Squash seed oil extraction and characterization

Extracción y caracterización de aceite de semillas de zapallo

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Abstract

In accordance with the ethereal extract (EE) of the seeds, 79 varieties of squash were selected for study. The EE was physically and chemically stable, with optimal organoleptic properties for edible oil: no rancidness; semi dried oil index was 122.90 mg KOH/g; acid index was 3.25 mg KOH/gm typical of edible oil. The iodine presumptive test revealed the presence of polyunsaturation and the absence of crystals. The composition of fatty acids was: Palmitic acid C16:0 (25.11 – 36.94%); Stearic acid C18:0 (10.79 – 13.37%); linoleic acid C18:2 (48.23 – 62.41%); linolenic acid C18:3 (0.66%); arachidic acid C20:0 (0.53 – 0.78%). The extracted oil from squash seeds contained 55.28% of unsaturated fatty acid with an appreciable amount of linoleic acid (55.11%). The squash oilseed cake presented a protein level of 51.11 ± 0.95%, and energy level of 4604. 66 ± 134.08 kcal/kg.

Key Words: *Cucurbita moschata,* Cucurbitacea, seed, vegetal oil, fatty acids.

Resumen

Se seleccionaron 79 introducciones de zapallo (*Cucurbita moschata* Duch.*)* teniendo en cuenta el contenido de extracto etéreo (EE) de las semillas. El EE fue estable física y químicamente, con propiedades organolépticas óptimas de aceite comestible, no presentó características de rancidez; aceite semisecante (122.90 mg/g de KOH.); índice de acidez 3.25 mg/g de KOH; la prueba presuntiva de yodo supone presencia de polinsaturación y sin formación de cristales. La composición de ácidos grasos mostró: palmítico C16:0 (25.11 – 36.94%); esteárico C18:0 (10.79 – 13.37%); linoleíco C18:2 (48.23 – 62.41%); linolénico C18:3 (0.66%) y araquídico C20:0 (0.53 – 0.78%). El aceite de semilla de zapallo contiene 55.28% de ácidos grasos insaturados con una cantidad apreciable de linoleíco (55.11%). La torta de semilla presentó proteína (51.11 ± 0.95%) y energía (4604. 66 ± 134.08 kcal/kg).

Palabras clave: *Cucurbita moschata*, Cucurbitacea, aceites vegetales, semillas oleaginosas, ácidos grasos.

Introduction

The squash (*Cucurbita moschata* Duch) is a horticultural crop native to the Americas (Whitaker & Bemis, 1975). In Colombia it is widely cultivated, particularly in home gardens. The majority of the genetic material planted is native, and presents considerable variation in size, form, fruit color, pulp texture and weight, and seed color and size (Montes et al., 2004). The product obtained is destined for the national market for fresh consumption, with the seed and the peel discarded at the point of consumption (Espitia, 2004).

The squash seed has been used as a diuretic, vermifuge, stomach tonic, anthelmintic, and to cure bronchial asthma, or certain skin diseases (Pérez, 1978). Due to its ethereal extract (EE) content (50.81 ± 5.17) it constitutes a source of oil (Achu et al., 2005).

The present study had as objective the characterization of the yield and the physiochemical properties of the ethereal extract and the oilseed cake of squash material in the collection of the Horticultural Program of the National University of Colombia, Palmira campus, and the identification of pristine varieties (Vega, 1988) that will form the base for future studies of genetic improvement in squash for the production of oil seed.

Materials and methods

Laboratory studies were carried out in the Palmira and Medellín campuses of the National University of Colombia. 79 squash accessions (*C. moschata* Duch) were studied. These were collected by Montes (2004) y selected by Ortíz (2006) (Box 1).

The seeds were milled and submitted to extraction with petroleum ether under reflux in a Soxhlet extractor at 180 °C for 8 h. The solvent was evaporated at 105 °C for 12 h. The following characteristics of the oilseed cake were determined: crude protein (nitrogen x 6.25); total ashes; crude fat remaining in the soluble fraction (EE); crude fiber (FB); and nitrogen-free extract (ELN) (Nielsen, 1998).

The accessions were ordered according to their yield of crude fat (AOAC, 1995). In order to identify the accessions that best combined the yield components of: seeds per fruit; weight of 100 seed; seed dry material; and EE (Ortiz, 1997), weighted analysis was employed (WSI) (Ceballos, 2006; Ortíz, 2006) so that the value could be selected as Aggregated progenitor (Falconer & Mackay, 1996).

Box 1. Squash (*Cucurbita. moschata* Duch.) accessions selected from the germplasm bank of the Horticultural Program of the National University of Colombia, Palmira campus.

Department	Locality	Introductions
Valle del Cauca South	Restrepo	1
	Pradera (La Tupia)	2, 3. 4. 5. 6. 7. 8. 10. 11
	Pradera (Lomitas)	12
	Candelaria (La Regina)	14. 15
	Candelaria (El Cabuyal)	16. 17. 18.19
	El Cerrito (Costa Rica)	20
	Palmira	144
Valle del Cauca North	Toro	41
	Roldanillo	42. 43
	Bolívar	45
	Alcalá	53
	La Unión	54. 55
Cauca	Santander de Quilichao	21. 22. 23. 24
	El Patía	25. 26. 27. 28. 29. 31. 32. 33. 34. 35. 36. 37
	El Bordo	38. 40
Quindío	Calarcá	46
	Armenia	47
	Quimbaya	48. 49. 50. 51. 52
Risaralda (Caldas)	Marsella	56. 57
	La Violeta	58
	Neira	59
	Hoyo Frío	60
Magdalena	Santa Marta	64. 65. 66. 69. 70. 71. 73. 75. 76. 77. 78. 79. 80. 81. 82. 84. 86. 88
Atlántico	El Banco	100. 101
	Magangué	108
	Barranquilla	118
	Campo La Cruz	120
	El Banco	122
Total accessions		79

To refine the EE the protocol of Madrid et al. (1998) was used. For the physiochemical characterization of the oil, the indices of rancidness, saponification, iodine, acidity and the cold test were employed (Gaviria & Calderón, 1988). In the gas chromatography mass spectrometry (GC-MS) of the methyl esters of fatty acids, diethyl ether and hydroxytetramethylamine were used. This analysis was carried out using a Shimadzu GC-R1A chromatograph, equipped with a mass detector at 280 °C, a splitless injection door at 175 °C, with an injection volume of 1 µl. A capillary column of HP-5MS (0.25 mm x 30 m x 0.25 microns) was used with a maximum temperature of 325 °C, in the presence of helium as the run gas, with a flow of 1.0 ml/min and a velocity of 37 cm/seg. The qualitative analysis was carried out using mass spectrometry with a Shimadzu RPR-G1 integrator. As a reference pattern for the results of the composition analysis of the fatty acids, a standard mixture was used (SIGMA Chemical Co., Oil Reference

Standard, AOCS (American Oil Chemists Society) no.2 (Gaviria, 2007)[1]. The yield of EE and the nutritive value of the oilseed cake were submitted to descriptive statistical analysis (Muñoz & Baena, 1988).

Results and discussion

Box 2 presents the squash accessions selected for their content of EE, dry material, weight of 100 seeds, and weight of seed per fruit. The coefficient of variation showed a high variability for carrying out selection from the 79 freely pollinated accessions (S_0), completely heterozygote for the character (Ceballos, 1998; Vega, 1988).

The high content of EE and the low coeficient of variation (Box 2) indicate that genotypes may be selected for the improvement of this character, after analysis of Combining Ability. The content of EE is equal or greater than that of soya (20%) (Younis et al. , 2000; Becerra, 2004); sunflower (36%) (Agüero et al., 1999); maize (5%); and linssed (20.7 - 55.3%) (Roy, 2000).

Based on the weighted selection index (WSI) (Box 2) the accessions 28, 36, 33, 6 and 79 were selected as valuable contributions to a gene pool forming the population base for recurrent selection (Hallauer & Miranda, 1988; Ceballos, 1989) to improve EE yield from squash seeds, and generate high value progenitor populations (Falconer & Mackay, 1996).

Box 2. Squash accessions selected for high seed weight per fruit, weight of 100 seeds, seed dry material and seed ethereal extract.

Introduction (No.)	Seed weight / fruit (g)	Weight 100 seeds (g).)	Seed dry material (%)	Seed EE (%)	WSIa
79	43.03	17.86	90.0	43.03	1.29
6	59.93	16.43	77.0	43.62	1.03
33	44.77	14.40	85.0	43.38	0.99
36	49.11	15.36	93.0	40.93	0.99
28	68.03	17.70	94.0	38.50	0.95
27	61.61	16.13	94.0	38.92	0.87
41	40.00	16.10	93.0	40.34	0.77
75	38.38	13.80	91.0	41.34	0.77
34	38.67	13.86	94.0	40.15	0.65
Mean of 79 Accessions.	45.11±24.5	15.0±1.99	92.79±0.25	36.55±3.25	
CV (%)	55	14.21	2.69	8.89	

a. WSI = Weighted selection index.

Oil Characteristics

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The color of the oil varied between red and yellow, being clear and free of sediment; the aroma and taste were normal with a negative rancidness index (Box 3); palatable rancidness was not observed. The acidity index was high, and neither disassociation of fatty acids, nor hydrolytic rancidness were observed.

The oil, definitely liquid and negative in the cold test (without water), was characterized by a low sapinification index, indicating the presence of saturated fatty acids or impurities. However, the iodine index (g of I/100 g) of between 97 and 132, denoted the presence of unsaturated fatty acids, allowing this oil to be described as semidry, or oxygen absorbing on exposure to air.

Box 3. Comparison of the physical and chemical properties of squash seed oil, compared with other species[a].

Parameters	Introductions (No.)						
	6	28	34	75	Index	Seeds of:	Source
Acidity Index	3.19	1.62	4.10	4.10	2.88	*Cucurbita* sp.	El-Adawy & Taha, 2001
					0.66	*C. pepo*	Younis et al. 2000
					0.3150	Peanut	Pascual et al, 2006
Saponification Index	112.88	122.90	104.17	118.69	189.7	Watermelon	Younis et al. (2000)
					188.0	Maize	Younis et al. (2000)
					206.0	*Cucurbita* sp.	El-Adawy & Taha, 2001
Iodine Index	132.7	129.0	114.9	97.01	99-119 110-126 118-145 107-135	Algodón Canola Sunflower Maize	http://portal.aniame.com/uploads/losaceitesvegetales.pdf. (08/08/2007
Rancidness Index[a]	(-)	(-)	(-)	(-)			
Cold test[a]	(-)	(-)	(-)	(-)			

a Qualitative tests.

Composition of fatty acids

The predominant fatty acid in the four accessions studied was linoleic acid, with levels between 48% and 62%, which, by nature of its polyunsaturated nature (two double bonds) and its inclusion in the omega-6 group (first hydrogen bond at carbon 6) gives the oil its liquid nature (Box 4). The palmitic and stearic acids varied between 25.11 and 36.94%, and between 10.79 and 13.37%, respectively. The arachidic acid (C20:0) observed in the accessions 28, 34 and 75 was significantly low (< 1%), which is positive, as, due to its saturated nature, the low concentration increases the value of this oil. Linolenic acid was only seen in accession 75, however, other researchers have not detected it in squash seeds (Younis et al., 2000). The concentration of this acid was relatively high, compared with, for example, the 0.35% seen in water melon seeds (El-Adawy & Taha, 2001) (Box 4).

Characteristics of the oilseed cake

The content of dry material was similar to that of the peanut seed cake. The crude protein was high compared with peanut, rapeseed and cotton (Chatterjee & Walli, 2000) (Box 5). The mineral content, represented by the ash, was also high compared with peanut seed (Pascual et al, 2006). The fibrous portion of the cake was represented in the neutral detergent fiber (NDF) with 39.98 ± 1.95%, which is considered optimum for dairy cow feed (NRC, 1989).

Box 4. Fatty acid composition (%) in squash seed oil.

Fatty Acids	Introductions (No.)						
	6	28	34	75		Seeds of	Source
Palmitic (C16:0)	25.11	33.66	33.50	36.94	13.4 11-14 11	*Cucurbita* sp. *C. pepo.* *Glicyne max*	El-Adawy & Taha, 2001 Younis et. al. (2000) Wikipedia, 2007
Stearic (C18:0)	12.47	11.14	10.79	13.37	10.2 9.6 8.0	*C. lanatus* *Cucurbita* sp. *C. pepo.*	 El-Adawy & Taha, 2001 El-Adawy & Taha, 2001 Younis et. al. (2000)
Arachidic (C20:0)	–	0.54	0.53	0.78	—	—	—
Linoleic (C18:2)	62.41	54.65	55.17	48.23	43-53 54.0 55.4	*C. pepo* *G. max* *Cucurbita* sp.	Younis et. al. (2000) Wikipedia, 2007 El-Adawy & Taha, 2001
Linolenic (C18:3)	–	–	–	0.66	0.35	*C. lanatus*	El-Adawy & Taha, 2001
Total Saturated	37.58	45.34	44.82	51.09	—	—	
Total Unsaturated	62.41	54.65	55.17	48.89	—	—	

Box 5. Dry material (DM) content, crude protein (PC), ash, neutral detergent fiber (NDF), nitrogen-free extract, and crude energy in oilseed cake from squash seeds.

Component	Accessions (No.)							
	6	28	34	45	mean		Seed cake of	source
DM (%)	89.48	89.75	89.97	89.89	89.77±0.21	89.1	Peanut	Pascual et al. (2006)
PC (%)	51.37	50.71	52.31	50.07	51.11±0.95	34.5 29.5	Rapeseed Cotton	Chatterjee & Walli, 2000
Ash (%)	3.3	3.8	4.3	4.3	3.9±0.47	2.6	Peanut	Pascual et al. (2006)
NDF (%)	40.85	40.81	37.06	41.22	39.98±1.95		—	—
N-free extract (%)	7.45	8.09	10.19	8.28	8.5±1.17		—	—
Crude energy (kcal/kg)	4504.9	4702.7	4474.4	4736.5	4604.6±134.0		—	—

Conclusions

- The squash seeds in this study can be considered as oilseeds, containing up to 43% of crude oil.
- The oil derived from squash seeds is physically and chemically stable, with saturated fatty acids varying between 37.5% and 51% and unsaturated between 48.8% and 62.4%. Amnogst the latter, linoleic acid was observed in quantities up to 62.41% , with lesser levels of palmitic (25.11%), stearic (10.79%), arachidic (0.53-0.78%) and linolenic (0.66%) acids.
- The squash oilseed cake could be used as a food supplement for dairy cows, due to its high protein content(52.31%) and crude energy (4.736.56 kcal/kg).

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