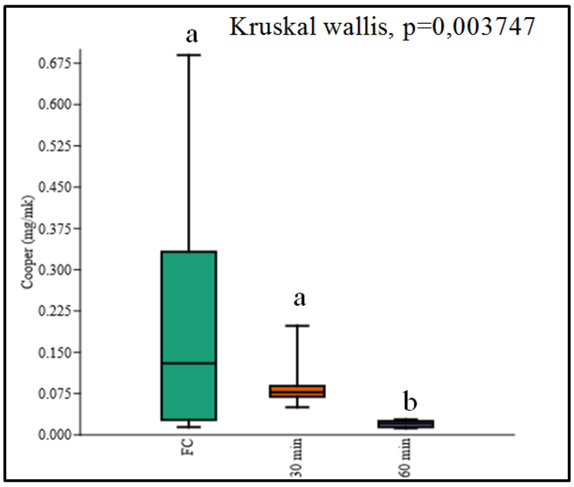

Figure 10: Effect of cooking time on copper content in *A. achatina* snail meat.

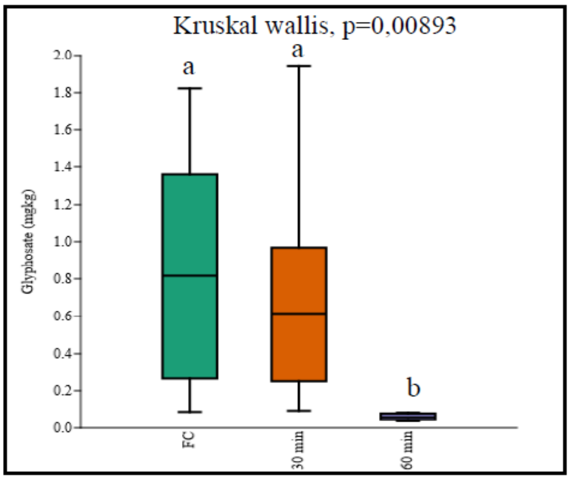

Figure 11: Effect of cooking time on glyphosate content in *A. achatina* snail meat.

DISCUSSION

The cadmium concentration decreases by 70% at 60°C and 50% at 90°C. On the other hand, this concentration exceeds that initially recorded (flood) from 120°C onwards, i.e. a 100% increase. These results corroborate those obtained by.[22] Indeed, during their research on the effects of processing on the proximity and metal content of three species of fish, these authors concluded that boiling and frying lead to a 100% increase in the concentration of Cd, while roasting increased the concentration of Cd by two times. We deduce from these obtained results that the high temperature increase would not only increase the cadmium concentration but also the health risk for consumers. The increase in cadmium concentration could be due to the loss of water resulting from frying and boiling, according.[23,24] These results are in agreement with those obtained by.[25] Indeed, during their research on the effect of two cooking methods (boiling and frying) of cephalopod snails of species *Archachatina marginata* and *Limicolaria spp*, the concentrations of cadmium in the flesh of *Archachatina marginata* species increased from 0% to 119% after boiling and from 144 to 219% after frying in vegetable oil at 180°C for 4 min. Cooking methods (boiling, steaming, frying, and others) can alter toxic metal concentrations through a variety of means, including evaporation of water and volatile components, solubilization of the element, as well as through metal binding to other macronutrients in the food such as carbohydrates, lipids, and proteins.[26] However, our results are contrary to those obtained by[27] who conducted research in which they reported that the cadmium content of crayfish decreased upon cooking. In sum, the loss of water and weight during heat treatment results in changes in inorganic[28] and organic chemicals and contaminants such as glyphosate. The effect of time on the content of pollutants differs from one element to another. First, copper and glyphosate are strongly degraded with increasing cooking time. Increasing cooking time is inversely proportional to copper and glyphosate concentrations in *Achatina achatina* snail. Indeed, reductions of 90% and 92,95% of copper and glyphosate concentration are respectively obtained after sixty minutes of cooking against 55% and 16,47% during thirty minutes of cooking. The strong degradation of glyphosate could be explained by the sensitivity of the bonds of the atoms constituting the glyphosate molecule in relation to the prolongation of the cooking time leading to the rupture of these bonds. In other words, the longer the cooking time, the greater the reduction in glyphosate content. As for copper, it is composed of only one type of atom and therefore combines with many elements. The transformation of copper into an oxide in the form of cupric ions with the formula Cu^{2+} or cuprous ions (Cu^{+}) could explain the strong degradation of this atom. Like the increase in temperature, cooking time reduces the content of lead in the cephalopod of the snail *A. achatina*. The cooking time has a weak effect in the degradation of cadmium and zinc concentrations.

CONCLUSION

In summary, the snail cephalopod *A. achatina* concentrates heavy metals and glyphosate. The evaluation of the effect of the cooking time on the content of pollutants revealed a reduction on the whole of the pollutants of which the most important were recorded at the level of glyphosate and copper. The effect of the cooking temperature has an impact on the degradation of the pollutant content except for cadmium for which an increase in temperature is proportional to the concentration in the cephalopod. The pollutants emitted into the environment are sooner or later found in our plates. Future studies will focus on the exact temperatures and cooking times at which the concentrations of heavy metals and active substances increase and/or decrease in food.

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