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Chemical Composition and Preservative Effect of Turkish Propolis on Egg Quality Durig Storage

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The present study was carried out to investigate chemical composition and preservative effects of Turkish propolis on quality of fresh eggs. For this purpose, different concentrations of ethonolic extract of propolis (5, 8, 10 % EEP) were prepared. Total of (9 period × 5 group × 10 n) 450 egg were used during experiment. The eggs were randomly divided into 5 experimental groups. The first, second and third group eggs covered with 5, 8, 10 % EEP, fourth group with alcohol control and fifth group as a control (uncovered), respectively. Ten eggs in each group were drawn randomly and examined each week between 17 March-12 May 2006. Chemical analysis of propolis extracts indicated that the propolis samples had high concentrations of the aromatic acids, esters and other derivatives which are responsible for the antibacterial, antifungal, antiviral, antiinflammatory and anticancer properties of propolis such as benzyl cinnamate, methyl cinnamate, caffeic acid, cinnamyl cinnamate and cinnamoylglcine besides the most common compounds as fatty acid, terpenoids, esters, alcohols hydrocarbons and aromatic acids. It was found that ethanolic extract of propolis improved interior egg quality.

Key Words: Bee product propolis, Eggs quality, Preservation, Haugh unit, Yolk index, Albumen index.

INTRODUCTION

Propolis is plant resin collected by bees for use in and around the hive. Much work has been conducted on the chemistry and properties of propolis. Hundreds of chemical compounds have been identified from propolis. The main chemical classes present in propolis are flavonoids, phenolics and various aromatic compounds. Propolis also contains some volatile oils, terpens and beewax. Flavonoids are well know plant compounds that have antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory and preservation properties. Other properties of propolis are used as a local anesthetic, reducing spasm, healing gastric ulcer and strengthening capillaries[1-3]. Recent investigations have indicated that the interest for natural preservative had increased. The used of propolis that is non-toxic as alternate preservative agent has been considered by consumers as safe[3-5]. Silici *et al.*[6] reported that propolis was worthy of further study as a natural preservative for foods susceptible to fungal spoilage.

Soylu *et al.*[7] reported that fruits damaged during postharvest handling might be protected by high concentrations of propolis successfully. However, propolis could not control green mold once fruit is infected. Ozdemir *et al.*[8] covered mandarin with propolis in order to prevent weight loss. Present workers reported that propolis is succesfully prevent mandarin weight loss during 3-3.5 month. Propolis has anti-bacterial effect that use in different agricultural products which need storage before consumption. For example, propolis has been used with alcohol on strawberry to inhibit *Boytris cinerae pers* development[9]. Propolis has wax and antibacterial properties. It may be important to coat the table egg shell with propolis extract during storage because the egg shell coating limits water loss through pores to restrict gas diffusion.

Eggs are expensive source of high quality protein and other nutrients. However, eggs are highly perishable and could rapidly lose their quality[10]. Losses to the egg industry as the result of problems with egg and eggshell quality have been estimated to be in excess of ten million dollars annually[10].

The major factors in determining egg quality are egg storage time, conditons, strain and age of hen[11]. Haugh unit, albumen and yolk indices are at maximum when the eggs are laid and decrease with increased storage time[12,13]. After ovopositon is the albumen pH value between 7.6-7.9 and this leads to a rise 9.7 during storage[14-18]. pH increases during storage because of carbon dioxide loss through the porous shell[14-18].

Eggshells are breathable material therefore they allow moisture and carbon dioxide to permeate through the shell. The permeation causes physical and chemical changes as albumen and yolk as well as weight loss. The pores on eggshell need to be sealed to reduce evaporation and escape of carbon dioxide[10,18]. The precautions for increased egg storage time are retarded go to stale because of prevention CO_2 and water loss from egg and protection the nature form of egg[17]. Some protection methods such as egg shell coating minimize interior egg quality loss[18,19]. The edible films, which are not detrimental on human health, have a barrier property against oxygen, carbon dioxide and humidity movement from eggs[20,21]. Although some storage methods to protection of interior egg quality used to such as oil coating[22], dipping, in low temperature storage, freezing, high temperature and drying[17], the coating of egg shell with chitosan, whey protein and shellac during storage, these are not detrimental affect on human health[10].

There has been increasing interest in using propolis as a food preservation method as well as a means to enhance food qualtity, safety and sustainability. In this study, the aim is to determine the effect of the interior egg quality of shell coated eggs with propolis in storage condition (5 ºC) and determined chemical composition of Turkish propolis.

EXPERIMENTAL

This research was carried out by using white-shell, fresh hen eggs which coated with ethanolic extract of propolis. Eggs were obtained from a local eggs producer

in Hatay and from hens 54 weeks old hens. After oviposition and equal weight eggs were collected and brought in study place for coating of various dosage ethanolic extract of propolis. Treatments consisted of eggs coated with 5, 8 and 10 % ethanolic extract of propolis. Uncoated eggs were control. Measurement about egg quality was carried out at during 9 weeks in 5 ºC. For each group (eggs per group totally 450 eggs), 50 eggs per week measured for determination of albumen height, albumen length, albumen width and yolk width for each experimental groups.

Preparations of 5 % ethanolic extract of propolis: Propolis was collected from honey bees in Hatay 2006 and exracted according to the method suggessted by Krell[23]. A 5 % ethanolic extract of propolis was prepared by mixing 1950 mL 70 % ethonol and 50 g of propolis, a 8 % propolis by mixing 1920 mL 70 % ethonol and 80 g of propolis and a 10 % ethanolic extract of propolis were prepared by mixing 1900 mL 70 % ethonol and 100 g of propolis. They were kept in a container, sealed the top and shaken twice daily for one week. It was filtered and kept in a clean, dark bottle at 4 ºC until it was used.

Chemical analysis of propolis: A GC-MS (Hewlett Packard Gas Chromatograph 6890 Series plus linked to Hewlett Packard 6890 Mass Spectrometer) system was used for the chemical analysis. The capillary column (25 µm thickness, 0.25 mm diameter, 30 m length) and Helium carrier gas (31 mL/min linear velocity, 20:1 split ratio and 230 ºC temperature) were used on the GS-MS system.

Coating of eggs with coating ethanolic extract of propolis and alcohol: Eggs were immersed in the 5, 8 and 10 % ethanolic extract of propolis and alcohol by hand for 1 min and this process repated once more and then dried at ambient temprature. Egss were stored around 5 ºC during 9 weeks during the experiment. Sample were divided into 5 groups, one of them for control and alcohol (as uncoated) and the others used for the coating by ethanolic extract of propolis (5, 8, 10 %). Fifty eggs (each group 10 × 5 group) were drawn each week during the 9 weeks for measurement.

Haugh unit (HU) and Yolk Index (YI): Individual Haugh unit[24] score was calculated using the egg weight and albumen height[25]. The Haugh unit values were calculated for individual egg using the following formula:

$$\text{Haugh unit (HU)} = 100 \log (H + 7.57\text{-}1.7G^{0.37})$$

where H is the height of the thick albumen in millimetres and G is the mass of the whole egg in grams. The parameter H was estimated by tripod micrometer. Egg mass measurements were recorded to within ± 0.001 g.

Yolk index (YI) was calculated as follows:

$$\text{Yolk index (YI)} = \frac{\text{Yolk Height (mm)}}{\text{Yolk diameter (mm)}} \times 100$$

Yolk height was measured by tripod micrometer and yolk width was measured with digital calliper.

Albumen index (AI): Eggs were broken on a flat surface where the height of the albumen was measured, half way between yolk and edge of the inner thick albumen using a standard tripod micrometer as mm. Albumen index (AI) was calculated as follows:

AI=Albumen height (mm)/[Albumen length (mm) + Albumen width (mm)] × 100

During measurement of albumen length and albumen width were used the nearest 0.1 mm a steel vernier calliper.

Data analysis: This study evaluated the combined effect of propolis and storage time on the properties of eggs. Analysis of variance was carried out on all the measurement parameters among the control, alcol and with propolis coaeted eggs during the storage time. The data were subjected to a GLM using software of SPSS 10[26], with treatments and storage time. When main effects were significant at $p < 0.05$, means were separated using Duncan's multiple range test[27].

RESULTS AND DISCUSSION

The chemical composition of the alcohol extracts of propolis from Hatay is summarized in Table-1. As seen from Table-1, propolis from Hatay region had high concentrations of the aromatic acids, esters and other derivatives such as benzyl cinnamate (4.43 %), methyl cinnamate (3.55 %), caffeic acid (4.37 %), cinnamyl cinnamate (6.95 %) and cinnamoylglycine (1.21 %). Sahinler and Kaftanoglu[3] was also reported that benzyl cinnamate (9.39 %), methyl cinnamate (6.23 %), caffeic acid (5.98 %), cinnamyl cinnamate (27.99 %) and cinnamoylglycine (0.83 %) in Hatay propolis.

TABLE-1
CHEMICAL COMPOSITION OF ETHANOLIC EXTRACTS OF PROPOLIS FROM HATAY

Substances	Area (%)	Substances	Area (%)
Aromatic acids		**Fatty acids**	
Benzyl cinnamate[3]	4.43	Hexacosanoic acid	2.45
Methyl cinnamate[3]	3.55	Octacosanoic acid[28]	1.79
Caffeic acid[3,4,28,30]	4.37	Triacontanoic acid	2.25
Cinnamyl cinnamate[3]	6.95	Butanedioic acid	1.32
Cinnamoylglcine[3]	1.21	Eicosanoic asit	1.50
Terpenoids		Docosanoic acid[28]	2.12
α-Pinene[3,29]	0.42	Tetracosanoic acid[28]	2.71
Indolin, 2-methylen[3]	2.31	9,2,15-Octadecatrienoic asit	1.25
Cyercene[3]	4.45	Octadecanoic asit	2.18
1S-*cis*-Calamenen[3]	0.25	9,12-Octadecanoic asit	1.72
α-Copaene[3,28]	3.43	**Hydrocarbons**	
β-Maaliene[3]	1.75	Nonacosane[3,28]	0.75
α-Elemene[3]	3.51	Heneicosane[3]	1.22
β-Eudesmol[3]	7.61	Triacosane[3]	1.75
α-Eudesmol[3]	4.51	Hexacosane[3,28]	0.78
α-Bisabolol[3]	1.25	**Ketonlar**	
Geranyl acetate	1.19	Pentadecanone	0.25
Calarene	0.92	2-Nonadecanone	1.73

Hydrocarbons, which are not associated with any of the reported biological activities of propolis were identified the varieties and rates in propolis extract from Hatay are given in Table-1. Only 4 hydrocarbons were determined in Hatay propolis. Greenaway *et al.*[28] was also reported nonacosane and hexacosane in these samples. Giuseppina *et al.*[31] reported that the botanical origin of the samples and genetic factors of the colonies have major influences on the composition of propolis resin in terms of hydrocarbon patterns. Total, 10 fatty acids were determined. Fatty acids determined in studies of Greenaway *et al.*[28], of Velikova *et al.*[30] were given in Table-1 as 1 and 3 numerals, respectively.

Preservative effect of propolis on egg quality: The effects of the interior egg quality (*i.e.*, Haugh unit, yolk index, albumen index and albumen height) of shell coated eggs with different concentration of ethanolic extracts of propolis in storage time was statistically significant all measure ($p < 0.01$). All the parameters were decreased with storage time all groups (I, II, III, IV, V) but changes were less gourps I, II, III than others groups (IV, V).

Haugh units (HU): HU is a very important indicator for egg interior quality determination. HU is related to albumen quality and is often measured as a function of the inner thick albumen height and egg weight. If HU value is more than > 79 maintained eggs grade 'AA' as perfect, between 55-78 grade 'A' as good, between 31-54 grade 'B' as bad and < 30 grade 'C' as very bad[10,25]. After 9 weeks, the HU decreased in all groups and the difference of HU value between groups was statistically significant ($p < 0.01$) (Table-2). The HU of I, II, III, IV and V groups were 74.26 ± 1.09, 75.28 ± 1.16, 74.98 ± 1.16, 68.02 ± 1.45 and 68.16 ± 1.50 in groups, respectively (Table-3).

TABLE-2
GRADE OF PROPOLIS COATED EGGS DURING 9 WEEKS OF STORAGE BASED ON THE HAUGH UNIT

Weeks	Treatments				
	1	2	3	4	5
1	AA	AA	AA	AA	AA
2	AA	AA	AA	AA	AA
3	AA	AA	AA	AA	AA
4	A	A	AA	A	A
5	A	A	A	A	A
6	A	A	A	A	A
7	A	A	A	A	A
8	A	A	A	B	A
9	A	A	A	A	A

AA = Perfect, A = god, B = Bad.

The HU decreases higher in I, II and III groups than in IV and V groups after one week storage. At the first-third weeks, all groups grade 'AA' eggs, at the fourth week grade 'A' all groups except group 3 (AA) and between 5-8 weeks all groups 'A' grades except four groups (B) (Tables 2 and 3). The eggs of 3 groups protected

TABLE-3
GRADE OF PROPOLIS COATED EGGS DURING 9 WEEKS OF STORAGE BASED ON THE HAUGH UNIT

Weeks	Treatments				
	1 $(\overline{X} \pm S_{\overline{X}})$	2 $(\overline{X} \pm S_{\overline{X}})$	3 $(\overline{X} \pm S_{\overline{X}})$	4 $(\overline{X} \pm S_{\overline{X}})$	5 $(\overline{X} \pm S_{\overline{X}})$
1	91.18±1.75	87.46±1.41	79.99±3.36	86.05±2.00	85.59±2.10
2	80.05±1.72	82.28±3.52	86.23±1.65	81.71±2.42	83.81±1.92
3	80.05±1.72	82.28±3.52	86.23±1.65	81.71±2.42	83.81±1.92
4	74.17±1.42	76.81±3.18	78.59±2.57	63.21±3.30	69.02±2.70
5	72.95±2.00	77.76±2.12	76.26±1.89	65.42±2.44	63.74±1.84
6	69.07±2.65	71.91±2.70	69.68±1.71	61.23±2.28	61.22±2.89
7	68.34±2.68	70.37±1.94	72.93±2.43	60.64±2.97	56.46±2.48
8	62.80±1.97	65.31±2.54	57.55±0.79	53.37±1.78	54.79±2.04
9	67.36±2.05	63.31±1.35	67.40±2.52	57.54±2.35	55.00±3.03
Means	74.26±1.09	75.28±1.16	74.98±1.16	68.02±1.45	68.16±1.50

The grade A.B or C is given an egg based upon interior and exterior quality not size.
AA = grade > 79. A ranges from 55 to 78; B ranges from 31 to 54; C ranges from < 30.

the same grade place for along time. This result is agreed with Allenoni and Antunes[32] and Caner[10] studies who exposed the HU value of coated eggs at 4th week higher than of uncoated eggs.

Albumen index (AI): Albumen index is a significant indicator in determination of interior egg quality. Albumen index value of all treatment eggs was decreased by increased storage time (during 9 weeks) (Table-4). In the beginnig study albumen index value in group I, II, III, IV, V 10.50 ± 0.58, 9.81 ± 0.35, 8.32 ± 0.55, 9.59 ± 0.71, 9.28 ± 0.57, respectievly. After 9 week storage time, while albumen index values of I, II, III, IV, V groups 5.37 ± 0.13, 4.84 ± 0.19, 4.69 ± 0.16, 4.55 ± 0.61, 4.43 ± 0.31, respectively (Table-4) ($p < 0.01$). It was found that the rate of decreasing in group I; II; II; IV; V 48 %, 50.6 %, 43.62 %, 52.55 %, 52.26 %, respectievly.

TABLE-4
EFFECT OF PROPOLIS COATING ON VALUE OF ALBUMEN-INDEX (%)
1 = %10 propolis, 2 = %8 propolis, 3 = %5 propolis, 4 = Alkol Kontrol, 5 = Kontrol grubu

Weeks	Treatments				
	1 $(\overline{X} \pm S_{\overline{X}})$	2 $(\overline{X} \pm S_{\overline{X}})$	3 $(\overline{X} \pm S_{\overline{X}})$	4 $(\overline{X} \pm S_{\overline{X}})$	5 $(\overline{X} \pm S_{\overline{X}})$
1	10.50±0.58	9.81±0.35	8.32±0.55	9.59±0.71	9.28±0.57
2	8.05±0.36	9.11±0.82	9.24±0.40	8.32±0.51	8.92±0.46
3	8.08±0.31	8.40±0.65	9.18±0.38	8.26±0.46	8.84±0.40
4	7.07±0.23	7.18±0.47	7.79±0.42	5.47±0.37	6.19±0.34
5	6.93±0.35	7.48±0.41	7.57±0.30	5.71±0.30	5.70±0.28
6	5.96±0.37	6.38±0.43	5.92±0.22	5.74±0.45	6.05±0.52
7	5.70±0.34	6.41±0.23	6.40±0.49	4.96±0.32	4.51±0.24
8	4.85±0.19	5.21±0.38	4.18±0.16	4.08±0.20	4.39±0.25
9	5.37±0.13	4.84±0.19	4.69±0.16	4.55±0.61	4.43±0.31
Means	7.00±0.21	7.20±0.23	7.03±0.22	6.31±0.25	6.48±0.24

These results agree with the result of Tilki and Saatci[33], they found in alectoris graeca eggs albumen index was at the beginning of storage 7.39 and this value decreased after 4 weeks to 2.81 and after 5 weeks to 1.99.

Albumen height (AH): Albumen height could be influenced with hen age, storage and strain of layer hen. Albumen height decreases with increased strain age and storage time[34]. The mean albumen height values were significantly different between all of treatment eggs ($p < 0.01$) with higher values in I (6.01 ± 0.15) and II (6.17 ± 0.16) groups than in III (6.14 ± 0.15), IV (5.46 ± 0.17) and V (5.51 ± 0.18) groups (Table-4). Albumen height of I, II, II, IV and V groups was 8.60,8, 7.02, 7.72, 7.70 mm, respectively at the beginning of storage. At the end of 9th week, albumen height of I, II, III, IV and V groups were 5.08, 4.55, 5.10, 4.68 and 3.89 mm, respectively (Table-5). Albumen height in eggs more decreased V and IV than I, II and III groups. This result agrees with the results of Silversides and Budgel[34] that albumen height decreased from 8.45 to 4.10 for 10 d storage. Results showed that the albumen height during storage time significantly decreased. In terms of albumen height differences between these groups were found statistically significant ($p < 0.01$).

TABLE-5
EFFECT OF PROPOLIS COATING ON ALBUMEN HEIGHT (mm)

Weeks	Treatments				
	1 $(\bar{X} \pm S_{\bar{X}})$	2 $(\bar{X} \pm S_{\bar{X}})$	3 $(\bar{X} \pm S_{\bar{X}})$	4 $(\bar{X} \pm S_{\bar{X}})$	5 $(\bar{X} \pm S_{\bar{X}})$
1	8.60±0.32	8.00±0.24	7.02±0.41	7.72±0.34	7.70±0.34
2	6.66±0.25	7.19±0.55	7.77±0.25	6.96±0.37	7.35±0.33
3	6.66±0.25	7.19±0.55	7.77±0.25	6.96±0.37	7.35±0.33
4	5.97±0.17	6.34±0.40	6.59±0.31	4.68±0.32	5.29±0.32
5	5.85±0.25	6.40±0.31	6.23±0.23	4.87±0.23	4.68±0.15
6	5.29±0.31	5.66±0.33	5.21±0.19	5.14±0.35	5.37±0.41
7	5.11±0.29	5.43±0.18	5.64±0.32	4.32±0.27	4.10±0.22
8	4.47±0.18	4.81±0.28	3.94±0.09	3.69±0.09	3.87±0.17
9	5.08±0.24	4.55±0.12	5.10±0.24	4.68±0.31	3.89±0.23
Means	6.01±0.15	6.17±0.16	6.14±0.15	5.46±0.17	5.51±0.18

a.b,c and d = In the table followed by different letters differ significantly ($p < 0.01$).

Yolk-Index (YI): Yolk index was measured by using the yolk height and width in fresh egg. Yolk index indicates a progressive deterioration of vitellin membranes and liquefaction of the yolk caused by diffusion of water from the albumen[12,35]. A fresh and good quality egg contains *ca.* 0.45 yolk-index[36]. The difference of YI value between treatment groups was statistically significant ($p < 0.01$). At the beginning of storage, YI value agreed with those of Senkoylu[36] who reported YI value for a fresh egg as 45 %. The effect of storage time on YI was significant ($p < 0.01$). In the beginnig study YI value in group I, II, III, IV, V 43.82 ± 1.78, 48.29 ± 0.79, 46.52 ± 0.80, 45.51 ± 1.13, 43.74 ± 2.26, respectievly. After 9 week storage time, while YI values of I, II, III, IV and V groups, 38.20 ± 0.73, 38.21 ± 0.52, 38.15 ±

1.08, 31.28 ± 3.52, 33.99 ± 0.87, respectively (Table-6) ($p < 0.01$). It was found that the rate of decreasing YI in group I; II; II; IV; V, 12 %, 20.87 %, 17.99 %, 31.26 %, 22.29 %, respectievly (Table-6).

TABLE-6
EFFECT OF STORAGE ON YOLK-INDEX

Weeks	Treatments				
	1 $(\bar{X} \pm S_{\bar{X}})$	2 $(\bar{X} \pm S_{\bar{X}})$	3 $(\bar{X} \pm S_{\bar{X}})$	4 $(\bar{X} \pm S_{\bar{X}})$	5 $(\bar{X} \pm S_{\bar{X}})$
1	43.82±1.78	48.29±0.79	46.52±0.80	45.51±1.13	43.74±2.26
2	46.22±0.66	44.28±1.11	44.79±0.56	41.50±2.21	44.22±0.81
3	41.79±0.81	44.10±0.98	43.06±0.48	44.35±0.96	40.90±0.91
4	40.68±0.70	42.62±1.03	43.79±1.19	38.12±0.63	38.80±0.67
5	42.38±0.38	40.47±0.71	42.58±1.06	38.73±0.77	39.40±0.84
6	38.85±0.70	37.17±0.90	38.57±0.78	35.77±0.62	33.89±0.84
7	39.75±1.15	38.93±0.54	40.90±1.04	35.74±0.70	34.94±0.95
8	36.76±0.81	37.87±0.48	35.90±0.61	35.91±1.09	34.79±0.68
9	38.20±0.73	38.21±0.52	38.15±1.08	31.28±3.52	33.99±0.87
Means	41.03±0.43	41.34±0.46	41.66±0.45	38.54±0.69	38.30±0.53

a, b, c and dc = In the table followed by different letters differ significantly ($p < 0.01$).

Conclusion

This study suggested that: (i) Effects of different concentrations of propolis treatments on albumen height were important at the 9th week. During storage, deviations in albumen height and albumen index values of high level of propolis coated eggs were less than low level of propolis coaled eggs. (ii) Haugh unit in group 3 (10 % ethanolic extracts of propolis) the first-fourth weeks grade 'AA' eggs, group 1 (5 % ethanolic extracts of propolis) and 2 (8 % ethanolic extracts of propolis) the first-three weeks grade 'AA'. (iii) During table egg storage at 5 ºC temperature, the coating of egg shell with propolis, as a nature product, decreases the CO_2 loss of albumen and lowered the changes of interior egg quality. (iv) Effective use of propolis will be possible at the shelf life length of table eggs (Table-7).

TABLE-7
EFFECT OF COATING ON THE EGG QUALITY OF TREATMENTS

Properties	Treatments					
	1 $(\bar{X} \pm S_{\bar{X}})$	2 $(\bar{X} \pm S_{\bar{X}})$	3 $(\bar{X} \pm S_{\bar{X}})$	4 $(\bar{X} \pm S_{\bar{X}})$	5 $(\bar{X} \pm S_{\bar{X}})$	Significant level
Albumen index (%)	7.00±0.21	7.20±0.23	7.03±0.22	6.31±0.25	6.48±0.24	**
Albumen height (mm)	6.01±0.15	6.17±0.16	6.14±0.15	5.46±0.17	5.51±0.18	**
Haugh unit	74.26±1.09	75.28±1.16	74.98±1.16	68.02±1.45	68.16±1.50	**
Yolk index (%)	41.03±0.43	41.34±0.46	41.66±0.45	38.54±0.69	38.30±0.53	**
Yolk height (mm)	17.95±0.14	17.97±0.15	17.98±0.15	16.95±0.20	16.73±0.18	**

(v) The interior egg quality of 10 % propolis coated eggs was higher than those of other groups. The egg shell coating with propolis above 10 % concentration can be studied in further studies.

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