

## Evaluation of groundnut genotypes from China for quality traits

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### Abstract

Quality analysis of seed samples of 152 groundnut genotypes from China showed that the protein, oil and sucrose content, oleic acid and linoleic acid content, as percentage of total fatty acids, ranged from 18.93 to 30.22%, 37.42 to 55.69%, 2.73 to 14.65%, 20 to 80.51% and 2.91 to 41.82%, respectively. Correlations between these quality attributes were also analyzed. Protein content was not correlated with oil content; however, both of them were negatively related to sucrose content. The study also indicated the feasibility for developing high protein, oil or sucrose groundnut cultivars with high oleate to linoleate ratio as this ratio is not correlated with protein, oil or sucrose content. Germplasm lines with high protein, oil, sucrose or oleate to linoleate ratio were identified, providing materials for studying genetics of quality traits and breeding quality groundnut cultivars.

### Introduction

Groundnut has been the main oilseed crop in China and other developing countries for several decades and will continue to be so in the future. Globally, groundnut (*Arachis hypogaea*) production and area during 1998–2007 averaged 34.97 million tons and 23.04 million ha (nearly 94% of the area was from developing countries), respectively (Wang 2009); at present, about 47% of the produce is crushed for edible oil (Wang 2009). In developing countries, the proportion for oil is generally higher than 50%. It is estimated that each 1% increase in oil content would raise the processor's benefit by 7% (Liao and Holbrook 2005). The oil content in seed of groundnut cultivars for commercial production is generally around 50%, while some germplasm accessions have been found to contain more than 55% oil (Liao and Holbrook 2005).

In the western world, most of the groundnut goes into food uses where groundnut butter, roasted groundnut and salted groundnut are preferred food for consumers

(Ahmed and Young 1982). There is an increasing need for high protein and low oil groundnut as these traits add to the confectionary quality of groundnuts.

High oleate to linoleate (O/L) ratio has been associated with prolonged groundnut shelf life and decreased tendency toward rancidity (Braddock et al. 1995). High oleate groundnut diet lowers total cholesterol and decreases bad low density lipoprotein (LDL) cholesterol, maintains beneficial high density lipoprotein (HDL) cholesterol, and helps to maintain good flavor (O'Byrne et al. 1997). In USA, Norden et al. (1987) first identified a groundnut line with an O/L ratio of 40. Since then, several high oleate groundnut cultivars have been released (Kassa et al. 2009). In China, the first high oleate groundnut variety Huayu 32 was released for commercial production in Shandong province in March 2009.

In comparison with the abundant information on groundnut protein, oil and fatty acid contents, there are only a limited number of reports regarding sugars. The sweet attribute of groundnuts is a heritable trait (Pattee et al. 2000). It is believed that the sweetness found in groundnuts is mainly due to the presence of large amount of free sucrose (Mason et al. 1969). Previous studies indicated a close relationship between the intensity of flavor of roasted groundnuts and the sucrose content of groundnut kernels (Zhang 1999). The eating quality is more closely correlated with sweetness ( $r=0.88$ ) than hardness of seed ( $r=0.64$ ). The trend is particularly evident when the sucrose content in seed exceeds 5% (Gocho 1992). Groundnut with  $\geq 6\%$  sucrose content is highly acceptable for early season consumption when boiled in-shell (Luo et al. 2004). Oupadissakoon et al. (1980) reported that the sucrose content of five groundnut cultivars tested at three digging dates ranged from 2.7 to 3.7%. Luo et al. (2004) analyzed 13 groundnut cultivars, mainly of Spanish type, and found the sucrose content varied from 5.1 to 5.9%. Zhang et al. (2003) stated that groundnut kernels harvested from clay soil contained more sucrose and total sugars than those from

loamy and sandy soil. We identified one groundnut genotype with 6.1% sucrose content (Wang et al. 2007). Sugar content was higher in groundnut grown in Argentina than that from USA and China (Bett et al. 1994). Low sucrose content of commercial groundnut seed is the limiting factor for exportation of Chinese groundnut into Japan (Ling Jia Huang, personal communication).

To broaden the narrow gene base of the cultivated groundnut and to achieve high and stable yields, researchers from the Biotechnology Division, Shandong Peanut Research Institute (SPRI), Qingdao, China have conducted studies on remote hybridization for over 20 years and on chemical mutagenesis for over 10 years, and have created a large number of elite lines. Huayu 31, the first large-seeded groundnut cultivar derived from an intersectional cross developed by us was released in March 2009 (Wang et al. 2009). Nevertheless, the chemical quality of most of the lines created along with landraces and cultivar releases collected and preserved by us is still unknown.

The present study was intended to evaluate the chemical composition of groundnut seeds in relation to protein, oil, fatty acid and sucrose contents of these materials, so as to provide quality parental lines for breeding and genetic research and lay foundation for the establishment of NIRS (near infrared spectroscopy) calibration equations for major quality attributes of groundnut, including sucrose.

## Materials and methods

A germplasm collection of 152 accessions preserved at the SPRI Biotechnology Division was sown with a population of 150000 hills per ha (2 seeds per hill) under polythene (with Acetochlor) mulch in unreplicated nursery at the SPRI Experimental Station at Laixi, Shandong, China on 26 April 2008. The collection includes 38 released cultivars, 34 landraces, 15 breeding lines, 36 interspecific derivatives, both of compatible and incompatible species, and 29 mutants. Large- and small-seeded genotypes with different seed coat color were represented. Standard cultural practices were employed in growing the collection (Wan et al. 2003). Groundnut was harvested on 10 September 2008.

Moisture content of groundnut seed samples was determined based on China National Standard GB/T 5497-85 for grain and oilseeds (SAP 1985). The sample was dried in the oven (model DHG-9243BS-III, Shimo Medical Machinery Co. Ltd, Shanghai, China) at 105°C until constant weight was reached. The loss in sample weight as percent of the initial weight was reported as the moisture content of the sample.

Protein content of groundnut seed samples was analyzed according to China National Standard GB 2905-82 (SAP 1982a). The samples were digested with concentrated sulfuric acid (E Merck, Darmstadt, Germany), hydrogen peroxide (Fisher Scientific, NJ, USA), and Kjeldahl catalyst reagent, and the total nitrogen amount in the sample was determined by using an automated Kjeldahl Analyzer (model 2300 II, Foss, Sweden). A conversion factor of 5.46 was used to convert the amount of nitrogen to amount of protein in the samples.

Oil content of groundnut seed samples was analyzed using the Soxhlet method as described in GB 2906-82 (SAP 1982b).

To analyze the fatty acids, 2 ml of sodium methoxide ( $0.5 \text{ mg L}^{-1}$ ) were added to groundnut sample (0.1 g), and esterification reaction was conducted in a water bath at 30°C. Fatty acid profiles were determined using an Agilent Model 7890 gas chromatograph equipped with a flame ionization detector (FID) and a Hp-INNOWAX capillary column ( $30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \text{ }\mu\text{m}$ ). Column temperature was programmed from 100°C (held for 1 min) to 225°C ( $15^\circ\text{C min}^{-1}$ ), and to 250°C ( $15^\circ\text{C min}^{-1}$ , held for 7 min). Gas flow rates were 2, 60 and  $100 \text{ ml min}^{-1}$  for nitrogen (carrier gas), hydrogen and air, respectively. A split ratio of 50:1 at an inlet temperature of 250°C was employed. Fatty acid methyl ester standard (Sigma-Aldrich) was run to use retention times in the identification of individual peaks.

To determine sucrose content, 100 mg of defatted groundnut flour was placed into a 10 ml volumetric flask, to which 9 ml of ethanol (75%) was added. The mixture was treated with ultrasound for 30 min, and brought to a volume of 10 ml using 75% ethanol. After thoroughly mixing, 1 ml of the extraction mixture was transferred into a clean test tube and centrifuged for 15 min at 14000 rpm. Then 0.5 ml of supernatant was placed into the HPLC sample bottle for analysis. Sucrose content was determined using KONIK HPLC 560A compact HPLC system (The KONIK Group, Spain) equipped with a differential refractive index detector and a KONIK NH2 column ( $250 \text{ mm} \times 4.6 \text{ mm} \times 5 \text{ }\mu\text{m}$ ). Column temperature was kept at 35°C. The mobile phase (acetonitrile/water=63/37, v/v) flow rate and run time were  $1 \text{ ml min}^{-1}$  and 10 min, respectively.

Moisture, protein, oil and sucrose contents and fatty acid content were estimated as averages of two replications. Data analysis was conducted using the DPS 7.05 package (Tang and Feng 2006).

## Results and discussion

The range of variation, average and standard deviation of protein, oil and sucrose contents, fatty acid composition

(in %, on a dry basis) and O/L ratio in the 152 lines from the SPRI Biotechnology Division's germplasm collection are listed in Table 1. Oil was the main component of groundnut seed, followed by protein and sucrose. Oleic (C18:1), linoleic (C18:2) and palmitic (C16:0) acids were major fatty acids in groundnut seed; the first two constituted about 80% of the fatty acids of groundnut seed. Behenic (C22:0), stearic (C18:0), arachidic (C20:0), eicosenoic (C20:1), lignoceric (C24:0),  $\gamma$ -linolenic (C18:3), erucic (C22:1) and myristic (C14:0) were also present, though in smaller amounts (Table 1).

Ten groundnut genotypes contained  $\geq 27\%$  protein, with J124 being the highest (30.22%); six groundnut genotypes had more than 54% oil, with J28 being the highest (55.69%) (Table 2). Eighty-four groundnut genotypes had O/L ratio over 1.50, 12 among which possessed O/L ratio more than 2.0. J123 had the highest O/L ratio (27.69), followed by J147 (4.78).

Of all the genotypes tested from our germplasm collection, 74 (48.68%) contained less than 5% sucrose, and 27 (17.76%) accessions were found to contain  $>6\%$  sucrose. According to Title 21 of the Code of US Federal Regulations (21CFR101.62), a product can be labeled reduced fat only if it has 1/4 less fat than the normal product. Groundnuts with less than 37.5% oil may qualify as having reduced fat (Isleib et al. 2004). Among these lines, J81 had the highest sucrose (14.65%), the lowest oil (37.42%) and acceptable O/L ratio (1.63), living up to the standard for reduced fat. In literature, the highest sucrose content in groundnut seed was around 9% (Gocho 1992). High sucrose in J81 was not expected; hence it had been confirmed by 7 repetitions and results

from total soluble sugar (TSS) analysis (TSS = 19.98%). This genotype is of some value in breeding, considering the fact that mulching cultivation lowers sucrose content in groundnut seed (Yu et al. 2008). Other lines with  $>6\%$  sucrose content were J119 (8.23%), J13 (7.14%), J106 (7.05%), J44 (6.96%), J56 (6.93%), J117 (6.91%), J65 (6.83%), J14 (6.72%), J66 (6.62%), J141 (6.60%), J104 (6.54%), J83 (6.53%), J133 (6.49%), J115 (6.49%), J116 (6.42%), J108 (6.35%), J105 (6.32%), J7 (6.30%), J58 (6.27%), J96 (6.25%), J71 (6.14%), J22 (6.12%), J92 (6.10%), J20 (6.04%), J33 (6.02%) and J85 (6.01%).

The results from the present study were the outcome of one year evaluation. It is generally believed that evaluations carried out across years (at least two) derive reliable conclusions on the range of the quality traits measured for each entry. For instance, the variations in oil content over the years may vary to an extent as high as 5% for the same entry. Anyway, our results provide some useful information for genetic improvement of the cultivated groundnut.

Protein content was not significantly correlated with oil content ( $r=0.069$ ); however, negative correlation between protein and oil content was earlier reported by Dwivedi et al. (1990), possibly reflecting differences in genetic background of the groundnut materials used. Both protein and oil content were negatively related to sucrose content, with the Pearson's correlation coefficients being  $-0.462$  and  $-0.789$ , respectively (significant at 0.01 level), indicating the possibility of breeding groundnut varieties with high oil and protein, or high sucrose and low oil.

Table 3 shows the Pearson's correlation coefficients among fatty acid components of the 152 groundnut genotypes. Oleic acid was negatively correlated with linoleic and palmitic acids, suggesting that it was probable to raise oleic acid content while lowering linoleic and palmitic acid contents. The O/L ratio was not significantly correlated with protein ( $r=0.051$ ), oil ( $r=0.074$ ) or sucrose ( $r=-0.074$ ) contents, an indicator of the feasibility for developing high protein, oil or sucrose groundnut cultivars with high O/L ratio.

In conclusion, we evaluated 152 Chinese groundnut seed samples of diverse origins for nutritional quality, and identified germplasm lines with  $\geq 27\%$  protein (10 accessions),  $>55\%$  oil (2 accessions), 6% sucrose (27 accessions) or O/L ratio over 2 (12 accessions). The variation range of protein, oil and O/L ratio does not go beyond the value in literature (Xiao et al. 1999, Ng et al. 2008). One genotype, J81, low in oil (37.42%), was uncommon in sucrose content (14.65%), representing a historical high value in groundnut studies. No doubt that the frequency for  $>10\%$  sucrose in groundnut is low, but the limited number of groundnut genotypes evaluated in the past is also considered to be the main cause for the

**Table 1. Protein, oil and sucrose contents and fatty acid composition (in %, dry basis) and O/L ratio of the groundnut genotypes tested.**

Components	Minimum	Maximum	Mean	SD
Protein	18.93	30.22	24.46	1.70
Oil	37.42	55.69	50.20	2.46
Sucrose	2.73	14.65	5.10	1.27
C14:0	0.01	0.05	0.03	0.01
C16:0	5.25	13.51	10.59	0.98
C18:0	1.93	8.50	3.49	0.85
C18:1	36.38	80.51	46.85	6.17
C18:2	2.91	41.82	32.52	5.08
C18:3	0.04	0.18	0.07	0.01
C20:0	0.98	3.02	1.50	0.28
C20:1	0.58	1.77	0.94	0.20
C22:0	1.86	5.07	2.64	0.54
C22:1	0.04	0.23	0.09	0.03
C24:0	0.90	1.73	1.30	0.18
O/L	0.87	27.69	1.65	2.17

**Table 2. Main quality attributes of groundnut genotypes with high protein/oil content.**

SPRI BD accession no.	Protein (%)	Oil (%)	Sucrose (%)	O/L ratio
<b>Genotypes with <math>\geq 27\%</math> protein</b>				
J124	30.22	48.86	4.35	1.02
J125	29.60	48.68	3.82	0.96
JW152	28.66	51.82	4.09	1.07
JW151	28.52	50.13	3.94	0.94
J130	28.10	51.78	3.55	1.55
J142	27.76	50.48	4.51	1.12
J143	27.15	51.24	4.78	1.21
J144	27.09	50.98	4.18	1.91
J93	27.06	49.79	4.12	1.24
J122	27.00	49.01	5.51	1.10
<b>Genotypes with <math>&gt; 54\%</math> oil</b>				
J28	20.36	55.69	3.83	1.54
J88	24.06	55.07	4.13	1.17
J38	26.51	54.84	3.18	1.26
J49	24.31	54.40	3.02	1.45
J50	24.13	54.10	3.42	1.40
J27	22.43	54.05	3.99	2.02

**Table 3. Correlation coefficients among fatty acid components of the 152 groundnut genotypes<sup>1</sup>.**

Components	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	C22:1
C16:0	0.430**									
C18:0	-0.282**	-0.005								
C18:1	-0.241**	-0.746**	-0.343**							
C18:2	0.326**	0.744**	0.187*	-0.975**						
C18:3	0.217**	0.178*	-0.248**	-0.118	0.095					
C20:0	-0.383**	-0.075	0.936**	-0.354**	0.176*	-0.152				
C20:1	-0.210**	-0.336**	-0.584**	0.372**	-0.371**	0.371**	-0.361**			
C22:0	-0.352**	-0.056	0.453**	-0.328**	0.158	0.304**	0.699**	0.290**		
C22:1	0.057	-0.126	-0.473**	0.291**	-0.290**	0.376**	-0.365**	0.645**	0.111	
C24:0	-0.110	-0.140	-0.270**	-0.043	0.003	0.342**	-0.015	0.733**	0.529**	0.474**

1. \* = Significant at 0.05 level (2-tailed); \*\* = Significant at 0.01 level (2-tailed).

slow process in identifying elite groundnut lines with high sucrose content. The largest number of groundnut seed samples used in previous studies on sucrose was less than 60 samples. In literature, this figure was generally between 10 and 20. Large-scale screening for sucrose content in groundnut seed may result in additional high sucrose groundnut genotypes.

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