The influence of carotenoid and chlorophyll content on the oxidative processes in the selected vegetable oils

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ABSTRACT

Purpose: More than 100 plant species have been classified as oil products, but only a few of them are used in industrial production. In the available literature, there are no studies that would describe the relationship between the content of plant dyes and their impact on auto-oxidative processes. Therefore, this study aimed to determine dye composition and to define their effect on the acid value, peroxide value and quality assessment of selected refined and unrefined oils.

Materials and methods: Twenty samples from different manufacturers were evaluated. Oils were purchased from retail trade of the Bialystok city. The total colour, acid, and peroxide values were determined in accordance with the Polish Standards PN-ISO 3960: 1996, PN-A-86934: 1995 and PN-ISO 3960: 1996, respectively.

Results: Statistically significant differences of total colour values between both groups were found (p=0.002). The acid value of refined oils was lower than in an unrefined group (p=0.02). A positive statistically significant correlation was noticed between the total colour value and the acid value in the refined group (R=0.65, p=0.04). No significant effect of plant dyes on the acid or peroxide value of unrefined oils was observed.

Conclusions: Refined and unrefined oils purchased in the city of Bialystok mostly met the standard values with the exception of cold-pressed oil from black cumin seeds, where the acid and peroxide value exceeded the values set in *Codex Alimentarius.*

Keywords: Vegetable oils, total colour, acid number, peroxide number

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INTRODUCTION

A large number of research shows that biologically active compounds contained in vegetable oils, such as fatty acids, dyes, sterols, fat-soluble vitamins and essential oils, affect the proper functioning of the human body [1-4]. Saturated and monounsaturated fatty acids and cholesterol under physiological conditions can be synthesized from *de novo* precursors; however, polyunsaturated fatty acids should be systematically delivered to the body, along with the diet, due to human inability to introduce double bonds in the n-3 and n-6 positions to the carbon chain during metabolic processes [5]. Over 100 plants species have been qualified for oilseeds, unfortunately only a small number of them are used in the production of oil on an industrial scale. Edible oils are mainly pressed from the seeds of palm, sunflower, cotton, rape, soy, olive pulp and nuts. Chlorophylls, commonly referred to green dyes, are found in plant plastids and together with proteins and lipids forms complexes. 4 pyrrole rings are the main part of the complex. They are connected by covalent and coordination bonds with a centrally located magnesium atom. This system is substituted with methyl, ethyl, ethylene groups and acetic and propionic acid residues. The protein-lipid complexes of plastids also include carotenoids - dyes of aliphatic or acyclic structure, usually consisting of eight isoprene residues, with numerous double bonds. Both chlorophylls and carotenoids perform important physiological functions - chlorophylls participate in photosynthesis, exhibit pro-oxidative properties, while carotenoids protect plants against oxidation processes. A characteristic feature of green and orange dyes are their hydrophilic properties, hence their source may be vegetable oils [6]. In addition to the mentioned substances, antioxidant activity is also exhibited by tocopherols, sterols and phenolic compounds [7].

Fats, as one of the main components of the daily food ration, have a significant impact on the correct body functioning and development (the construction of biological membranes, hormones, vitamin carriers and energy source in metabolic processes). Therefore, selected and consumed products should be of good quality. Unfortunately, oxidation processes especially in unrefined vegetable oils are already initiated during production [8]. The durability and properties of oil are determined by their fatty acid profile [9,10].

During auto-oxidation, in addition to peroxides, hydroperoxides - aldehydes, acids and ketones may form. These compounds show very harmful biological activity: they damage intracellular structures and cytoplasmic membranes and possess cytotoxic, atherogenic and enzymatic inhibition activity. Most secondary oxidation products are mutagenic. Malonic dialdehyde creates covalent bonds with DNA (deoxyribonucleic acid), phospholipids, proteins, which affects their mutation [8]. According to the Commission Regulation (EC) No 1881/2006, the maximum permissible oil concentration of benzo(a)pyrene is 2 μg/kg, whereas the sum of dioxins and polychlorinated biphenyls is 1.5 μg/g [3].

The protection of oil against unfavourable decomposition processes is of great importance. The ranges of auto-oxidation and hydrolysis reactions are influenced by raw material's access to water, light and oxygen, storage temperature and type of packaging. A bottle of dark glass or a varnished, zinc-coated metal can were shown to reduce rancidity processes. Unfortunately, most of the packages are plastic bottles that do not constitute an appropriate external barrier [8].

Due to the numerous health and sensory values, unrefined oils like pumpkin seeds oil, hemp, black cumin seeds or milk thistle, are gaining on popularity. On the other hand, the biological properties and oxidative stability of these products are not fully known. In the available literature there are no studies that would describe the relationship between the content of plant dyes and their impact on auto-oxidative processes. Therefore, the aim of this study was to determine the content of carotenoid and chlorophyll dyes and to define their effect on the value of the acid number, peroxide value and quality assessment of selected oils.

MATERIALS AND METHODS

The material for the study consisted of 10 vegetable oils samples not subjected to the process of extraction and purification (unrefined) and 10 refined, various Polish and foreign producers.

The following unrefined oils were analysed: evening primrose oil (*Oenothera biennis L.*), linseeds oil (*Oleum Lini*), sunflower seeds oil (*Helianthus annuus*), rapeseeds oil (*Brassica napus L.*), milk thistle seeds (*Silybum marianum (L.) Gaertner*), hemp seeds (*Cannabis sativa L.*), black cumin seeds (*Nigella sativa L.*), coconut (*Cocos nucifera L.*), pumpkin seeds oil (*Cucurbita pepo*), sesame oil (*Sesamum Indicum*). The refined group included: sunflower (*Helianthus L.*), maize (*Zea mays*), rapeseeds oil (*Brassica napus L.*), olive pomace oil (*Olea europaea L.*), roasted sesame seeds and soya beans (*Sesamum indicum L., Glycine max L.*), rice oil (*Oryza L.*), grape seeds (*Vitis L.*), arachid peanuts (*Arachis hypogaea L.*), coconut (*Cocos nucifera*) and soya beans oil (*Glycine max*).

All products were purchased in the city of Bialystok. The tested samples had a valid expiration date. The samples were carried out in triplicate immediately after opening the packages.

Total color

The spectrophotometric method involves the measurement of light absorbance (Abs, A). To determine the carotenoid dyes, the wavelength λ=442 nm, whereas for the chlorophyll dye λ=668 nm was used. The obtained Abs values where then added up and presented as the total oil colour in the form of an integer without the unit [B = 1000 x (A442 + A668)]. The total colour was determined in accordance with Polish Standard PN-A-86934: 1995 [11].

Peroxide number (Lea number)

The Lea number is a measure of peroxide content and is defined as the ml number of 0.001 mol/L disodium trioxide solution used to titrate iodine separated from potassium iodide as a result of peroxides contained in 1 g of oil. The content of peroxides was determined in accordance with the International Organization for Standardization PN-ISO 3960: 1996 [12].

The acid number and acidity

The acidity value is the ml number of 1 mol sodium hydroxide needed to neutralize free fatty acids contained in 100g of oil. The acid number determines the amount of free fatty acids, expressed in mg of potassium hydroxide needed to neutralize free fatty acids in 1 g of oil. The value of the acid number and acidity were determined in accordance with the PN-ISO 660: 1998 [13].

Statistical analysis

The obtained results were subjected to statistical analysis using the computer program Statistica 12.0., Poland. The minimum and maximum values, upper and lower quartiles as well as median values were calculated. In addition, correlation tests were carried out using the Spearman rank factor. The quantitative variables between the groups, the non-parametric U Mann-Whitney test were analysed. The $p<0.05$ was considered to be statistically significant.

RESULTS

Among unrefined oils, the highest value of the total colour was obtained from hemp seeds oil (4464), in which chlorophyll dyes predominated (85.3%). The high value of this parameter was also characteristic of pumpkin seeds oil (4049), where carotenoids accounted for as much as 89.9%. The lowest values of the total colour were shown for sesame oil, sunflower seed oil and linseeds oil (55.5, 118.0, 232.5, respectively). In the studied group of unrefined oils, the share of carotenoid and chlorophyll dyes was variable (Table 1A).

Table 1A. The carotenoids, chlorophylls content and the total colour of unrefined oils

unrefined oil	carotenoids (%)	chlorophylls (%)	total colour
evening primrose	438 (14.0)	2682 (86.0)	3120
black cumin seeds	330 (37.8)	543 (62.2)	873
pumpkin seeds	3639 (89.9)	410 (10.1)	4049
hemp seeds	657 (14.7)	3807 (85.3)	4464
coconut	211.5 (38.1)	343 (61.9)	554.5
linseeds	180.5 (77.6)	52 (22.4)	232.5
sunflower seeds	37 (31.4)	81 (68.6)	118
sesame	30.5 (55.0)	25 (45.0)	55.5
milk thistle seeds	119 (35.6)	215 (64.4)	334
rapeseeds	217 (73.1)	80 (26.9)	297

Table 1B. The carotenoids, chlorophylls content and the total colour of refined oils

refined oil	carotenoids (%)	chlorophylls (%)	total colour
coconut	4 (16.7)	20 (83.3)	24
olive pulp pomace	40 (53.0)	35.5 (47.0)	75.5
arachid peanuts	2 (10.0)	18 (90.0)	20
sunflower seeds	21.5 (42.2)	29.5 (57.8)	51
maize	8 (34.0)	15.5 (66.0)	23.5
rice	29.5 (74.7)	10 (25.3)	39.5
roasted sesame seeds and soya beans	378.5 (92.7)	30 (7.3)	408.5
grape seeds	47 (66.2)	24 (33.8)	71
soya beans	50.5 (46.3)	58.5 (53.7)	109
rapeseeds	7 (58.3)	5 (41.7)	12

Refined oils were characterized by significantly lower total colour values. The most intense colour was obtained from roasted sesame seeds and soya beans, followed by: olive pomace oil <grape seeds<sunflower seeds<rice< coconut< maize<arachid peanuts and rapeseeds (Table 1B). Statistically significant differences of total colour values between both groups were found (p=0.002). The total colour of unrefined oils was 9 times greater than the refined ones, Me=444.2, Q1=232.5, Q3=3120 vs. Me=45.2, Q1=23.5, Q3=75.5, respectively (Table 2).

The median of carotenoid pigments in unrefined oils was 214.2. The richest source of carotenoids was pumpkin seeds oil (3639), and the poorest one - sesame oil (30.5). There were statistically significant differences in the carotenoid content between the analysed groups (p=0.006). Among the unrefined oils, these values were 8 times higher, Me=214.2, Q1=119, Q3=438 vs. Me=25.5, Q1=7, Q3=47, respectively (Table 2).

Table 2. The minimum, maximum, median, Q1 and Q3 values of carotenoid, chlorophyll and the total colour in the studied oils

unrefined vs. refined oils	n*	range		median	lower quartile (Q_1)	upper quartile (Q_3)
		min	max			
carotenoids	10	30.5 vs. 2	3639 vs. 378.5	214.2 vs. 25.5	119 vs. 7	438 vs. 47
chlorophylls	10	25 vs. 5	3807 vs. 58.5	279 vs. 22	80 vs. 15.5	543 vs. 30
total colour	10	55.5 vs. 12	4464 vs. 408.5	444.2 vs. 45.2	232.5 vs. 23.5	3120 vs. 75.5

n-number of samples in each group

Analysis of chlorophyll content also showed statistically significant differences between groups - unrefined oils had more than 12.5 times higher chlorophyll content (p <0.001). The lowest value was obtained by rapeseed oil (5) (Table 1B). The highest acid number (AN) for unrefined oils, was characterized by black cumin seeds oil (7.416 mg KOH/g), which was more than 7 times higher than the result of coconut oil (0.112 mg KOH/g). Cannabis seeds oil (3.202 mg KOH/ g) and milk thistle seeds oil (2.921 mg KOH/g) had also high acid values (Table 3A, 3B).

Table 3A. The Lea number, acidity, acid number and acidity percentage value of unrefined oils

unrefined oil	Lea number [meq O_2/kg]	acidity value	acid number [mg KOH/g]	acid value [%]
evening primrose	1.904	1.400	0.787	0.395
black cumin seeds	59.734	13.200	7.416	3.726
pumpkin seeds	2.010	2.400	1.348	0.678
hemp seeds	5.764	5.700	3.202	1.609
coconut	0.049	0.200	0.112	0.056
linseeds	0.427	0.600	0.337	0.169
sunflower seeds	2.964	1.200	0.674	0.339
sesame	0.145	2.700	1.517	0.762
milk thistle seeds	0.907	5.200	2.921	1.468
rapeseed	1.355	1.500	0.843	0.423

Table 3B. The Lea number, acidity, acid number and acidity percentage value of refined oils

refined oil	Lea number [meq O_2/kg]	acidity value	acid number [mg KOH/g]	acid value [%]
coconut	0.000	0.400	0.225	0.113
olive pulp pomace	3.277	0.600	0.337	0.169
arachid peanuts	5.279	0.200	0.112	0.056
sunflower	3.768	0.400	0.225	0.113
maize	2.527	0.400	0.225	0.113
rice	2.286	2.500	1.404	0.706
roasted sesame seeds and soya bean	2.240	1.600	0.899	0.452
grape	3.179	0.400	0.225	0.113
soya beans	4.356	0.400	0.225	0.113
rapeseed	2.146	0.300	0.169	0.085

In the refined oil group the highest AN values were found for rice oil (1.404 mg KOH/g) and roasted sesame seeds and soya beans oil (0.899 mg KOH/g). The lowest result was found in arachid peanuts oil (0.112 mg KOH/g). Coconut, sunflower, maize, grape seeds and soya beans oils had an equal AN values - 0.225 mg KOH/g. There were statistically significant differences in the AN values between the studied groups (p=0.02). In the group of unrefined oils, the values were 4.5 times higher, Me=1.096, Q1=0.674, Q3=2.921 vs. Me=0.225, Q1=0.225, Q3=0.337, respectively (Table 4).

Approximately half of the unrefined oils obtained the value of the Lea number ≥1.630 meq O2/kg. What's more almost, 75% of unrefined oils did not exceed the value above 2.964 meq O2/kg, only black cumin seeds oil, hemp seeds and sunflower seeds oils obtained higher values. The Lea number in the majority of refined oils reached the range of 0.427- 2.964 meq O2/kg. A strong (R=0.65, p=0.04) positive correlation, between the total colour and the acid number in the refined group was found. No such relationship for unrefined oils was observed (p=0.35, R=0.34). No statistically significant differences were noticed in the Lea number between groups (p=0.19). In unrefined oils, black cumin seeds oil score of 59.734 and strongly differed from the upper quartile designated for this group (Table 4).

Table 4. The minimum, maximum, median, Q1 and Q3 values of the Lea number, acidity, acid number and acidity value of studied oils

unrefined oils vs. refined oils	**n***	**range**		**median**	**lower quartile (Q_1)**	**upper quartile (Q_3)**
		min	**max**			
Lea number [meq O_2/kg]	10	0.049 vs. 0.000	59.734 vs. 5.279	1.630 vs. 2.853	0.427 vs 2.240	2.964 vs. 3.768
acidity number	10	0.200 vs. 0.200	13.20 vs. 2.500	1.950 vs. 0.400	1.200 vs 0.400	5.200 vs. 0.600
acid number [mg KOH/g]	10	0.112 vs. 0.112	7.416 vs. 1.404	1.096 vs. 0.225	0.674 vs 0.225	2.921 vs. 0.337
acidity value [%]	10	0.057 vs. 0.056	3.726 vs. 0.706	0.551 vs. 0.113	0.339 vs 0.113	1.468 vs. 0.169

n-number of samples in each group

DISCUSSION

The content of chlorophyll and carotenoid pigments in vegetable oils is determined via maturity of the raw material, botanical variety and production technology used. Interestingly, plant dyes have both antioxidant and pro-oxidative activity. The presence of chlorophyll dyes in unrefined oils, affects the initiation of photochemical processes. Chlorophylls facilitate the conversion of oxygen into a singlet form that causes oxidation of unsaturated fatty acids, whereas carotenoids occur to be natural preservatives [14].

Wroniak et al. [14], noticed that in unrefined oils, the ratio of carotenoids and chlorophylls is variable. In our study, a similar trend was observed, but the share of chlorophyll dyes was slightly higher. In our study, among all analysed products, pumpkin seeds oil (4049), was the second, next to the hemp seeds oil (4464), which had the highest total colour value. Kruszewski et al. [15] analysed the AN in unrefined coconut and linseeds oils and values were as follows: 0.56 and 1.42 mg KOH/g, in our own research, the AN values were lower: 0.112 and 0.337 mg KOH/g. A similar relationship was observed in the Lea number 1.22 meq O2/kg 2.67 meq O2/kg vs. 0.049 and 0.427 meq O2/kg, respectively.

The majority of refined oils met the requirements of *Codex Alimentarius* (Lea number 10≥meq O2/kg, acid number 0.6≥mg KOH/g). Arachid peanuts oil slightly exceeded the permissible Lea number of the Polish Standard (5.279 meq O2/kg vs. 5 meq O2/kg) [11], [12] the acid numbers of olive oil 0.6 mg KOH/g, rice oil 1.404 mg KOH/g and oil from roasted sesame and soya beans (0.899 mg KOH/g) also exceeded *Codex Alimentarius* and the Polish Standard (0.3 mg KOH/g). Among the refined oils, the lowest AN values were found in arachid peanuts oil and rapeseeds oil. It should be noted that low AN values of rapeseed oil were associated with a high % share of monounsaturated fatty acids that are oxidatively stable [1-4,16].

In the Zychnowska et al. study [17], the research material was refined and unrefined rapeseeds oils. As in our own study, over 4 times

higher total colour values were found in unrefined oils. In addition, it was noticed that rapeseeds oils are predominantly rich source of carotenoid pigments, which is consistent with our own observations. The values of the acid number of unrefined rapeseed oils varied between 1.35-5.17 mg KOH/g, while refined oils ranged from 0.22-0.34 mg KOH/g [17]. In the own study, the AN values of rapeseeds oil - refined and unrefined were within the defined limits. It was noted that AN is significantly lower in the case of refined oils. A similar tendency was observed for the Lea number 1.355 meq O2/kg vs 2.146 meq O2/kg. The Lea number did not exceed the Polish standard (10 meq O2/kg) and *Codex Alimentarius* (15 meq O2/kg) [11,18].

In the study of Derewiak et al. [19], unrefined linseeds oils obtained the AN in the range of 0.5-2.85 mg KOH/g. All oils met the quality requirements described in *Codex Alimentarius* [18]. In addition, the Lea values were in the range of 0.98-2.92 meq O2/kg. All studied unrefined oils did not exceed both standards. The Lea values of cold pressed linseed oil obtained by other researchers, including Cichosz et al. and Tańska et al. was 1.8, 1.24 meq O2/kg, respectively [8,20]. In the own study, the values of linseed oil parameters were lower than the values set by Derewiak et al. [19]. In the Babatunde et al. [21] study, the AN of rafined arachid oils peeled between 0.39-0.86 mg KOH/g, while the Lea value was in the range of 1.87-3.20 O2 meq/kg. Unfortunately, no information was given regarding the methods of oils production. In our own study the arachid refined oil had the AN of 0.112 mg KOH/g and was slightly lower than range determined by Babatunde et al. [21]. The peroxide value was higher and amounted to 5.279 O2 meq/kg. For unrefined evening primrose oil Skolimowska et al. [22], obtained the following parameters: AN-2.6 mg KOH/g, and Lea value-2.2 O2 meq/kg, both within the norm. In our own research similar low values were observed 0.787 mg KOH/g and 1.904 O2 meq/kg.

Preschy et al. [23], analysed unrefined sesame oils, where the AN was within the range of 0.02 - 0.20 mg KOH/g and the Lea number of 0.70-2.20 O2 meq/kg. The value shown in own study was much higher in case of acid number (1.517 mg KOH/g), but lower in relation to Lea number (0.145 meq O2/kg). Unrefined sunflower oils tested by the same team reached the value of the acid number in the range of 0.14-0.23 mg KOH/g, while the peroxide value was 2.26-6.27 meq O2/kg. The AN of sunflower oil in the own study was higher than the upper value of the range marked in the compared study (0.674 mg KOH/g). The peroxide value of sunflower oil in own research was within the range specified by the cited authors (2.964 meq O2/kg). In the comparison study, unrefined oils from pumpkin seeds obtained AN in the range of 0.14-0.46 mg KOH/g and 2.10-9.43 meq O2/kg per Lea number. The values of the acid and peroxide number set by our team were high: 1.348 mg KOH/g and 2.010 meq O2/kg. Interestingly, in hemp seeds oil, both the AN and the Lea value were higher in our own study (3.202 mg KOH/g, 5.764 meq O2/kg) than in the Perschy et al. (0.10-0.39 mg KOH/g, 2.63-4.55 meq O2/kg). The milk thistle seeds oil was characterized by the acid number in the range from 0.44 to 1.06 mg KOH/g, and the peroxide value from 1.04 to 5.43 meq O2/kg. In own study the AN of the milk thistle seeds oil was 2.921 mg KOH/g and the peroxide value - 0.907 meq O2/kg.

Stec et al. [24] evaluated the quality of refined grape seeds oils. The AN and the Lea number were 0.137 mg KOH/g and 1.958 meq O2/kg, respectively. In the own study the AN was slightly lower (0.225 mg KOH/g), while the peroxide value was significantly higher (3.179 meq O2/kg). The parameters tested in both works did not exceed the established standards.

Among all tested oils the coconut one was characterized by the lowest values of the peroxide number. Coconut oil had high oxidative stability due to saturated fatty acids (> 91%). Tested by Prasanth Kumar et al. [25], refined coconut oils as in the own study were characterized by zero peroxide number. Whereas the peroxide value of unrefined coconut oil in the Prasanth Kumar et al. study ranged from 0.0 to 2.7 meq O2/kg, the values obtained were within the normal range. In this study, the value of peroxide number of crude oil (0.049 meqO2/kg) fit in the norm.

In another study, Stec et al. [26] determined the average acid and Lea value of refined soya beans oils to be 0.212 mg KOH/g and 2.520 meq O2/kg - both values did not exceed the norm. Refined rice oils had an average acid value of 0.167 mg KOH/g, and peroxide value - 1.967 meq O2/ kg. The value of AN of soya beans oils in the own study was similar (0.225 mg KOH/g), while the peroxide value was more than twice as high (4.356 meq O2/kg). In case of rice oil, the AN value as well as the peroxide value exceeded the norm (1.404 mg KOH/g, 2.286 meq O2/kg). It was noted that rice oil had lower peroxide values compared to soya beans oil. In the Kaleem A et al. [27] study, refined pomace oil (3.2 meq O2/kg) obtained almost the same value as in the own study (3.277 meq O2/kg).

In the scientific community, interest in black cumin seeds oil is constantly growing. It seems that its composition contains a number of substances with health-promoting effects, including anticancer as well as antibacterial properties. The composition of fatty acids in the seeds of black cumin is as follows: polyunsaturated fatty acids - 50-60% linoleic acid, monounsaturated fatty acids - 20% oleic acid, 3% eicosanamide, saturated fatty

acids - 30% palmitic and stearic acids. It has been shown that the method of obtaining oils from seeds affects the percentage of free fatty acids (FAA). It was shown that cold oil pressing, causes 0.9% occurrence of FAA, while during extraction using organic solvents, this value increases up to 2.3%. In a study conducted by Bourgou et al. [28] and Ali et al. [29] the Lea number for black cumin seeds oil may exceed 10, suggesting high oxidative sensitivity of this oil. Solvent extracted oil from Morocco was characterized by a value of 11.4 meq O2/kg, while obtained by cold stamping obtained the value 3.4 meq O2/kg. In this work, cold-pressed oil had the highest values of the acid and peroxide number (7.416 mg KOH/g, 59.734 meq O2/kg), exceeding the values set in standards, which may indicate poor grain quality or inadequate storage methods.

CONCLUSION

Refined and the unrefined oils purchased in the city of Bialystok largely corresponded to the established norms, with the exception of cold-pressed oil from black cumin seeds, where the acid and peroxide values exceeded the permitted values. Significant differences of total colour values between both groups were found (p=0.002). The acid value of refined oils was lower than in unrefined group (p=0.02). It was observed a positive statistically significant correlation between the total colour value and the acid value in the refined group (R=0.65, p=0.04). There was no correlation between the total color and the peroxide value in the group of unrefined oils, in which the share of carotenoid and chlorophyll dyes was variable. No significant effect of plant dyes on the acid value or peroxide of unrefined oils was noticed.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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