REFERENCES

1. The Wealth of India, Raw Materials, Vol. 2, Council of Scientific and Industrial Research, New Delhi, 1950, p. 20.
2. The Wealth of India, Raw Materials, Vol. 1, Council of Scientific and Industrial Research, New Delhi, 1948, p. 9.
3. The Wealth of India, Raw Materials, Vol. 5, Council of Scientific and Industrial Research, New Delhi, 1959, p. 88.
4. Official and Tentative Methods of the American Oil Chemists' Society, 3rd edn., 1958 (revised 1973), AOCS, Champaign, IL.
5. Folch, J., M. Lees, and G.H. Sloane-Stanley, J. Biol. Chem. 226:497 (1957).
6. Schneider, E.L., S.P. Loke and D.T. Hopkins, JAOCS 45:585 (1968).
7. Lakshminarayana, G., JAOCS 45:523 (1968).
8. Fioriti, J.A., A.P. Bentz and R.J. Sims, JAOCS 43:487 (1966).
9. Nunn, J.R., J.Chem. Soc. 313 (1952).
10. Joshi, S.S., and R.K. Shrivastava, Curr. Sci. 45:450 (1976).
11. C.H.W. Hirs, J. Biol. Chem. 219:611 (1956).
12. Berry, S.K., J. Sci. Food Agric. 31:657 (1980).
13. Husain, S., M. Babu, M.U. Ahmad, A.A. Ansari and S.M. Osman, Fette, Seifen, Anstrichm. 82:29 (1980).
14. Babu, M., S. Husain, M.U. Ahmad and S.M. Osman, Ibid. 82: 63 (1980).
15. Berry, S.K., JAOCS 59:57 (1982).

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⁂Phospholipid Composition of Some Plant Oils at Different Stages of Refining, Measured by the Iatroscan-Chromarod Method

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ABSTRACT

Although the phospholipid composition of crude plant oils has been well studied, not much is known about the effect of the different refining processes on the individual phospholipids. This information is useful to the manufacturer to optimize the refining process. In this study corn, sunflower seed and peanut oils, at different stages of refining, were analyzed with the Iatroscan-chromarod method. The total phosphorus content of the samples was also determined with a classical method. The Iatroscan gave results of acceptable accuracy for the analysis of crude oils with phospholipid-phosphorus values between 145 and 536 ppm. However, for oils at further stages of refining, with phospholipid-phosphorus values between 1 and 10 ppm, less accurate results were obtained. For these oils, the Iatroscan results had to be supported by conventional thin layer chromatography. Degummed oils contained phosphatidylcholine (1.1–22 ppm P), phosphatidylethanolamine (1–2 ppm P), phosphatidylinositol (trace–10 ppm P) and phosphatidic acid (trace–5 ppm P). Further refined oils contained no phospholipids with the exception of two samples. Bleached sunflower oil contained about 1 ppm phosphatidylinositol and bleached peanut oil contained ca. 1 ppm phosphatidylethanolamine and 1 ppm phosphatidylcholine. Fully refined edible oils contained no phospholipids.

INTRODUCTION

Phospholipids occur in crude plant oils at levels ranging between 0.1 and 1.8% (1). The composition and characteristics of phospholipids in crude oils have been thoroughly studied (1, 2); however, very little is known about the phospholipid composition of oils at different stages of refining. Some phospholipids act as antioxidant synergists with the tocopherols in plant oils (1, 3, 4), but the general practice is to remove the phospholipids from the oils prior to neutralization (alkali refining) or steam distillation (physical refining (5).

Phospholipids cause losses of neutral oil during neutralization (6, 7) and their presence leads to oil discoloration during deodorization and steam distillation (5).

The phospholipids are largely removed from the crude oils by water or acid degumming. This process reduces the phospholipid content of sunflower seed oil about eight times, and that of soy oil 70 times (3). The total phosphorus content of oils is monitored to give a general indication of phospholipid removal.

Very little is known about the effect of degumming and the successive refining steps on the individual phospholipids. The main reason for the lack of information is the unavailability of suitably sensitive methods for phospholipid analysis in oils.

High performance liquid chromatography offers a limited solution. Ultraviolet detection at 200–210 nm proved to be useful for qualitative evaluation in our laboratory (Y. Totani, personal communication), but not for quantitative analysis of phospholipids (8). In this investigation, the Iatroscan thin layer rod technique was used to study the phospholipid changes in oils that underwent alkali and physical refining.

EXPERIMENTAL PROCEDURES

Instruments and Materials

The Iatroscan TH 10 TLC analyzer Mk III (Newman-Howells Ass. Ltd., England) was used, coupled to a Hewlett Packard 3390A integrator. Hydrogen flow rate was 175 mL/min and air flow rate 1,850 mL/min. Chromarods SII were used for separations. Merck (Darmstadt) silica gel 60 TLC plates (without fluorescence indicator, Art. 5721); silica gel 60 for column chromatography (70-230 mesh, Art. 7734); AR grade chloroform, methanol, acetone and glacial acetic acid were used. Tertiary butylhydroquinone (TBHQ) was supplied by Eastman Products (Kingsport, TN).

The following phospholipid standards were obtained from Sigma Chemical Co. (St. Louis, MO): L-α-phosphatidic acid, sodium salt (PA) no. P9511, L-α-phosphatidyl-

ethanolamine (PE) no. P3511, L-α-phosphatidylcholine (PC) no. P5388, L-α-phosphatidyl-dl-glycerol, ammonium salt (PG) no. P0514, phosphatidylinositol 50% pure (PI) no. P6636, and cardiolipin, sodium salt (DPG) no. C1649.

Samples

Corn, sunflower and peanut oils were supplied by four local oil processing companies. Company E conducted a phosphoric/citric acid degumming, caustic refining, bleaching and deodorization (5). Companies N and V used water degumming followed by caustic refining, bleaching and deodorization (5). Company S applied water degumming, bleaching, winterizing and physical (steam) refining (5).

For the purposes of this paper, the end product of all these processes is described as a fully refined edible oil.

Methods

In order to obtain accurate and reproducible results, it is important to comply with the recommended procedures of the manufacturers of the Iatroscan apparatus. Full details conerning the handling, cleaning, conditioning and development of the rods are given in the instruction manual (9).

A variation of Borgström's method (10) was used for the isolation of total phospholipids from plant oils. Glass columns (38 cm × 2 cm) fitted with sintered glass outlets and 250 mL reservoirs were packed with a suspension of 5 g silica in chloroform. A 5–10 g oil sample was mixed with 50 mL chloroform and loaded onto the column. The neutral lipids were eluted with 150 mL chloroform, followed by 150 mL methanol to elute the phospholipids. One microgram TBHQ was added to the methanol fraction to protect the phospholipids against oxidation. The methanol was evaporated below 40 C and the residue dissolved in 1–10 mL chloroform. One μL samples were applied to the chromarods.

After sample application, a first development was done in acetone for 30 min (11). The rods were dried at 60 C for 2 min and scanned at rate 4 up to the band of polar lipids remaining at the origin of the rod. The rods were then placed in an atmosphere of 65% humidity for 10 min, hung in the vapor of the Innis (12) mobile phase (chloroform/methanol/water 80:35:3, v/v/v) for 10 min, and finally developed for 1 hr. The rods were dried at 60 C for 3–4 min and scanned fully. In order to calculate the quantities of individual phospholipids in the different oils, standard solutions ranging from 1 to 10 μg phospholipid were analyzed by Iatroscan chromatography. A standard curve of peak area vs μg phospholipid was obtained for each individual phospholipid. Each point on a standard curve represents the mean of 10–20 replicate analyses.

The phosphorus content (μg P/g oil) for individual phospholipid peaks on the chromarods was calculated using the formula:

$$\mu g\ P/g\ oil = \frac{\text{area PL} \times 5\ (\mu g) \times \text{tot vol}\ (\mu L)}{\text{area std} \times \text{spot vol}\ (\mu L) \times \text{sample weight (g)} \times \text{PL factor}}$$

where area PL = area of phospholipid peak; tot vol = volume of chloroform used to dissolve phospholipids after methanol evaporation; area std = area of 5 μg of the appropriate standard; and PL factor = ratio between molecular weight of phospholipid and atomic weight of phosphorus in the molecule.

To calculate the factor for each phospholipid, the average molecular weight of the fatty acids on the phospholipids was taken to be 280 ($C_{18}H_{32}O_2$), resulting in the factors shown in Table I.

TABLE I

PL Factors for Phospholipids

Phospholipid	Mol weight	Factor
PA	696	22.5
PC	781	25.2
PE	739	23.8
PG	770	24.8
DPG	1448	23.4
PI	858	27.7

A phospholipid mixture containing PA, PE, PI and PC was isolated from raw peanuts by chloroform/methanol (2:1, v/v) extraction and purified by silica gel chromatography (13) for recovery studies. Aliquots of the mixture in oil-free medium, as well as aliquots dissolved in 10 g refined sunflower seed oil, were subjected to the complete experimental procedure. Peak areas of the individual phospholipids of these aliquots were compared to the corresponding peak areas of the phospholipids in aliquots not subjected to all the steps of the method. The same phospholipid mixture was chromatographed ten times to determine the coefficient of variation of the rod chromatography alone. All samples were also analyzed for total phosphorus content by means of an ashing-spectrophotometric method (16).

Selected samples were applied to thin layer plates (TLC) and developed in chloroform/methanol/acetic acid (62:25:8, v/v/v) (15). Phosphate esters were visualized with the spray reagent of Dittmer and Lester (16). The limit of detection for each phospholipid on TLC was obtained by visual comparison with standards.

RESULTS AND DISCUSSION

Calibration curves for phospholipid standards are presented in Figure 1. The equations for the different curves are as follows:

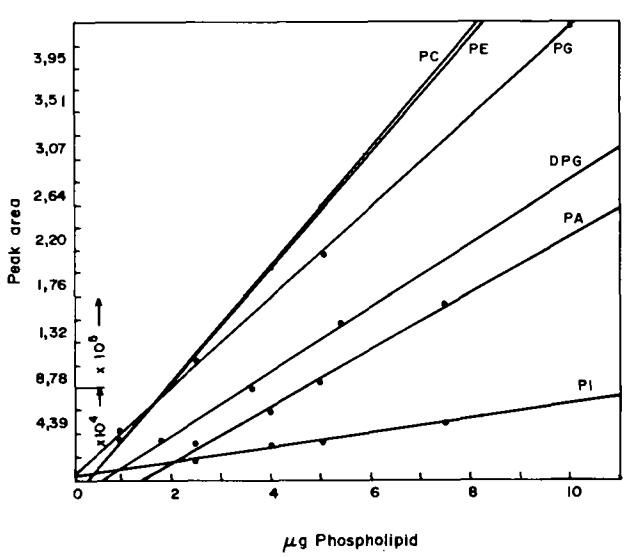

FIG. 1. Response of phospholipid standards.

DPG: $-y = -19558 + 30797x$ $R = 0.99711$
PG: $-y = 4229 + 43431x$ $R = 0.99996$
PA: $-y = -38794 + 23237x$ $R = 0.98997$
PE: $-y = -17174 + 55631x$ $R = 0.99906$
PI: $-y = 1943 + 7270x$ $R = 0.99872$
PC: $-y = 17212 + 55846x$ $R = 0.99888$

The observation that PE and PC respond almost identically is verified by the results of Takemoto (17). PI has a very poor response. The response of compounds in the flame ionization detector is a complex matter (18) depending largely on the ratio of carbon and oxygen atoms in the molecule (19).

Recovery figures are given in Table II. The coefficient of variation for the chromatography of individual peanut phospholipids was found to be: PA 15%; PE 7.6%; PI 10.7% and PC 7.1%. The larger variation in the case of PA and PI

TABLE II

Percentage Recovery of Phospholipids[a]

PA	PC	PE	PI
Phospholipid mixture in oil-free medium			
92	93	93	90
Phospholipid mixture added to 10 g refined oil			
105	89	93	96

[a]Mean of four replicates.

may be attributed to their smaller and less symmetrical peaks (Fig. 2). To improve the accuracy of the method, duplicate column chromatographic isolates of phospholipids from oil samples were each chromatographed five times.

Chromatograms of corn oil at different stages of refining are shown in Figure 2. Typical retention times are: DPG 0.04; PG 0.10; PA 0.16; PE 0.19; PI 0.23 and PC 0.39. A phosphatidylserine standard chromatographed between PI and PC.

Results of the analyses are shown in Table III. In these oils the lower limit for reliable determination was found to be: 2 ppm phosphatidylethanolamine phosphorus (PE-P) and PC-P; 9 ppm PA-P and 7 ppm PI-P. Where lower values are reported in Table III, these values were verified by means of thin layer chromatography (TLC) and the specific spray reagent. In this way, 0.5 ppm PE-P and PC-P; 2 ppm PI-P and 1.5 ppm PA-P could be determined.

In crude oils, a fair agreement was found between the total phosphorus determined by the Iatroscan method and the spectrophotometric method, although the former tends to give slightly lower results in most cases. For degummed and more refined oils, the difference increased, with the chromarod method detecting much less phosphorus than the spectrophotometric method.

Bleached, winterized and deodorized oils contain very little, if any, phospholipids. After bleaching, very low levels of PC, PE and PI were found in sunflower seed oil and peanut oil only.

The difference between the two sets of results suggests that the spectrophotometric method measures non-PL-P, or additional PL-P present in compounds not investigated in this study.

Goh and coworkers (20) also found the total phosphorus

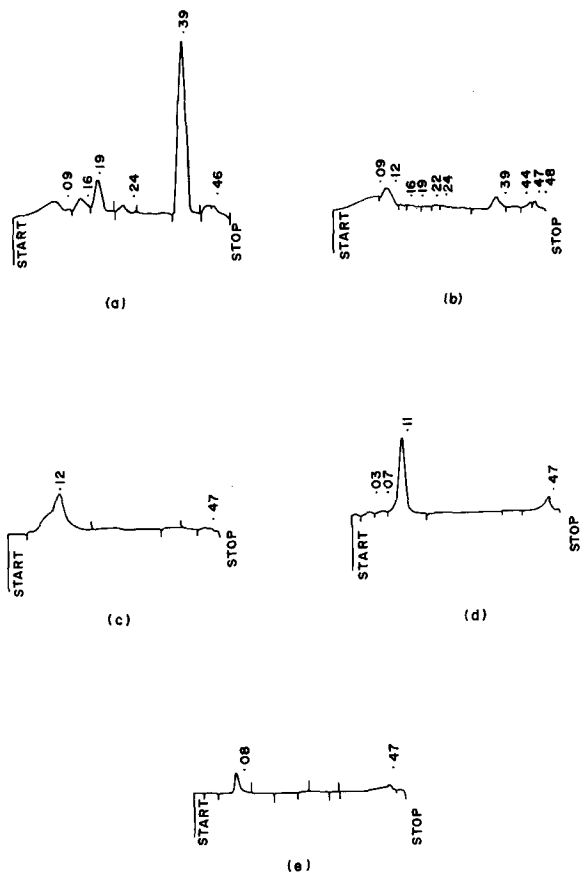

FIG. 2. Chromatograms of corn oil at different stages of refining. (a) Crude corn oil; (b) degummed corn oil; (c) bleached corn oil; (d) winterized corn oil; (e) fully refined edible corn oil. RT 0.16: PA; 0.19: PE; 0.23: PI; 0.39: PC; 0.44: lyso-PC.

in palm oil to be considerably higher than the PL-P, and attributed the difference to inorganic phosphates. It is also well known that phytin occurs in seeds (21) and since phytoglycolipids have been indicated in corn oil (22), compounds of this nature may be responsible for the observed difference in PL-P and total P.

In the oil samples investigated, PG and DPG could not be confirmed beyond any doubt. The separation of PG, DPG and PA standards on chromarods is shown in Figure 3. In most samples exposed to processing, significant peaks with the same retention times as PG were observed (Fig. 2b–e and Fig. 3); and in some cases, minor peaks with the same retention times as DPG. However, with the aid of TLC (Fig. 4), these components proved not to be DPG or PG.

On the chromarods the retention times of the unidentified P-containing components observed by TLC and shown in Table III, are unknown.

Table IV presents a comparison of the percentage composition of the phospholipids of crude corn oil, crude sunflower seed oil and crude peanut oil, determined with the Iatroscan-chromarod method, and literature values (23–26). In general, good agreement was obtained.

ACKNOWLEDGMENTS

R. Bagnari made the phosphorus determinations. P.J. van Niekerk prepared the standard curves.

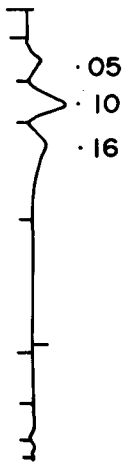

FIG. 3. Separation of DPG, PG and PA standards. RT 0.05: DPG; 0.10: PG; 0.16: PA.

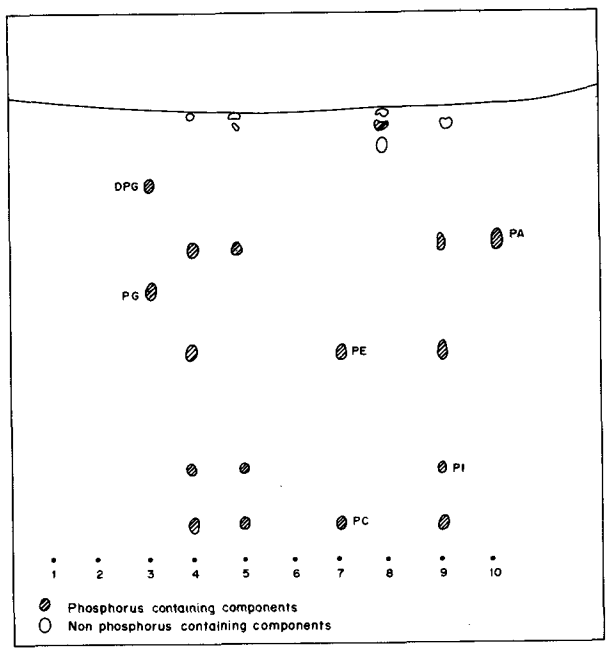

FIG. 4. Thin layer chromatogram of phospholipid standards and oil samples. (1) Bleached corn oil; (2) winterized corn oil; (3) standards; (4) crude sunflower seed oil; (5) degummed corn oil; (6) fully refined edible corn oil; (7) standards; (8) degummed sunflower seed oil; (9) crude peanut oil; (10) standard.

TABLE III

Phospholipid Composition of Oils

Oil	Source	Iatroscan method (ppm P) PA	PE	Lyso-PE	PI	PC	Lyso-PC	Total	Spectrophotometric method (ppm P)
Corn									
Crude	(S)	72	29	+[a]	55	205	+	361	375
	(E)	15	9	+	38	83	+	145	124
	(V)	16	47	+	23	159	+	245	296
	(N)	38	13	+	32	125	+	209	233
		6	23	–[b]	37	185	–	251	310
		9	25	+	75	146	+	255	263
		15	26	+	55	159	–	255	238
Degummed	(S)	4	1.5	+	6	4	+	15.5	31.8[c]
	(E)	5	2	–	10	22	+	39	73.6
	(V)	2.2	–	–	1.6	1.1	–	4.9	9.3
	(N)	–	–	–	3	2	–	5	10.4
Bleached	(S)	–	–	–	–	–	–	0	4.4
Winterized		–	–	–	–	–	–	0	4.6
Refined[e]		–	–	–	–	–	–	0	3.4
Bleached	(E)	–	–	–	–	–	–	0	0.9
Refined[e]		–	–	–	–	–	–	0	4.3
Refined[e]	(V)	–	–	–	–	–	–	0	2.5
Refined[e]	(N)	–	–	–	–	–	–	0	1.3
Sunflower									
Crude	(S)	28	81	+	59	252	+	420	452
	(E)	TLC[d]	9.2	+	5.3	15.7	+	30.2	77[c]
Degummed	(S)	TLC	1.1	–	2.5	1.9	–	5.5	17.9
	(E)	–	–	–	–	–	–	0	10.1[c]
Bleached	(S)	–	–	–	1	–	–	1	5.9
Winterized		–	–	–	–	–	–	0	4.8
Refined[e]		–	–	–	–	–	–	0	4.3
Bleached	(E)	–	–	–	–	–	–	0	0.5
Refined[e]		–	–	–	–	–	–	0	2.5
Peanut									
Crude	(S)	51	63	+	109	313	+	536	543
Degummed		TLC	1	–	TLC	2.1	–	3.1	35
Bleached		TLC	1	–	TLC	1.1	–	2.1	24
Refined[e]		–	–	–	–	–	–	0	27

+[a]Detected, not quantified.

–[b]Not detected.

[c]Unidentified P-containing components with TLC.

[d]Detected with TLC only.

[e]Fully refined edible oil.

TABLE IV

Percentage Composition of Phospholipids in Crude Oils

Oil	N-acyl PE	PE	Lyso-PE	PG	PA	PC	Lyso-PC	PS	PI	
Corn		10.2	+[a]	–[b]	9.8	61.8	+	–	18.3	Iatroscan
	2.9	4.8	+	2.0	15.1	43.4	3.5	1.5	21.1	Weber (23)
Sunflower seed		19.3	+	–	6.7	60	+	–	14	Iatroscan
		19.7			2.2	52			26	Chapman (24)
		23			4	51			22	Shustanova (25)
Peanut		11.8	+	–	9.5	58.4	+	–	20.3	Iatroscan
		18			4	44			24	Wagner (26)

+[a]Detected.

–[b]Not detected.

REFERENCES

1. Sonntag, N.O.V., in Bailey's Oil and Fat Products, 4th edn., Vol. 1, edited by D. Swern, John Wiley and Sons, New York, 1979, p. 49.
2. Strickland, K.P., in Form and Function of Phospholipids, 2nd edn., Vol. 3, edited by G.B. Ansell, J.N. Hawthorne and R.M.C. Dawson, Elsevier Pub. Comp., Amsterdam, 1973, p. 9.
3. Du Plessis, L.M., CHEMSA 8(11):150 (1982).
4. Pongracz, G., Int. J. Vitam. Nutr. Res. 43:517 (1973).
5. Davies, M.G., I.M. Jawad and G.S.D. Weir, Scientific and Technical Surveys No. 112 (1979), Leatherhead Food Res. Ass., Leatherhead, Surrey, UK.
6. Norris, F.A., in Bailey's Industrial Oil and Fat Products, 4th edn., Vol. 2, edited by D. Swern, John Wiley and Sons, New York, 1982, p. 255.
7. Young, V., Chem. Ind. (London) 18:692 (1978).
8. Yandrasitz, J.R., Varian Instrum. Appl. 15(3):14 (1981).
9. Iatroscan TH-10 Instruction Manual, Distributors Newman-Howells Ass. Ltd., Winchester, Hampshire, UK.
10. Borgström, B., Acta Physiol. Scand. 25:101 (1952).
11. Ackman, R.G., and J. Woyewoda, J. Chromatogr. Sci. 17:514 (1979).
12. Innis, S.M., and M.T. Clandinin, J. Chromatogr. 205:490 (1981).
13. Choudhury, R., and L. Arnold, JAOCS 37:87 (1960).
14. Boltz, D.F., and M.G. Mellon, Anal. Chem. 19:873 (1947).
15. Nichols, B.W., Biochim. Biophys. Acta 70:417 (1963).
16. Dittmer, J.C., and R.L. Lester, J. Lipid Res. 5:126 (1964).
17. Takemoto, Y., Kawasaki Med. J. 6:1 (1980).
18. Ackman, R.G., in Methods in Enzymology, Vol. 72, Part D, edited by J.M. Lowenstein, Academic Press, New York, 1981, p. 213.
19. Sipos, J.C., and R.G. Ackman, J. Chromatogr. Sci. 16:445 (1978).
20. Goh, S.H., H.G. Khor and P.T. Gee, JAOCS 59:296 (1982).
21. Lawton, K., in Phosphorus and its Compounds, Vol. 2, edited by J. R. van Wazer, Interscience Publishers Inc., New York, 1961, p. 1463.
22. Carter, H.E., W.D. Celmer, D.S. Galanos, R.H. Gigg, W.E.M. Lands, J.H. Law, K.L. Mueller, T. Nakayama, H.H. Tomizawa and E. Weber, JAOCS 35:335 (1958).
23. Weber, E.J., JAOCS 58:898 (1981).
24. Chapman, G.W., Jr., JAOCS 57:299 (1980).
25. Shustanova, L.A., A.U. Umaron and A.L. Markham, Khim. Prir. Soedin. 3:292 (1970).
26. Wagner, H., and P. Wolff, Fette, Seifen, Anstrichm. 66:425 (1964).

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