"Okara" a New Preparation of Food Material with Antioxidant Activity and Dietary Fiber from Soybean

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1. Introduction

Okara (OC) is a byproduct of the production of soybean foods such as tofu and soy milk. It is a nutrient-rich product, containing about 25% protein, 20% fat and 33% dietary fiber on a dry basis. Approximately 700,000 tons of okara are produced in Japan each year, some of which has been utilized as a feed for domestic animals and as a fertilizer (O'Toole, 1999). Most of it, however, is discarded as industrial waste because is perishable and other uses for it have not been identified (Ohno & Shoda, 1993), which has created social and environmental problems. Although many papers have reported methods for fermentation (Matsuo, 1997; Jiang et al., 2005; Mizumoto et al., 2006), extraction (Quitain, 2006) and digestion (Kasai et al., 2004), development of effective methods for OC utilization remain an important and difficult challenge. So, as part of a study increase utilization of OC, we have developed a new food product by combining fermented OC with fruits, especially banana. We investigated two properties of this food, reactive oxygen scavenging activity, the effect of including it as part of diet therapy for obesity using dogs as a model.

2. Reactive oxygen scavenging activity of the new food material from OC

Several investigators and some individual enterprises have been studying how to use OC as a new type of food, or in another industrial fields. For example, Maeda Y. developed a method of extracting water-soluble polysaccharides from OC and used them as an emulsifer or viscoelastic reagent (Maeda, 1992). Matsuo M. investigated the composition and properties of OC fermented by *Aspergillus oryzae* (*A. oryzae*) and *Rhizopus oligosporus* (*R. oligosporus*) and reported that the fermented OC could be a useful high-fiber, low-energy food material (Matsuo, 1989a; 1989b). Tempeh, a soy-based food originating in Indonesia, also is fermented by *R. oligosporus*. Today, it is still the most popular soy-based protein food in Indonesia (Golbitz, 1995). The antioxidant activity of OC fermented by *A. oryzae* also has been evaluated, and the 80% methanol extract of it showed high antioxidant activity (Matsuo, 1997).

Today, many foods are imported to Japan from all over the world. Approximately 1,700,000 tons of fruits imported each year (http://www.kanbou.maff.go.jp /www/jk/kajitu/ 15kajitu.pdf; The Ministry of Agriculture. Forestry and Fisheries of Japan, published results, 2003), including one million tons of banana imported from Philippine and some other countries. Banana is one of the most popular fruits in the world, and represents ca. 50% of all fruits imported into Japan. More that 10% of the imported volume of banana are wasted, however, because of inadequate quality for the retail market. We have a thought that this is quite large volume, which also creates social problems, also might be recycled as a food. Bananas are known to contain antioxidant compounds such as vitamins, flavonoids and phenolic compounds, and the antioxidant activity of banana is higher than that of other fruits. Using photon emission scavenging of reactive oxygen by XYZ system, Someya S. et al. determined that the antioxidant activity of banana was approximately 85-fold and 510-fold greater than that of grapefruit and lemon, respectively (Someya et al., 2003). In addition, these researchers found that the antioxidant activity of banana peel was ca. 1.2-fold that of banana pulp. Based on this information, we have tried to prepare a new food material with high reactive oxygen scavenging activity (ROSA) using fermented OC and banana.

Lately, many people have an interest in reactive oxygen and antioxidant because of the positive relationship between some diseases and reactive oxygen species. The ROSA of cereals and the synergistic effects of combinations of materials have been investigated by XYZ system. Akiyama Y. et al. found a synergistic effect on ROSA between rice and soybean, and between rice and green tea (Akiyama et al., 2002). In their report, they presumed that the thiamin in rice, catechins in green tea, and isoflavones in soybean were the major constituents contributing to the synergistic effects. However, a synergistic effect of fermented OC and banana on ROSA has not previously been reported.

Here, we present a new product created by combination of fermented of OC (OT) and banana, and investigate the synergistic effects of OT and banana on ROSA using photon emission scavenging reactive oxygen by XYZ system.

2.1 Materials and methods

2.1.1 OC fermentation

The method of fermentation of OC described by Aoki H. et al. was utilized with some modifications (Aoki et al., 2003). OC (Food Chemifa Co., Ltd.) was soaked in 0.2% sodium citrate (Wako Pure Chemical Industries) at room temperature for 60 min, and then steamed at 121°C for 20 min. The steamed OC was then cultured with *R. oligosporus* (Akita Konno Co., Ltd.) at 30°C for 24 h under aerobic conditions. After incubation, the fermented product, OT, was lyophilized.

2.1.2 Extraction of ethanol-soluble and water-soluble fraction

Extraction of ethanol-soluble and water-soluble fraction of the mixture of OT and banana (OTB) was based on methods described by Mizuno M. et al. (Mizuno et al., 1998), Nakamura T. et al. (Nakamura et al., 2004) and Matsuo M. (Matsuo, 2005). Lyophilized OTB powder (5 g) was extracted with 80% ethanol (50 mL) at 45°C for 24 h. After extraction with 80% ethanol, the remaining residue was extracted with ultra pure water (50 mL) at room temperature for 24 h. The 80% ethanol-soluble fraction, water-soluble fraction, and the residue after extraction with 80% ethanol and water were lyophilized to powder.

2.1.3 Measurement of reactive oxygen scavenging activity

ROSA was measured using the XYZ system as described by Okubo K. (Okubo, 2002). The lyophilized sample (30 mg), saturated KH_2CO_3 in 10% (Vol/Vol) acetaldehyde (1000 µL), and 0.6% H_2O_2 (1000 µL) were mixed in a 24-well micro titer plate and photon emission was monitored with a Bio-Emission Detector Model Bio-ED21 (E&T Corporation).

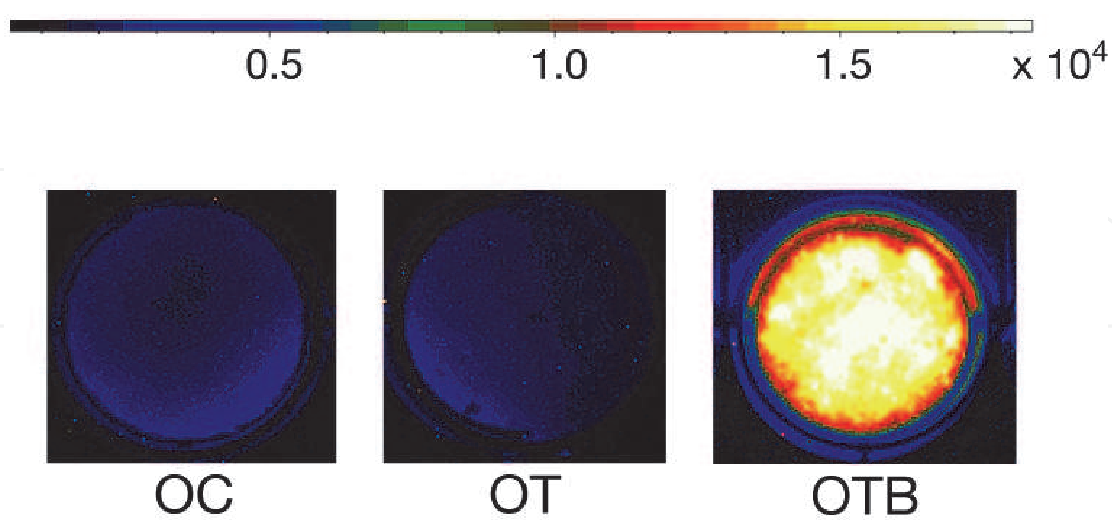
2.2 Results and discussion

2.2.1 Photon emission scavenging reactive oxygen of OTB by XYZ system

The XYZ system to measure scavenging activity for reactive oxygen was developed by Arai S. et al. in the process of research on soybean saponins (Arai et al., 2001). The characteristics of this system are (1) short measuring time, (2) simultaneous measurement of 10-20 samples at once, (3) simplified measurement technique, and (4) applicability to liquid and solid samples (Yoshiki et al., 2004). In the case of the ferric thiocyanate method or DPPH radical scavenging method, only liquid extraction samples can be measured. In contrast, the photon emission scavenging reactive oxygen by XYZ system permits analysis of materials of diverse form such as solid extraction residues as well as liquid extraction samples. Researchers in this field would like to have an experimental system able to measure the antioxidant activity of materials of diverse from such as solid type sample like extraction residues for bioresource science.

We next attempted to determine the ROSA of lyophilized OC, lyophilized OT, and lyophilized OTB. OT and banana (B) were mixed (OT:B ratio 1:1 by weight) and the mixture was lyophilized. The photon emission images, obtained using a charge-coupled device camera, are shown in Fig. 1. In this system, photon emission is observed when H_2O_2 reacts with antioxidants and mediators. The intensity of photon emission increases with the radical scavenging ability of the sample.

The photon emission intensity revealed that of OC had almost the same ROSA as that of OT, 1,093 cd/m² and 1,266 cd/m², respectively. The photon intensity activity of OTB, however, was 34,405 cd/m², ca. 30-fold stronger that of either OC or OT alone.

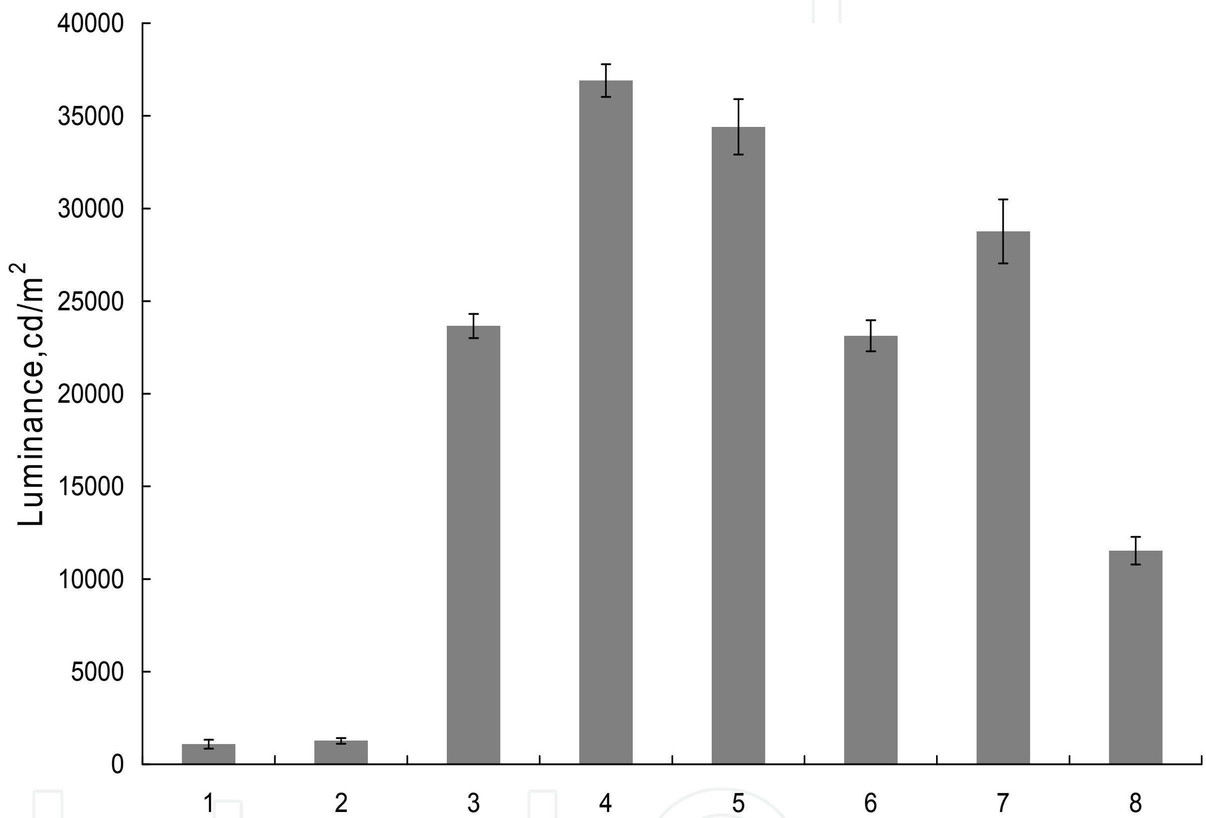


Detection image of photon emission from OC, OT and OTB using a charge-coupled device camera. OC, lyophilized okara; OT, lyophilized okara fermented by R. oligosporus; OTB, lyophilized OT-banana combination. The lyophilized sample (30 mg), saturated KH2CO3 in 10% (Vol/Vol) acetaldehyde (1000 µL), and 0.6% H2O2 (1000 µL) were mixed in a 24-well micro titer plate.

Fig. 1. Photon emission of OC, OT and OTB.

2.2.2 Effect of mixing ratio of OT and banana on photon emission scavenging reactive oxygen by XYZ system

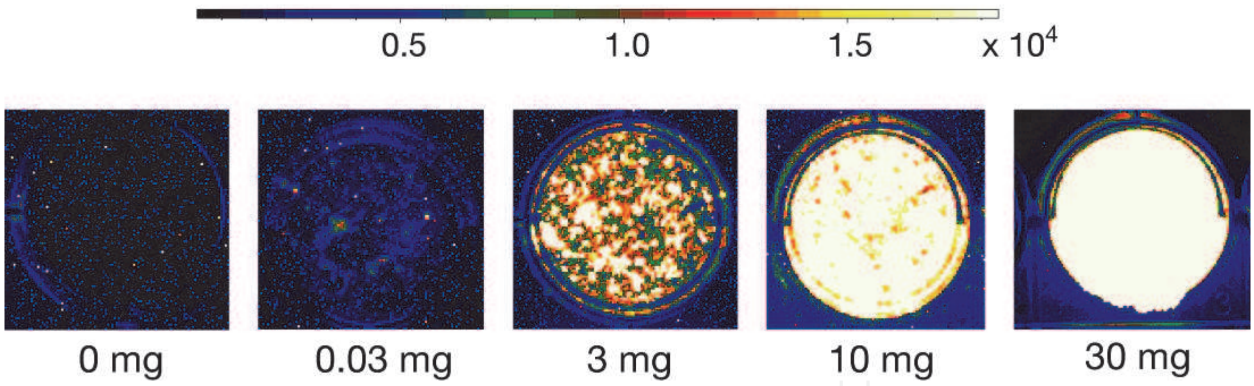
To investigate the relationship between the ROSA and the mixing ratio of OT:B (by weight), mixtures of 4:1, 4:3, 1:1, 3:4, 1:4, and dry banana powder were prepared. All samples were lyophilized, and the ROSA was measured using the method of photon emission scavenging reactive oxygen by XYZ system (Fig. 2). All of the prepared OTB mixtures, exhibited a synergistic effect on ROSA. The photon emission intensity of OTB 4:3 was highest of all the OTB mixtures at 36,906 cd/m^2, some 3-fold and 30-fold more potent than that of dry banana powder (photon intensity: 11,531 cd/m^2) or OT (photon intensity: 1,266 cd/m^2), respectively.



1, lyophilized OC; 2, lyophilized OT; 3, lyophilized OTB 4:1; 4, lyophilized OTB 4:3; 5, lyophilized OTB 1:1; 6, lyophilized OTB 3:4; 7, lyophilized OTB 1:4; 8, lyophilized banana. The lyophilized sample (30 mg), saturated KH_2CO_3 in 10% (Vol/Vol) acetaldehyde (1000 μL), and 0.6% H_2O_2 (1000 μL) were mixed in a 24-well micro titer plate. Data represent the mean ± SD (n=5).

Fig. 2. Effect of mixing ratio (by weight) of OT and banana on photon emission intensity.

The effect of various concentrations of OTB 4:3 (0 mg, 0.03 mg, 3 mg, 10 mg, and 30 mg) on ROSA is shown in Fig. 3. The ROSA of OTB 4:3 increased with increasing OTB 4:3 concentrations. Gulcin I. investigated the effect of concentration of L-carnitine on antioxidant activity, and reported that the antioxidant power of L-carnitine increased with increasing concentration (Gulcin, 2006). There is also a report that the antioxidant effect of 3-hydroxyanthranilic acid (HAA) from soybean increased with increasing the added amount of HAA (Esaki et al., 1996). Our results were similar to that of those reports, and confirmed the reproducibility of ROSA of OTB 4:3.



Imaging detection of photon emission from OTB 4:3 using a charge-coupled device camera. The lyophilized OTB 4:3 (0 mg, 0.03 mg, 3 mg, 10 mg, and 30 mg), saturated KH_2CO_3 in 10% (Vol/Vol) acetaldehyde (1000 µL), and 0.6% H_2O_2 (1000 µL) were mixed in a 24-well micro titer plate.

Fig. 3. Photon emission of various concentration of OTB 4:3.

2.2.3 Photon emission scavenging reactive oxygen of OTB extraction by XYZ system

The 80% ethanol-soluble fraction and water-soluble fraction of OTB were extracted to investigate the ROSA of extracted OTB by XYZ system. In this experiment, the 80% ethanol-soluble and water-soluble fractions were extracted from OTB 4:3 (Fig. 4), because the ROSA of OTB 4:3 was greater than that of other OTB mixture (see preceding section and Fig. 2). The ROSA of whole OTB 4:3 (30 mg), 80% ethanol-soluble fraction (30 mg), water-soluble fraction (30 mg), and extraction residue after extraction with 80% ethanol and water were measured using XYZ system, and the results were shown in Fig. 5. Contrary to the prediction, the ROSA of 80% ethanol-soluble fraction and water-soluble fraction were lower than that of other samples. On the other hand, ROSA of the extraction residue of OTB 4:3 (photon intensity: 42,062 cd/m^2) was higher than that of both the 80% ethanol-soluble fraction (photon intensity: 312 cd/m^2) and the water-soluble fraction (photon intensity: 4,528 cd/m^2). From these results, it was concluded that the effective ROSA of OTB 4:3 was attributable to components in the residue left after extraction with 80% ethanol and water. Iwai K. et al. studied the antioxidant activity of blueberry using the XYZ-dish method and reported that the activity of the residue left after extraction with distilled water was greater than that of the water solution (Iwai et al., 2001b). Someya S. et al. investigated the chemiluminescence properties of banana extracts and extraction residue after extraction with water by XYZ system and demonstrated that the photon emission intensity of extraction residue was 5.7-fold that of extracts (Someya et al., 2003). Our results agree with the data by Iwai K. et al. and Someya S. et al.. The residue left after extraction with 80% ethanol and water of OTB 4:3 has many bioactive components, and dietary fiber may be one of the most important substances there. OC and OT contained dietary fiber at a level of ca. 50% of dry weight (Matsuo, 1989b). Approximately 2 g of dietary fiber is contained in 100 g of banana (Forster et al., 2002). The dietary fiber in foods has protective effects against cardiovascular disease and atherosclerosis (Anderson et al., 1990; Anderson, 1995; Rimm et al., 1996; Van Horn, 1997). In addition to these functions, dietary fiber may show the photon emission scavenging reactive oxygen by XYZ system. Prosky L. et al. reported the method of extraction and determination of insoluble, soluble, and total dietary fiber in foods and food products (Prosky, 1988). In the method, foods and food products were treated by thermostable α-amylase and amyloglucosidase for extraction of dietary fiber. The ethanol-soluble and water-soluble fraction of OTB 4:3 did not contain dietary fiber, because thermostable α-amylase and amyloglucosidase treatments were not done during OTB 4:3 extraction. The ROSA of the extraction residue of OTB 4:3 may have been higher than that of the 80% ethanol-soluble and water-soluble fractions because the dietary fiber may have remained in the residue after extraction with 80% ethanol and water. The antioxidant components in OTB and the mechanism of a synergistic effect of OT and banana on ROSA are still unclear, and we currently are examining these further.

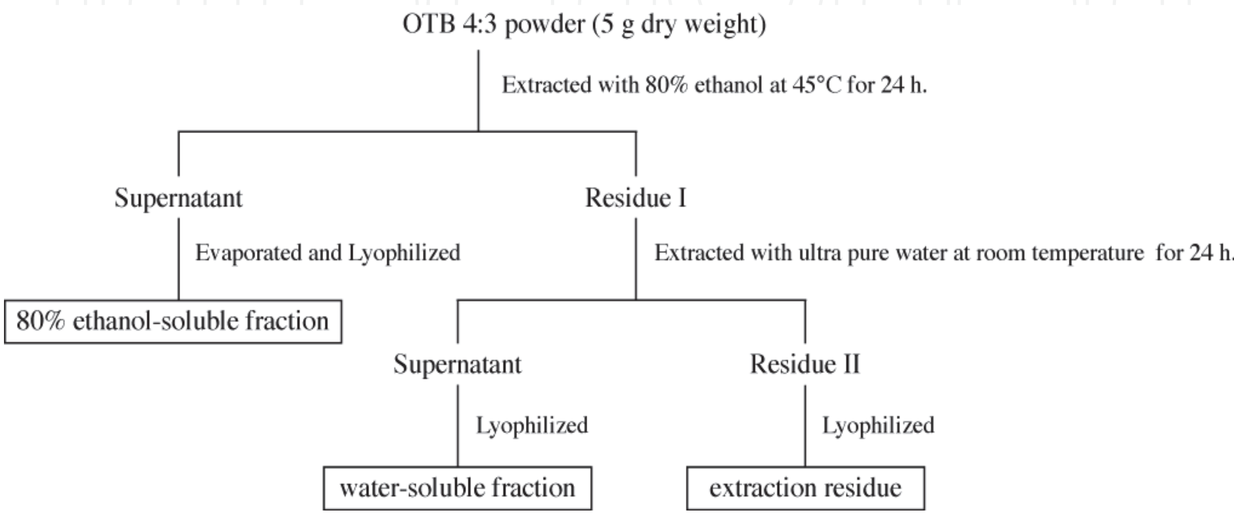
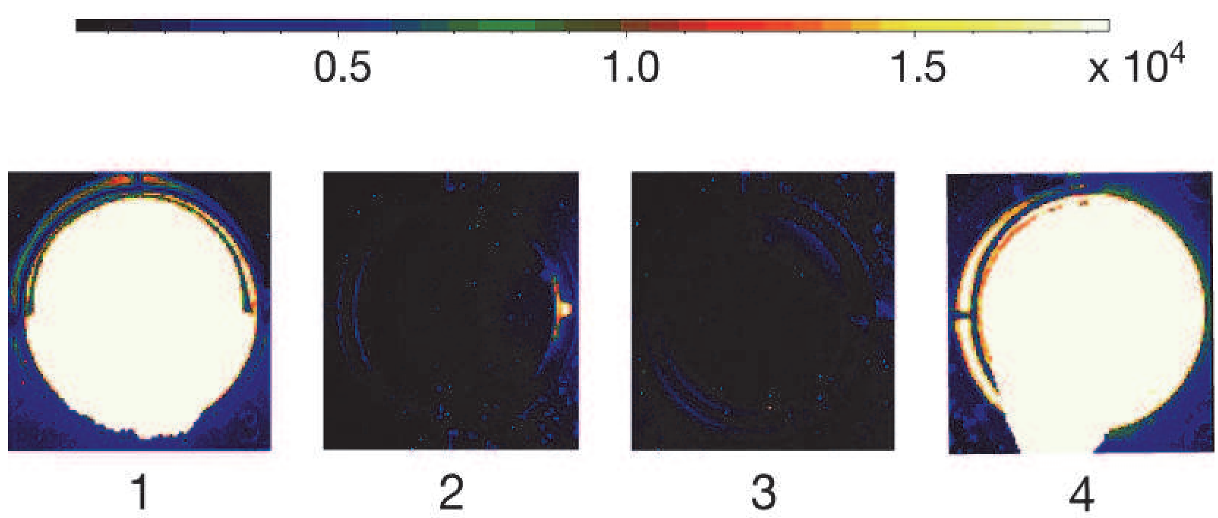


Fig. 4. Extraction procedure of ethanol-soluble and water-soluble fraction of OTB 4:3.

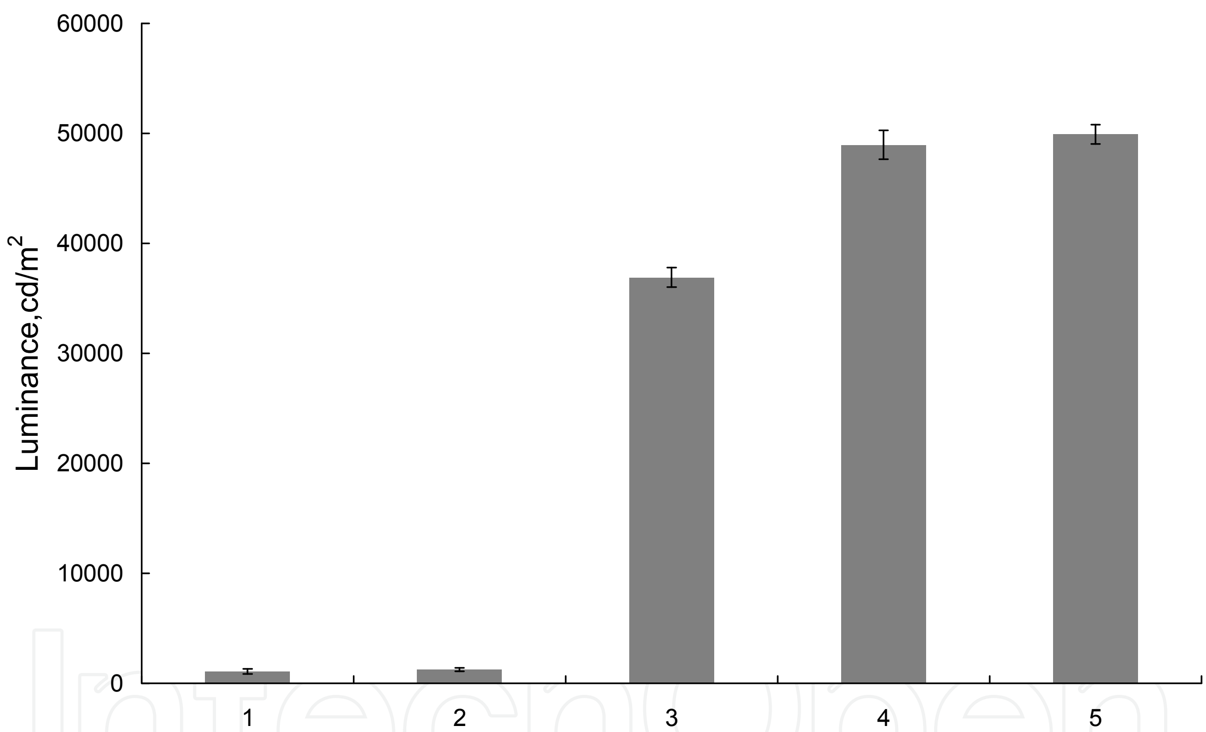


1, lyophilized OTB 4:3; 2, lyophilized 80% ethanol-soluble fraction from OTB 4:3; 3, lyophilized water-soluble fraction from OTB 4:3; 4, lyophilized extraction residue after extraction with 80% ethanol and water. Imaging detection of photon emission from OTB 4:3, 80% ethanol-soluble fraction, water-soluble fraction, and their residue using a charge-coupled device camera. The lyophilized sample (30 mg), saturated KH_2CO_3 in 10% (Vol/Vol) acetaldehyde (1000 μL), and 0.6% H_2O_2 (1000 μL) were mixed in a 24-well micro titer plate.

Fig. 5. Photon emission of OTB 4:3 (OTB 4:3) extractions.

2.2.4 Comparison of OTB with commercial organic green tea powder on photon emission scavenging reactive oxygen by XYZ system

Recently, many reports have appeared concerning about the antioxidant activity of various foods. One of the representative antioxidant foods is green tea, because ascorbic acid, α-tocopherol, and catechins are found at high concentrations in green tea. Iwai K. et al. investigated the antioxidant activity of commercially available tea and found that the antioxidant activity of tea infusions was stronger than that of canned tea (Iwai et al., 2001a). We compared the ROSA of OTB 4:3 powder with that of two kinds of commercially available organic green tea powder (Fig. 6), and found that the ROSA of OTB 4:3 was nearly equal to that of the tea samples (The photon intensity of lane 4 and lane 5 of Fig. 6 was 48,961 cd/m^2 and 49,914 cd/m^2, respectively). In addition, we had received data that showed the lipid peroxide concentration in the serum of mice that took the OTB 4:3 tended to decrease (Sugiyama H., School of Medicine, Akita University, personal communication). These data show that OTB 4:3 has high ROSA, and could be useful as a novel antioxidant food material and/or nutritional supplement.



1, lyophilized OC; 2, lyophilized OT; 3, lyophilized OTB 4:3; 4 and 5, two kinds of commercial organic green tea powder. The lyophilized sample (30 mg), saturated KH_2CO_3 in 10% (Vol/Vol) acetaldehyde (1000 μL), and 0.6% H_2O_2 (1000 μL) were mixed in a 24-well micro titer plate. Data represent the mean ± SD (n=5).

Fig. 6. Photon intensity of OTB 4:3 and commercial organic green tea.

3. Diet effect of the new food material from OC on overweight and/or obesity dogs

An increased prevalence of obesity recently has been reported worldwide, both in developed and developing countries. Overweight and obesity have been associated with several chronic diseases and disabilities, including type 2 diabetes, cardiovascular diseases, hypertension, certain types of cancer, and premature death (Chopra et al., 2002; Lew, 1985). Mokdad, A. H. et. al. reported that in Unites States, the number of patients with obesity and has been increasing in men and women of all ages, all races, all educational levels, and all smoking levels (Mokdad et al., 2003). Therefore, the health status and health problem related to obesity are becoming more and more important issues.

The prevalence of obesity is increasing in companion dogs as well as in human beings. Companion dogs are an indispensable part of people's lives, and many owners consider their dogs to be part of their family. Several investigators have reported a relationship between health and obesity in dogs. Stone, R. et al. reported the higher serum concentrations of triglycerides and cholesterol in obese dogs suggesting an association between metabolic derangements and obesity in dogs similar to those observed in human beings (Stone et al, 2009). Increasing rates of obesity in dogs also have been associated with increased rates of osteoarthritis, insulin resistance and certain neoplasias (Laflamme, 2006). Heuberger, R. and Wakshiag, J. estimated that owners who ate nutrient-rich, calorie-poor diets had normal weight dogs, whereas owners who fed more table scraps had overweight dogs (Heuberger & Wakshlag, 2011). In addition, non-obese dogs consumed significantly more crude dietary fiber in their diets (Heuberger & Wakshlag, 2011).

As mentioned, OC contains a rather large amount of dietary fiber. In general, increasing dietary fiber intake promotes body fat loss (Roberts et al., 2002; Slavin, 2005). Matsumoto K et al. investigated the effect of OC on the prevention of obesity using a mouse model (Matsumoto et al., 2007). However, to our knowledge there is no published literature describing the effects of consumption of OTB on obesity in dogs. To begin to address this question, we conducted a pilot clinical research study to assess the effect of addition of OTB to the usual diet on overweight and obesity dogs.

3.1 Materials and methods

3.1.1 Case selection

Candidate dogs for weight loss study were selected from out-patient dogs that were relatively healthy except for overweight and/or obesity. The veterinarian estimated the body fat of the dogs using the Body Condition Score (BCS, Fig. 7), and diagnosed overweight (BCS=4) and/or obese (BCS=5) dogs admitted for usual care. For weight loss to succeed, one must select suitable candidate patient dogs and owners, those who concerned about "their"obesity and ready to make changes.

3.1.2 Weight loss study program

The diet effect of OTB paste (moisture: ca.60%) was examined in consultation with the pet owners about the obese state of their dogs (Fig. 8). Owners substituted OTB paste (OTBp) for 30% to 50% of the dog's usual food to maintain the volume of the food equivalent to the original by weight as shown in Fig. 9. The effectiveness of this addition was evaluated by periodic measurement of body weight. In some cases, laboratory examinations also were performed.

3.1.3 Blood biochemical clinical examination

Blood tests were performed on two of the six cases according to conventional methods. Blood samples were collected at the starting date of the study, and again at the end of the test. Blood tests were performed using a PocH-100iv, Sysmex, Tokyo, Japan, and blood biochemical examinations were performed using a Spotchem EZ #sp-4430 Arkray, Tokyo, Japan.

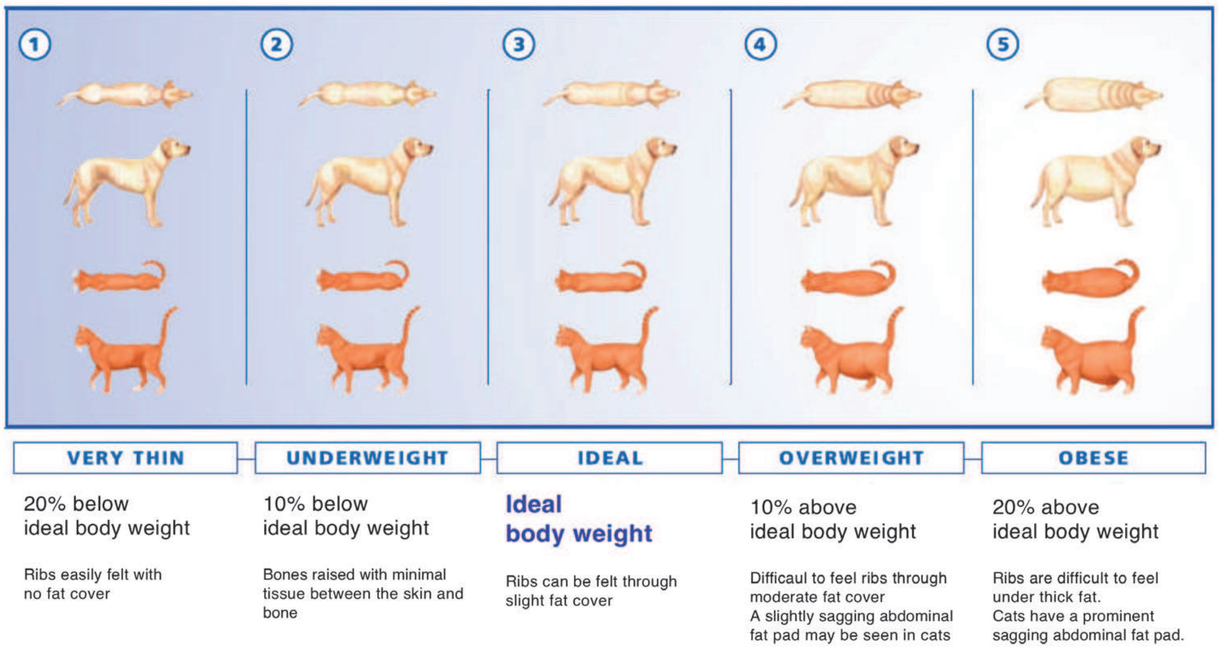


Fig. 7. Body Condition Score (BCS).

Fig. 8. The obesity dog (case No. 05709).

30 % to 50 % by weight of the daily ration of dog food was replaced with OTBp.

Fig. 9. The modified food used in weight loss study program.

3.2 Results and discussion

The nutrition facts of OTBp were shown in Table 1. As shown in the Table 1, OTBp contains only 137 kcal per 100 grams as fed.

Per 100 g edible portion

Nutrition Facts	
Energy	137 kcal
Water	63.8 g
Protein	3.8 g
Lipid	1.3 g
Ash	1.3 g
Carbohydrate	25.1 g
Fiber	4.7 g
Sodium	19.6 mg

Test Analysis by Japan Food Analysis Center

Table 1. Nutrition facts of OTB paste.

Prior to the weight loss study, we tested the response to OTBp of dogs that were selected at random from usual clinic patents. We found that 42 of 51 dogs, nearly 80%, ate the OTBp, suggesting good acceptance by dogs. Of the eight owners enrolled in the weight loss after oral informed consent, only two dropped out of the study because their dogs refused to eat the diet. The remaining six dogs completed the study. Average for the changes in body weight is shown in Fig. 10.

Patient No.	Canine Strain	sex	Age	BCS at start	Body weight (kg)[a] point 0	point 1	point 2	point 3	point 4	point 5	BCS at end	Max Reduce %	Period days	Satis-faction[b]
05803	Miniature Dachshund	♀	7.5	4	7.00	6.95	6.75	6.60	6.55	6.55	3	6.4%	75	excellent
					100.0%	99.3%	96.4%	94.3%	93.6%	93.6%				
03767	Miniature Schnauzer	♂spay	5.5	5	9.60	9.80	9.65	9.50	9.55	9.65	5	1.0%	37	good
					100.0%	102.1%	100.5%	99.0%	99.5%	100.5%				
05634	Miniature Pinscher	♀	3.5	5	5.75	5.70	5.65	5.60	5.45	---	3	5.2%	42	excellent
					100.0%	99.1%	98.3%	97.4%	94.8%					
05709	Miniature Dachshund	♀spay	12.3	5	8.85	8.30	8.20	8.10	7.85	7.80	3	11.9%	53	excellent
					100.0%	93.8%	92.7%	91.5%	88.7%	88.1%				
06081	Papillon	♂	4.5	5	6.50	6.55	6.40	6.40	6.36	6.34	4	2.5%	50	excellent
					100.0%	100.8%	98.5%	98.5%	97.8%	97.5%				
05645	Miniature Dachshund	♀spay	6.5	4	6.70	6.75	6.75	6.60	6.65	---	4	1.5%	37	fair
					100.0%	100.7%	100.7%	98.5%	99.3%					
	mean		6.63	4.67	100.00%	99.30%	97.85%	96.52%	95.61%	94.94%	3.67	4.75%	49.00	
	±SD		3.10	0.52	0.00%	2.91%	3.00%	2.98%	4.14%	5.36%	0.82	4.08%	14.35	

[a] Body weight of the dogs were measured every seven or ten days.
[b] Owner's satisfaction was as follows; excellent, good, fair and poor.

Table 2. Dog characteristics and body weight changes.

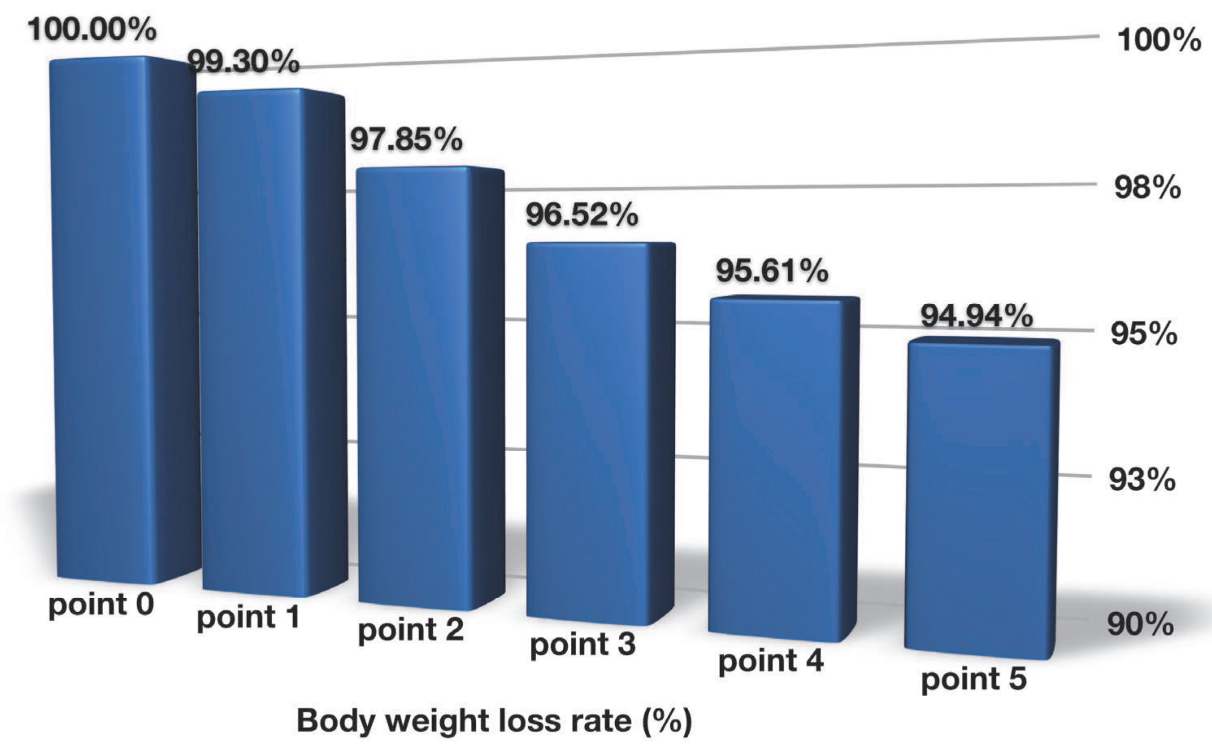


Fig. 10. Body weight loss rate (%) of dogs (n=6).

Lose of body weight was clearly seen in three cases, #05803, #05634 and #05709, during the study, and the dog's owners were satisfied with the program. In case of #06081, a body weight gain was observed between the beginning of the study and the seven day weight (point 1), but after that the body weight decreased and the owner was also satisfied with these results. On the other hand, in two cases (#03767 and #05645), the final body weight (point 5, at the end of the study) of the dogs was almost same as that of point 0, at the beginning of the study. In these cases, the body weight of the dogs increased up to point 2, decreased from point 2 to point 3, and increased again after point 3.

The mean percent loss of the body weight in the overwight and/or obesity dogs (n=6) is shown in Fig. 10. As can be seen in Fig. 10 and Table 2, feeding OTBp resulted in a average body weight loss of about 5%, and a body fat (BCS) loss of approximately 20% during the 35 days of the study. Although preliminary, these results suggest a beneficial effect of addition of OTBp to the diet on body fat loss in overweight and/or obese dogs.

Blood and serum biochemical evaluations were carried out in two cases, #03767 and #06081, and the results aree shown in Table 3. The results revealed that OTBp did not exhibit any adverse reactions or influences on health conditions of the dogs through out the period of study. In additiont, OTBp treatment lowered the alkaline phosphatase activity (ALP IU/L) of the dogs between the initial (day 0) and final measurements, suggesting that OTBp might have an improving effect on liver function. Nishi, S. et al. investigated the antiobesity effects of seaberry leaf polyphenol (SBLPP) juice using male mice (ddY) fed a high-fat diet. In the paper, they reported that the body weight of experimental mice fed a high-fat and SBLPP juice for eight weeks was significantly lower than that of control levels, and that the SBLPP juice also lowered the blood glutamic pyruvic transaminase (GPT) and ALP activities in the mice. (Nishi et al., 2007). Swaroop, A. et al. and Yang, Z. G. et al. reported that the SBLPP had potent antioxidant activity (Swaroop et al., 2005; Yang et al., 2007). Taken together, these results suggest that OTBp may improve liver function, possibly due to its potent antioxidant activity.

ITEM	#03767		#06081	
	DAY 0	DAY31	DAY 0	DAY50
WBC x10^2/μL	100.00	93.00	93.00	140.00
RBC x10^4/μL	845.00	889.00	916.00	938.00
HCT %	56.10	58.40	56.30	56.70
PLT x10^4/μL	46.80	48.40	36.20	22.40
Glu mg/dl	105.00	101.00	94.00	97.00
T-Cho mg/dl	> 400.0	> 400.0	> 400.0	> 400.0
BUN mg/dl	13.00	13.00	14.00	14.00
T-Bil	< 0.2	< 0.2	< 0.2	< 0.2
GOT IU/L	14.00	10.00	11.00	14.00
GPT IU/L	92.00	86.00	72.00	76.00
T-Pro g/dl	8.00	7.60	6.40	6.10
GGT IU/L	< 10.0	< 10.0	< 10.0	< 10.0
ALP IU/L	2115.00	1547.00	212.00	< 130.0

Table 3. The results of blood test and biochemical examination on case #03767 and #06081.

The results of this pilot study are consistent with those of other investigations of soybean components on obesity. For example, Jenkins, D. J. et al. reported that consumption of a low-energy diet containing soy protein had signnificant anti-obesity effects (Jenkins et al., 1989). Goodman-Gruen, D. and Kritz-Silverstein, D. investigated the beneficial effects of soy phytoestrogen, and isoflavones on excess body weight (Goodman-Gruen and Kritz-Silverstein, 2001). Moreover, in a study of genetically obese mice (yellow KK), Aoyama, T. et al. reported that soy-protein and its hydrolysate (active tetrapeptides) were effective for weight reduction (Aoyama et al., 2000). From these reports, we hypothesized that OTBp might contain effective components from the soybean residue, OC. OTBp also is dilute in energy, and contains a large amount of dietary fiber (Table 1). Some reports showed that the dietary fiber has an effect on reducing serum cholestrol concentrations by increasing fecal bile acid excretion (Andersson et al., 2002; Chau and Huang, 2005; van Benneleum, 2005). Ble-Castillo, J. L. et al. reported that addition of a banana supplement containing dietary fiber to the diet significantly lowered body weight and increased insulin sensitivity (Ble-Castillo et al, 2010). Slavin, J. L. et al. reported that increasing intake of dietary fiber resulted in decreased feelings of hunder, and played a role in control of energy balance (Slavin et al, 2005). Epidemiological research also has shown the effectiveness of dietary fiber intake on preventing obesity (Robert et al, 2002).

Many papers also have shown obesity to be associated with increased oxidative stress in human beings and mice, which could be associated with obesity-associated metabolic syndrome (Keaney et al., 2003; Vincent et al., 1999; Furukawa et al., 2004). Hogan, S. et al. demonstrated that grape pomace extract had significant antioxidant capacity, and exerted an anti-inflammatory activity in subjects with diet-induced obesity (Hogan et al, 2010). Shen, X. H. et al. reported that the antioxidant vitamin E could play an important role in the treatment of obesity-related diseases (Shen et al., 2010). Given the high antioxidant activity we found in OTB (see preceding section). The effects of OTBp on fat loss in overweight

and/or obesity dogs may have resulted from its combination of properties of low energy, presence of effective soybean compnents, amount of dietary fiber, and potent antioxidant activity.
Another important consideration for the success of diet therapy for weight loss is the attitude of to owner toward their pet, as shown in the recent report of the the relationship between feeding patterns and obesity in dogs by Heuberger, R. and Wakshlag, J. mentioned previously. Thus, we studied pets whose owners were conserned about their pet's weight and wanted to make a change. Under these circumstances, we found that addition of OTBp might be an effective treatment for dog owners as well as for their obese pets. The detailed mechanisms of the OTBp effect are still unclear because we have not yet conducted any laboratory experiments to clarify the diet effects of OTBp. We need basic experiments using obesity model mice.

4. Conclusion

In this paper, we have described our efforts to use OC to develop a new food material from OC by fermentation with *R. oligosporus* and combination with banana. We have investigated two activities, ROSA, and the effects of the new food material from OC on obese dogs. First, in the ROSA studies, a synergistic effect on ROSA between OT and banana was observed, and the activity of OTB was approximately 30-fold more potent than that of OC alone by XYZ system. We concluded that ROSA of OTB was attributable to components contained in the dietary fiber portion of the residue after extraction with 80% ethanol and water. This is the first report about the synergistic effect of fermented OC and banana on ROSA by XYZ system (Suruga et al., 2007). Secondary, OTBp showed a dietary effect on obesity in dogs. This clinical study was a part of application researches of the OTB. In the veterinary clinical study, OTBp reduced the body weight and BCS in the obese dogs in a time-dependent manner during the study. Moreover, the ALP activity of the two dogs was lowered than that of the control (0 day). This result indicated that OTBp could have improving effect on the liver function because of the potent antioxidant activity present in OTB. From these results, OTBp is thought to have an improving effect on liver function, and a diet effect on the body weight without any adverse reactions. OTB prepared from OC by fermentation and combination with banana could be developed as a useful, novel food material and/or nutritional supplement with both antioxidant and dietary effects. Further research and development is undergoing.

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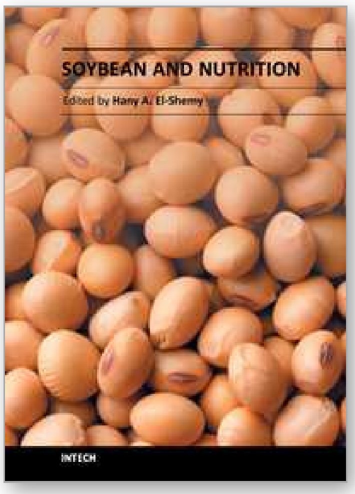

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