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FATTY ACID AND MOLECULAR WEIGHT DISTRIBUTIONS OF TRIACYLGLYCEROLS IN DEVELOPING WINGED BEAN SEEDS

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Key Word Index—*Psophocarpus tetragonolobus*; Leguminosae; winged bean; fatty acids; triacylglycerols; stereospecific analysis; development.

Abstract—The positional distribution of fatty acids (FA) and the molecular weight distribution of triacylglycerols (TG) in developing winged bean (*Psophocarpus tetragonolobus*) were studied. There were marked changes in the positional distribution of FA in the TG as well as in the molecular weight distribution of the TG during seed development. The levels of palmitic acid (16:0) and stearic acid (18:0) at the sn-1 position decreased whereas the levels of the same acids in the sn-3 position increased as the seed developed. Oleic (18:1), linoleic (18:2) and linolenic (18:3) acids were found in all three positions of the TG molecules but most were found in the sn-2 position. Behenic acid (22:0) was preferentially esterified to the sn-3 position even at the early stage (3 WAF) of seed development but its level in the sn-1 and sn-3 positions increased as the seed developed. The levels of 16:0 and 18:0 decreased while the level of 22:0 and the proportion of TG with larger molecular weights, that is, C_{56}, C_{58} and C_{60} also increased as the seeds developed. Our findings indicate that winged bean seed oil may be atherogenic as compared to peanut oil.

INTRODUCTION

The potential of winged bean as a source of protein and fats for human consumption in tropical countries has been widely recognised [1–5]. Winged bean seed oil is less unsaturated than soybean oil [4, 6]; but it tastes and smells like soybean oil which implies that it could probably be used as a substitute in many food uses [6]. The FA composition of winged bean seed oil is somewhat similar to that of peanut oil [4, 5], except that it contains three- to ten-fold more 22:0 than peanut oil [7–9]. In peanut oil, 22:0 acid is present at low level, 1–3% of the total FA; however, its presence has been implicated as the cause for the higher atherogenicity of peanut oil as compared to other oils in animal experiments [10–14]. Myher *et al.* [15] further demonstrated that peanut oil has a significantly higher proportion of 18:2 in the sn-2 position and arachidic (20:0), 22:0 and lignoceric (24:0) acids in the sn-3 position of its TG were more atherogenic. The high level of 22:0 in winged bean seed oil is thus causing much concern from the standpoint of nutrition and health. Furthermore, there is no information on the stereospecific distribution of FA in the winged bean TG. Hence, in this paper we report the stereospecific distribution of FA in the TG and the profiles of the TG molecules according to their M_rs or carbon number in developing winged bean seeds.

RESULTS AND DISCUSSION

The structure and FA composition of TG are important determinants in lipid nutrition [16], in oil stability and in its possible physiological effects [11, 15]. Hence, we have studied the FA distribution in the TG of developing winged bean seeds, with particular emphasis on the distribution of 22:0. The stereospecific distribution of FA in the TG of developing winged bean seeds is shown in Table 1. Marked changes were seen in the TG structures as the seed developed. The esterification of 16:0 and 18:0 to the sn-1 position decreased as the seeds matured, whereas the concentrations of these two FA in the sn-2 position remained fairly constant during seed development. On the other hand, in the sn-3 position, the level of 16:0 increased slightly but the level of 18:0 decreased as the seed developed.

The unsaturated FA were found predominantly in the sn-2 position of winged bean seed TG. 18:1 and 18:3 decreased but 18:2 increased during seed development. In the sn-1 and sn-3 positions, 18:1 initially increased and peaked at 4 WAF, after which its level gradually declined. On the other hand, the amounts of 18:2 in the sn-1 position increased as the seed developed but in the sn-3 position, its level fluctuated.

Long chain FA were found in all three positions of TG molecules but they showed preference for the sn-3 position. The proportion of 20:0 in the sn-1 and sn-3 positions did not alter much during seed development. In contrast, the proportions of 22:0 and 24:0 in the sn-1 and sn-3 positions increased as the seed developed. However, most of the 22:0 and 24:0 were esterified to the sn-3 position.

On the whole, the above results indicate a non-random distribution of FA in the sn-1, sn-2 and sn-3 positions of the winged bean seed TG and agree with previous findings on FA distribution in TG of other oilseeds such as soybean [17], peanut [15, 18, 19], safflower [20], rapeseed [21] and linseed [22].

The significance of our findings on the distribution of FA in the TG of winged bean seeds (Table 1) in relation to its nutritional value can be related to studies done on peanut oil TG. Myher *et al.* [15] and Manganaro *et al.* [23] reported that, out of several varieties of peanut oil,

Table 1. Positional distribution of fatty acids in winged bean triacylglycerols during seed development

		Fatty acids (mol %)										
WAF	PSN	14:0	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0
	1	0.7	28.2	9.3	32.7	19.7	1.1	1.3	1.8	4.3	—	0.9
3	2	0.3	2.8	1.8	47.3	42.7	3.2	—	0.8	1.1	—	—
	3	0.2	0.2	5.4	40.3	18.6	1.1	3.5	4.3	20.4	1.2	4.8
	1	0.2	22.7	9.2	37.5	18.9	1.2	1.7	1.4	6.0	—	1.2
4	2	0.4	3.5	1.1	46.6	43.8	2.3	—	0.4	1.1	0.5	0.3
	3	0.6	1.7	5.3	44.9	12.0	0.1	2.5	5.4	22.3	0.4	4.8
	1	0.4	18.1	7.8	35.5	23.7	0.6	1.0	2.3	8.1	0.3	1.5
5	2	0.4	3.2	1.0	43.9	47.9	2.1	—	0.5	0.6	0.1	0.3
	3	0.1	4.1	4.7	36.7	15.7	0.9	2.6	7.1	21.9	1.4	4.8
	1	0.4	14.8	5.6	34.7	26.9	1.0	1.2	2.6	10.4	0.8	1.6
6	2	0.1	4.8	1.2	40.4	48.9	1.7	—	0.5	1.5	0.4	0.5
	3	0.1	5.3	4.0	35.9	12.1	1.2	2.1	7.4	24.1	1.8	6.0
	1	0.4	15.5	6.0	33.8	27.0	1.0	0.8	2.0	11.6	0.6	1.3
7	2	0.3	3.5	1.4	40.6	46.4	1.7	0.7	1.8	2.6	0.4	0.6
	3	0.2	7.7	4.6	35.1	9.1	0.3	2.7	6.4	25.4	2.3	6.2

WAF = Weeks after flowering.
PSN = Stereospecific position.
— = not detected.

Table 2. Changes in triacylglycerol profiles during development of winged bean seeds

	Triacylglycerols by carbon number (mol %)*						
WAF	C48	C50	C52	C54	C56	C58	C60
3	0.42	6.19	28.02	41.05	10.36	12.26	1.64
4	t	2.62	18.00	40.66	13.41	22.12	3.15
5	t	1.53	15.49	42.44	14.71	22.88	2.91
6	1.15	4.10	17.67	35.68	15.84	22.06	3.31
7	t	3.26	17.69	31.68	17.87	26.03	3.38

*Each value is the average of two determinations.
WAF = Weeks after flowering.
t = trace, less than 0.1%.

the most atherogenic oil had the highest percentage of 18 : 2 in position 2 coupled with the highest percentage of long chain saturated FA in position 3 of the TG. Hence, it would appear that winged bean seed oil might be atherogenic in nature; but this property of the oil has to be further confirmed by biological testing.

Analysis of the TG according to their M_rs or by their carbon number by GC showed that winged bean seed oil TG were very different from those of peanut oil TG (Table 2). At maturity (7 WAF) *ca* 47% of winged bean seed oil TG have carbon numbers greater than 54 as compared to peanut oil TG which has only 18% or less of its TG having carbon numbers greater than 54 [15, 23]. Our results (Table 2) also show that the levels of C_{56}, C_{58} and C_{60} TG increased progressively, whereas those of C_{50}, C_{52} and C_{54} TG decreased as the seeds developed. The fact that there was no C_{66} type of TG detected in our analysis confirmed the findings of Omachi *et al.* [24] who used HPLC to analyse the molecular species of TG in winged bean seed oil and found no tribehenin. Therefore, the small amount of 22 : 0 in the sn-2 position as presented in Table 1 could be due to acyl migration during hydrolysis. The appearance of substantial amounts of the C_{58} type of TG at 3 WAF agreed with our previous findings [25] that 22 : 0 was synthesized and incorporated into TG very early in the development of winged bean seeds.

In the attempt to develop and promote the use of winged bean as a source of protein for human consumption in the tropical countries, the use of its seed oil, the main potential by-product, as a source of edible oil for food uses could be expected to increase as well. However, our results caution the extensive use of winged bean seed oil with high concentrations of 22:0. Although, the level

of 22 : 0 could be reduced substantially by physical refining of the oil or by agronomical selective breeding or genetic engineering to produce a low 22 : 0 variety.

EXPERIMENTAL

Materials. Winged bean [*P. tetragonolobus* (L.) D.C.] was grown in the field and seeds at different stages of development were harvested as described earlier [25, 26]. Porcine pancreatic lipase was obtained from Sigma and phospholipase A_2 (*Ophiophagus hannah* venom) was obtained from Ross Allen Reptile Institute Inc., Silver Spring, Florida).

Extraction, fractionation and isolation of lipids. Lipids of winged bean seeds at different stages of development were extracted with CH_2Cl_2–MeOH as described in ref. [25]. The seed total lipids were then fractionated into neutral and polar lipids by acid-treated Florisil CC [27]. TG were isolated from neutral lipids by CC on 7% H_2O-deactivated Florisil [28]. Purity of the isolated TG was further confirmed by TLC.

Stereospecific analysis of FA distribution. Analysis was carried out according to the method of ref. [17] with slight modification. Porcine pancreatic lipase 25 mg in 0.25 ml of H_2O was streaked across as a narrow band on a silica gel G-coated plate (20 × 20 cm, 0.4 mm thickness) which was impregnated with 2% boric acid. TG (25 mg) in 50 μl of hexane was overlaid as evenly as possible on the enzyme band. The plate was placed in an oven at 40°. After 3 min, the plate was exposed to conc HCl vapours in a closed chamber for 1 min to stop the lipase reaction. Excess HCl was removed by blowing a stream of N_2 across the plate for 1 min. The plate was then developed in Et_2O to a height of 2 cm above the origin in order to remove lipid materials from the enzyme band at the origin. Excess Et_2O was allowed to evaporate off the plate after which the plate was developed to the top with hexane–Et_2O–HCO_2H (4 : 1 : 1). Lipid bands were visualized by spraying with 0.02% 2,7-dichlorofluorescein in EtOH. Bands representing 2-monoacylglycerol (2-MG) and sn-1,2(2,3)-diacylglycerol (1,2-DG) were scraped from the plate and eluted with 2 × 15 ml of Et_2O. Pooled sn-1, 2-diacylglycerols were then phosphorylated as described in ref. [17]. Synthetic phosphatide was hydrolysed stereospecifically with phospholipase A_2 to remove FA at the sn-2 position. The FA, the lysophosphatidylphenol and the unhydrolysed phosphatide were isolated as described in ref. [17].

GC analysis of fatty acid Me *esters* (FAME). FAME of various lipids were prepared by refluxing with 5% MeOH–HCl for 2 hr [29]. FAME were extracted and purified as described in ref. [26]. Purified FAME were then analysed by GC, using a galss column (3 mm × 2 m) which was packed with 10% SP 2300 on Chromosorb Q. Analysis was carried out isothermally at 230° and the results were quantified with a data microprocessor.

GC analysis of TG. TG were isolated from seed neutral lipid fractions by CC on 7% H_2O-deactivated Florisil [28] and analysed by GC according to their carbon number or M_r using a glass column (0.5 m × 3 mm) packed with 1% Dexsil 300 on Chromosorb Q (100/120 mesh) and FID. Analysis was carried out by temp prog from 320 to 360° at 2°/min. Inj and detector temps were kept at 360°; N_2 flow rate was 100 ml/ min. Results were quantified using a data microprocessor and expressed in mol %.

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