Fatty Acid and Triacylglycerol Compositions of Seed Oils of Five *Amaranthus* Accessions and Their Comparison to Other Oils

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ABSTRACT: This paper reports the fatty acid and triacylglycerol (TAG) compositions of five *Amaranthus* accessions (RRC1011, R149, *A*.K343, *A*.K432, and *A*. K433) representing two species and a cross between one of these and a third species. Seed oils of these were analyzed by gas chromatography and reversed-phase high-performance liquid chromatography, and their compositional properties compared with buckwheat (*Fagopyrum esculentum*), corn (*Zea mays*), rice bran (*Oryza sativa*), soybean (*Glycine max* L. *M*err.), sesame (*Sesamum indicum*), quinoa (*Chenopodium quinoa*), and cottonseed (*Gossypium hirsutum*) oils. All *Amaranthus* accessions were relatively high in palmitic (21.4–23.8%) and low in oleic (22.8–31.5%) and linolenic (0.65–0.93%) acids when compared to most of the grain and seed oils. The fatty acid composition of *Amaranthus* accessions K343, K433, and K432 (group I) were different from R149 and RRC1011 (group II) in mono and polyunsaturated fatty acids, but the saturate/unsaturate (S/U) ratios were very similar. All *Amaranthus* accessions were similar in TAG type, but showed slight differences in percentage. High similarities in UUU, UUS, and USS composition were observed among *Amaranthus* K343, K433 and K432, and between R149 and RRC1011. The fatty acid compositions of *Amaranthus* oil (group I) and cottonseed oil were similar, but their TAG compositions were different. The grain and oilseed oils were different from each other and from the *Amaranthus* accessions oils in terms of fatty acid composition, S/U, and TAG ratios. The UUU, UUS, and USS percentages were very diverse in grain and seed oils. The percentages of squalene in the TAG sample from the *Amaranthus* accessions were 8.05% in K343, 11.10% in K433, 11.19% in K432, 9.96% in R149, and 9.16% in RRC1011. Squalene was also tentatively identified in quinoa and ricebran oils at levels of 3.39 and 3.10%, respectively.

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KEY WORDS: *Amaranthus* accessions, *Amaranthus* seed oils, buckwheat oil, cottonseed oil, fatty acid composition, grain oils, quinoa oil, sesame oil, soybean oil, squalene, triacylglycerol composition.

Amaranthus (a dicotyledonous pseudocereal) is one of the New World's oldest crops, having originated in Meso-America around 400 A.D. and then moving northward to the southwestern United States (1), where it was grown by the Arizona cliff-dwellers as early as 1350 A.D. (2). Presently, it is grown in many areas of the world, including Central and South America, Africa, India, China, and the United States (3). Popularity in the cultivation and consumption of *Amaranthus* seed in the modern era began in the mid-1970s with the rediscovery and promotion of amaranth due to its superior nutritional attributes as compared to cereal grains (1). In general, the protein content of *Amaranthus* seed has been shown to be substantially higher than that found in other cereal grains (4,5). Further, it has been well documented that amaranth protein is composed of relatively high levels of essential amino acids such as lysine and the sulfur-containing amino acids, methionine and cysteine, which are limited in common grains (1).

More recently, research activities have focused on examining and characterizing the lipid component of *Amaranthus* seed (3,6). Although the lipid content of *Amaranthus* seed is typically 6–9%, some species such as *A. spinosus* and *A. tenuifolius* have been reported to contain as much as 19.3% (3). Although *Amaranthus* seed is not considered a typical oilseed crop, it has been identified as one of the new crops that can be considered a rich source of squalene (2.4–8%) (2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene) (1,3,6). Squalene is an expensive terpenoid compound, derived primarily from shark *(Cantrophorus squamosus*) and whale liver (*Physeter macrocephalus*) oils. It is an important ingredient in cosmetics, skin penetrants, pharmaceuticals, and lubricants for computer disks (3,7). Owing to the fact that all squalene used in the United States is imported (200–300 metric tons per year) and to the concern for marine animal protection, attention has been focused on identifying crop sources of squalene (6). Therefore, amaranth may, potentially serve as a rich vegetable source of squalene as well as provide edible oil and protein for various specialized purposes.

Although some work has been performed to determine the fatty acid composition of the oils of various *Amaranthus* species (3), and their similarities to corn (*Zea mays*), buckwheat (*Fagopyrum esculentum*), and cottonseed (*Gossypium hirsutum*), no other work has been performed to confirm and to elucidate the extent of these observed similarities. The objective of this study was to examine and compare the fatty acid and triacylglycerol (TAG) profiles of various *Amaranthus* accessions with other seed oils.

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MATERIALS AND METHODS

Materials. Non-heat-treated amaranth seeds were obtained from the Agriculture Canada Delhi Research Station (Delhi, Ontario, Canada) and the Agricultural Research Service North Central Regional Plant Introduction Station of the U.S. Department of Agriculture (Ames, IA). The accessions included *A. cruentus* (RRC1011) (PI477913), *A. hypochondriacus* (R149), *A. hypochondriacus* × *A. hybridus* (K343), *A. hypochondriacus* × *A. hybridus*, (K432), and *A. hypochondriacus* × *A. hybridus* (K433). Soybean (*Glycine max* L. Merr.) cultivar corsoy 79 was obtained from the Harrow Research Station (Agriculture Canada, Harrow, Ontario, Canada). Buckwheat (*F. esculentum*), corn (*Z. mays*), sesame seed (*Sesamum indicum*), ricebran (*Oryza sativa*) and quinoa (*Chenopodium quinoa*) were obtained from the University of Guelph, Department of Crop Science; whereas, cottonseed (*G. hirsutum*) was obtained from Yazoo Valley Oil Mill Inc. (Greenwood, MS).

Methods. (i) Fat Extraction. The seeds were ground in a Micromill coffee grinder model 103 and passed through a 60-mesh screen for crude oil extraction by the Soxhlet procedure for 5 h. The petroleum ether was removed by rotary evaporation in a water bath set at 37°C.

(ii) Fatty acid analysis. The American Oil Chemists' Society (AOCS) Official Method (Ce 2-66) (8) with minor changes was used to prepare the fatty acid methyl esters (FAME). The 50-mL reaction flask was replaced with 15-mL screw-capped test tubes for the methylation step. A Shimadzu gas chromatograph, model GC-14 (Mandel Scientific Co. Inc., Guelph, Ontario, Canada), equipped with a split mode injection system, flame-ionization detector, and a 30 m × 0.25 mm i.d., 0.25 mm film thickness, SP2330 fused-silica capillary column (Supelco, Oakville, Ontario, Canada) was used for FAME analysis. The gas chromatographic conditions were: initial oven temperature (145°C), initial time (2.0 min), heating rate (6°C/min), final temperature (235°C), final hold time (10 min), injection port temperature (260°C), detector port temperature (260°C), hydrogen gas flow (30 mL/min), air flow rate (300 mL/min), and hydrogen gas carrier flow rate (1.0 mL/min). The injection volume was 0.1 mL, and the data were integrated with a Shimadzu model CR4A Chromatopac (Mandel Scientific Co. Inc.). The FAME were identified by comparing retention times to pure standards purchased from Sigma-Aldrich Co. (Oakville, Ontario, Canada).

Reversed-phase high-performance liquid chromatography (RP-HPLC) (iii). The AOCS Official Method (Ce 5b-89) (8) with slight modifications to the mobile phase was used to determine the TAG composition. The separation was performed on two Econosil C-18 columns (5 mm, 4.6 × 250 mm, Alltech, Deerfield, IL) in series. The analysis was carried out isocratically with a mobile phase consisting of 60:40 (vol/vol) acetone/acetonitrile.

Fat samples (5%) were dissolved in HPLC-grade acetone and 20 µL aliquots were automatically injected onto the column (Waters 700 Satellite WISP; Millipore, Milford, MA) and eluted at a flow rate of 1 mL/min. The column was equilibrated at 30°C and the effluent monitored with a Waters 410 RI detector (30°C and sensitivity 64). The TAG were identified by comparing retention times to pure standards purchased from Sigma-Aldrich Co.

Squalene was eluted from the C-18 column under the same conditions used for the TAG and was identified by comparing retention to pure squalene purchased from Sigma-Aldrich Co.

RESULTS AND DISCUSSION

Fatty acid composition. The fatty acid compositions of amaranth oil from five *Amaranthus* accessions were similar (Table 1). The major fatty acids were linoleic acid (39.4–49.1%), oleic acid (22.8–31.5%), and palmitic acid (21.4–23.8%). These oils were high in total unsaturates (71.4–73.2%). It appears that the genetic variation in five accessions of two *Amaranthus* species and a cross between one

TABLE 1
Fatty Acid Composition of Five *Amaranthus* Accessions[a] (RRC1011, R149 and *A.*K343, *A.*K432, *A.* K433).

	Fatty acid composition (%)				
	Group I[b]			Group II[b]	
Fatty acid	*A. hypochondriacus* K343	*A. hypochondriacus* K433	*A. hypochondriacus* K432	*A. hypochondriacus* R149	*A. cruentus* RRC1011
14:0	0.29	0.28	0.21	0.26	0.27
16:0	23.8	23.8	21.4	23.4	22.2
16:1	0.11	0.19	0.10	0.15	0.11
18:0	3.11	3.65	3.98	3.68	3.57
18:1	23.0	23.7	22.8	31.5	30.1
18:2	47.9	46.7	49.1	39.4	42.2
18:3	0.88	0.83	0.93	0.65	0.69
20:0	0.56	0.62	0.89	0.60	0.68
20:1	0.18	—	0.23	0.24	—
22:0	0.14	0.22	0.32	0.19	0.24
S/U[c]	0.39	0.4	0.37	0.39	0.37

[a]Representing two species and a cross between one of these and a third species.
[b]Groups are based on fatty acid compositions.
[c]S/U, saturate/unsaturate ratio.

TABLE 2
Fatty Acid Composition of Grain, Oilseed and Bran Oils

Fatty acid	Fatty acid composition (%)						
	Buckwheat	Corn	Ricebran	Soybean	Sesame	Quinoa	Cottonseed
14:0	0.24	—	0.51	0.12	0.02	0.32	1.00
16:0	19.5	11.6	18.6	12.7	9.51	11.4	25.7
16:1	0.25	0.14	0.23	0.11	0.11	0.07	0.53
18:0	2.18	1.91	1.75	3.96	5.41	0.79	2.45
18:1	37.1	27.8	42.4	21.7	40.0	25.6	17.7
18:2	35.5	56.5	34.8	53.9	43.5	52.8	52.1
18:3	1.93	1.65	1.10	7.23	0.33	7.00	0.22
20:0	1.49	0.40	0.49	0.28	0.60	0.29	0.25
20:1	—	0.05	—	0.04	0.18	1.00	0.05
22:0	1.31	0.05	—	0.05	0.14	0.24	0.05
22:1	—	0.05	—	—	—	0.52	0.05
24:0	0.51	0.05	—	—	0.09	—	—
S/U[a]	0.34	0.16	0.27	0.2	0.19	0.15	0.41

[a]S/U, saturate/unsaturate ratio.

of these and a third species did not dramatically alter their fatty acid composition. Only a small difference was noticed between amaranth R149 and amaranth RRC1011. These two accessions had higher oleic acid and lower linoleic acid levels compared to K343, K433, and K432.

The oils from the grain and oilseed samples were extracted with petroleum ether and the fatty acid compositions determined (Table 2). The fatty acid compositions were all within the range of published results (9,10). The major fatty acids were linoleic acid (34.8–56.5%), oleic acid (17.7–42.4%), and palmitic acid (9.51–25.7%). The total unsaturated fatty acid content ranged from 70.6–86.9% and the total saturated fatty acid content ranged from 13.1–29.4%. As expected, there were more variations in the composition in these oil samples than between the *Amaranthus* species. A comparison of the average fatty acid composition of the amaranth oil with individual grain and seed oils identified cottonseed and buckwheat oils as having similar profiles. Lyon and Becker (11) reported similar fatty acid compositional data for amaranth, cottonseed, and corn oils. The other four seed oils in this study differed from amaranth only in one or two fatty acids. Soybean and quinoa contained significant amounts of linolenic acid (7–7.23%) compared to amaranth (0.65–0.93%). Sesame and ricebran oil had higher levels of oleic acid (40–42.4%) compared to amaranth (22.8–31.5%), and three of these oils (quinoa, sesame, and corn) had lower palmitic acid (9.5-11.6%) levels compared to amaranth (21.4–23.8%).

Comparison of S/U (where S is saturated and U is unsaturated) fatty acid ratios showed cottonseed and buckwheat oil with ratios very close to that of amaranth (Tables 1 and 2). Corn, soybean, sesame, and quinoa on the other hand, had higher levels of unsaturated fatty acids (>82%) compared to amaranth (<74%) resulting in much smaller S/U ratios.

TAG composition. The fatty acid composition can be used to evaluate the stability and nutritional quality of fats and oils, but not always their functional properties. What is important is the type and the amounts of the various TAG species in the oil. It is the TAG composition that ultimately determine the final physical and functional properties of the oils. Table 3 shows the TAG composition of the oil from five *Amaranthus* accessions. Overall, the TAG compositions were similar, but much greater similarities were found among species K343, K433, and K432 (group I) and between species R149 and RRC1011 (group II). The main differences between the two groups were in the TAG LLL, PLL, and OOL (where L is Linoleic, P is Palmitic, and O is Oleic). It appears that the amaranth accessions can be split into two groups by either fatty acid composition or by TAG composition. The predominant TAG in the three *Amaranthus* species were PLL (14.1–20.3%), POL (16.5–18.7%), OLL (10.4–10.9%), OOL + PoOO (6.7–10.9%), LLL (5.4–8.6%), and a large unresolved peak containing a mixture of OOO/MSO/OOP/PSL/POP (3.1–17.2%), where M is myristic, S is stearic, and Po is palmitoleic acid (Table 3, Fig. 1). Figure 1 is a typical amaranth chromatogram of K432.

As was the case with fatty acid composition, the TAG compositions of the grain, bran, and seed oils were very diverse (Table 4). The predominant TAG were PLL (8.54–28.1%), OLL (9.42–20.4%), OOL (3.1–16.3%), LLL (2–20.5%), POL (9.5–15.6%), and a broad unresolved peak containing mixtures of OOO + MSO (1.99–11.3), OOP + PSL (1.7–10.94), and POP (0.02–2.31%) (Table 4 and Fig. 2). Figure 2 is a typical oilseed oil chromatogram (quinoa oil).

Differences in TAG compositions were much greater between the grain and seed oils than between the *Amaranthus* accessions (Tables 3, 4). Although cottonseed oil shows similarity in fatty acid composition with amaranth, the TAG compositions (i.e., LLL, PLL, OOL, PPL) were different in amaranth and cottonseed oil. The LLL levels in corn, soybean, and quinoa oils were higher (19.83, 20.5, and 19.22%, respectively) compared to the *Amaranthus* species (5.44–8.03%). The PPL, OLL, POL, OOO, and OOP levels also showed dramatic differences when comparing corn, soybean, and quinoa oils to *Amaranthus* oil. The OOL and PLL in ricebran and sesame oil were also different from those in *Amaranthus* accessions (Table 3 and 4).

TABLE 3
TAG Composition of Five *Amaranthus* Accessions[a] (RRC1011, R149 and *A.* K343, *A.* K432, *A.* K433).

	TAG composition (%)				
	Group I[b]			Group II[b]	
TAG profile	*A. hypochondriacus* K343	*A. hypochondriacus* K433	*A. hypochondriacus* K432	*A. hypochondriacus* R149	*A. cruentus* RRC1011
Mono- and diglycerides and unknown	1.92	1.84	2.00	2.72	2.19
LnLnL	0.34	0.65	0.91	0.31	1.11
LnLL	1.06	1.68	1.85	1.27	2.40
LLL	8.03	7.87	8.66	5.44	5.94
PLnL	0.98	1.10	1.34	0.85	1.10
OLL, OOLn	11.60	10.73	10.40	11.23	10.97
PLL, PLnO	20.33	18.33	18.65	14.08	14.48
OOL, PoOO	7.96	7.16	6.72	10.94	10.95
POL	17.21	16.87	16.47	18.67	16.69
PPL	8.84	8.76	9.00	7.42	7.01
MPO	0.70	0.51	0.83	0.35	0.58
OOO, MSO				OOO, MSO: 6.13	OOO, MSO: 4.82
OOP, PSL	15.62	16.86	17.23	OOP, PSL: 13.30	OOP, PSL: 11.80
POP	—	—	—	3.13	3.27
PPO	1.05	1.07	0.86	0.56	0.64
PPP	—	0.95	—	—	—
OOS	1.01	1.23	1.70	0.73	1.27
POS	0.77	—	1.49	0.86	0.49
PSS	0.15	—	—	—	—
SOS, SSO	0.55	—	—	—	—
UUU[c]	33.6	33	34.2	34.3	35.7
UUS	50.2	48.1	46.8	46.1	43.4
USS	11.9	10.6	11.3	13.2	12.5

[a]See Table 1, footnote *a*.
[b]Groups are based on triacylglycerol (TAG) composition. S, stearic acid; P, palmitic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid; Po, palmitoleic acid; M, myristic acid.
[c]UUU (triunsaturates), UUS (diunsaturates), USS (monounsaturate). The UUU and USS compositions in multiple bands were calculated on the basis of fatty acid/TAG balance.

By recalculating the data on the basis of triunsaturates (UUU), diunsaturates (UUS), and monounsaturates (USS), general similarities and differences between the five *Amaranthus* accessions and the seven grain and oilseed oils become apparent (Tables 3 and 4). The *Amaranthus* accessions contained more diunsaturates (43.4–50.2%) than triunsaturates (33–35.7%). The majority of the grain and seed oils were the reverse, with significantly more UUU than UUS. The exceptions were buckwheat and cottonseed, which had TAG (UUU, UUS, and USS) compositions very close to those of the *Amaranthus* accessions. On the basis of tri-, di-, and monounsaturates in the TAG composition, one would expect the amaranth oils to have functional properties similar to buckwheat and cottonseed.

The very polar materials, which were soluble in acetone, appeared at retention times before 17 min. These compounds have been reported to be mono- and diglycerides, phospholipids, and terpenoids (i.e., squalene) (6,12). Squalene is an unsaturated hydrocarbon with a higher polarity than TAG except for trilinolenin. Under the RP-HPLC conditions used to separate TAG, the squalene in all *Amaranthus* accessions, ricebran, and quinoa elutes between 16 and 17 min. Based on the peak area, the percentages of squalene in the TAG samples were calculated to be 8.05% in K343, 11.10% in K433, 11.19% in K432, 9.96% in R149, 9.16% in RRC1011, 3.39% in quinoa, and 3.10% in ricebran. The amount of squalene in 11 *Amaranthus* seed oil has been reported to be approximately 4.9–8.1% (4,6).

Although previous research has shown that *Amaranthus* may potentially serve as a rich vegetable source of squalene as well as provide edible oil, this study has indicated that genetic differences do exist between various *Amaranthus* accessions, which can be exploited for various specialized purposes. The study has also shown that *Amaranthus* fatty acid and TAG profiles are substantially different from other conventional and nonconventional oil seeds; therefore, they merit further investigation.

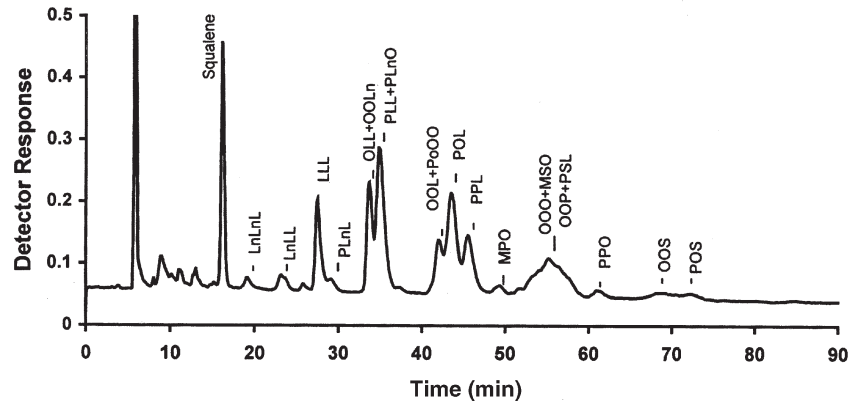

FIG. 1. Reversed-phase high-performance liquid chromatogram of *Amaranthus* accessions (K432), showing squalene and the triacylglycerol (TAG) peaks. The separation was performed on two Econosil C18 columns (Alltech, Deerfield, IL) in series, with a mobile phase consisting of 60:40 (vol/vol) acetone/acetonitrile, eluted at a flow rate of 1 mL/min, and monitored with a Waters 410 RI detector (WISP, Millipore Milford, MA) at 30°C and sensitivity 64 (S, stearic acid; P, palmitic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid; Po, palmitoleic acid; M, myristic acid).

Table 4
TAG Composition of Grain, Oilseed and Bran Oils[a]

	TAG composition in oil (%)						
TAG profile	Buckwheat	Corn	Ricebran	Soybean	Sesame	Quinoa	Cottonseed
Mono- and diglycerices and unknown	4.91	7.29	8.4	1.59	1.37	4.23	2.94
LnLnLn	—	0.91	—	0.38	—	—	0.32
LnLnL	1.13	0.47	0.26	1.58	—	1.09	—
LnLL	1.03	2.07	0.60	7.45	0.52	6.04	—
LLL	2.08	19.83	6.61	20.50	11.94	19.22	16.17
PLnL	1.15	0.70	0.50	3.98	—	2.86	1.66
OLL } OOLn }	9.42	20.45	13.91	14.95	17.89	21.09	9.95
PLL } PLnO }	11.75	12.09	9.71	14.94	8.54	11.34	28.08
OOL } PoOO }	13.83	11.37	16.21	7.37	16.29	12.78	3.08
POL	15.63	10.70	15.12	11.97	14.59	9.47	15.09
PPL	3.24	1.42	3.14	2.22	—	1.54	12.99
MPO	2.61	0.63	—	1.02	—	—	—
OOO } MSO }	9.27	3.28	11.32	4.33	8.88	1.99	5.08
OOP } PSL }	7.83	6.14	10.94	1.70	13.09	3.88	3.51
POP	0.96	2.10	2.31	—	0.02	1.97	—
PPO	3.19	—	—	—	0.24	0.61	—
PPP	—	—	—	—	—	0.34	—
OOS	2.61	—	—	2.93	4.10	0.41	1.02
POS	4.63	—	—	—	2.52	—	—
PSS	—	—	—	—	—	—	—
SOS } SSO }	—	—	—	—	—	—	—
	—	—	—	—	—	—	—
UUU	35.3	58.8	46.2	54.2	46.6	62.15	30.1
UUS	37.1	24.9	37.02	33.3	26.97	26.55	49.8
USS	17.1	3.05	7.7	3.94	6.2	5.68	16.9

[a]For abbreviations see Table 3.

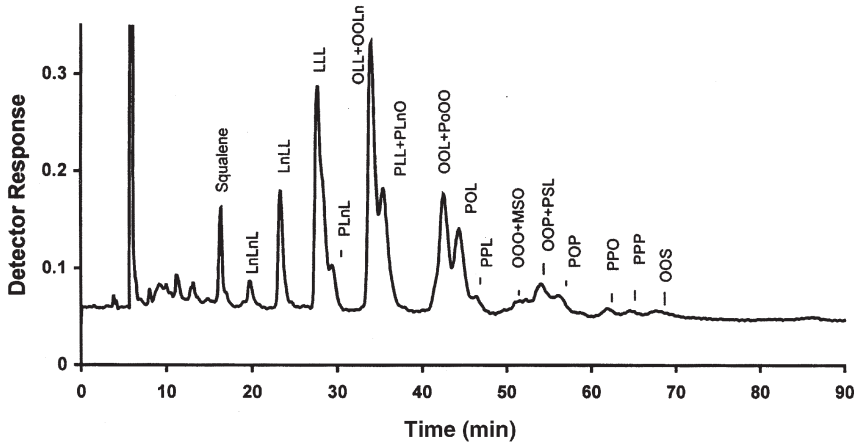

FIG. 2. RP-HPLC chromatogram of quinoa oil showing squalene and TAG peaks. Symbols and abbreviations as in Figure 1.

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