Proximate Composition, Amino Acid Profile, Fatty Acid Composition, and Mineral Content of Peanut Seeds Hydroponically Grown at Elevated CO_2 Levels

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Peanut plants (*Arachis hypogaea* L. cv. Georgia Red) were grown hydroponically using a recirculating nutrient film technique. The effect of CO_2 enrichment on nutritive composition of hydroponic peanut seeds was examined at two elevated CO_2 levels (700 and 1400 ppm) that simulate potential conditions in National Aeronautics and Space Administration (NASA) Controlled Ecological Life-Support Systems (CELSS) and compared to ambient CO_2 condition in hydroponics (the control). Plants were harvested at 97 days after planting, and the seeds were air-dried and analyzed for composition. Percentages of crude protein, crude fat, ash, and carbohydrate of hydroponic peanut seeds were around 30%, 30%, 3%, and 30%, respectively. The major amino acids were aspartic acid, glutamic acid, and arginine. The limiting amino acid of peanut, methionine, was 1.2%. Linoleic acid was the major fatty acid, followed by oleic and palmitic acids. The major mineral elements were K, P, Mg, and Ca. The results showed that certain peanut varieties can be grown hydroponically. The composition of the hydroponically grown peanuts is generally similar to reported peanut composition. The nutrient composition was not affected at the elevated CO_2 concentrations investigated.

Keywords: *CELSS; hydroponic; NFT; elevated CO_2 levels; peanut*

INTRODUCTION

As plans progress for the human habitation of the moon or Mars and manned space travel beyond Mars, investigations of bioregenerative life support systems will intensify to minimize the economic costs associated with resupply. The Controlled Ecological Life-Support System (CELSS) program therefore was initiated by the National Aeronautics and Space Administration (NASA) in 1978 (Averner, 1989) to address the need to increase self-sufficiency of life support systems and maintain an Earth-like living environment to promote human productivity and psychological well-being in longer duration manned space missions (Schwartzkopf, 1992).

There are five subsystems included in a CELSS: biomass production, food processing, waste treatment, atmosphere regeneration, and water purification (Fu and Nelson, 1994). For biomass production, photosynthetic plants generally are considered the primary candidates. NASA originally selected eight crops, including wheat, rice, white potato, sweetpotato, soybean, peanut, sugar beet, and lettuce, to provide a balanced diet in controlled environments for future long-duration human space missions (Tibbitts and Alford, 1982). Peanut (*Arachis hypogaea* L.) was chosen because of its high protein and oil contents.

As part of research supported at Tuskegee University's NASA Center for Food and Environmental Systems for Human Exploration of Space (CFESH), peanut seeds have been successfully grown in a recirculating hydroponic system using the nutrient film technique (NFT). NFT allows control of the root environment (Graves, 1983) and has been used successfully to grow root crops such as sweetpotato (Hill et al., 1989; Bonsi et al., 1992; Grant et al., 1993). However, only preliminary studies on the production of peanut seeds in NFT have been reported previously. Additionally, in a CELSS, the effect of carbon dioxide concentration on plant growth is important since it is critical to plant photosynthesis. This is especially true in a sealed environment where accumulation of volatile substances becomes an important issue. Carbon dioxide, being a major metabolic waste of crew members, may accumulate at high levels in a CELSS. High concentrations of CO_2 in turn may cause stress to plants in a CELSS, substantially affecting their performance. A previous work (Hill et al., 1992) described preliminary studies on hydroponically grown peanut at Kennedy Space Center but not under elevated CO_2. In this paper, we examined the effect of two elevated CO_2 levels that might simulate conditions to be expected in a CELSS on the nutritive content of peanut seeds grown hydroponically. Peanut seeds grown hydroponically at ambient CO_2 concentration were used as the experimental control for comparison.

MATERIALS AND METHODS

Peanut. Two-week-old seedlings of peanut (*A. hypogaea* L.) cv. Georgia Red were placed in the NFT growing chambers. Georgia Red, a Valencia-type peanut, was chosen for its compact bunch growth habit (Branch and Hammons, 1987) and because its fruit production is centered around the tap root, which has been found more suitable for hydroponics. In addition, Georgia Red is one of the few peanut cultivars tested successfully in hydroponic systems (Hill et al., 1992).

Growth Conditions and Nutrition. The study was conducted in NFT growing channels (Bonsi et al., 1992) in EGC15 Reach-In Growth Chambers (Environmental Growth Chambers Inc., Chagrin Falls, OH). The photosynthetic

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Table 1. Proximate Composition of Peanut Seeds (Cv. Georgia Red) Grown at Elevated CO_2 Levels Using Hydroponics[a]

growth condition	moisture (%)	crude protein (% N × 6.25)	crude fat (%)	crude ash (%)	carbohydrate[b] (%)
ambient[c]	5.79 ± 0.33	30.37 ± 3.27	30.77 ± 1.81	3.07 ± 0.02	30.00
700 ppm	5.80 ± 0.57	30.23 ± 3.27	29.12 ± 2.89	3.12 ± 0.18	31.73
1400 ppm	6.03 ± 0.16	30.38 ± 3.70	28.07 ± 2.23	3.07 ± 0.07	32.45

[a] No significant differences were noted among proximate compositions of peanut seeds produced in ambient or CO_2-enriched hydroponic environments. Values are expressed as means ± standard deviation ($n = 3$). [b] Percentage crude carbohydrate was estimated by subtracting average percentage moisture, crude protein, crude fat, and crude ash from 100%. [c] Ambient concentration of CO_2 was approximately 400 ppm (control).

photon flux (700 μmol m^{-2} s^{-1}) was supplied by a mixture of cool white fluorescent and incandescent lamps. The photoperiod was 12/12 h, temperature 28/22 °C (light/dark) with relative humidity held at 70 ± 5%. To investigate the effect of long-term elevated CO_2 exposure, CO_2 concentrations inside two growth chambers were maintained at 700 or 1400 ppm, respectively, through the use of an infrared gas analyzer, and the control growth chamber was at an ambient CO_2 level, ~400 ppm. A modified half-strength Hoagland solution (Hoagland and Arnon, 1950) was used in which $Ca(NO_3)_2$ was replaced by $CaCl_2$, and the solution was changed biweekly. The electrical conductivity was maintained at 1.2 dS m^{-1}. The solution pH was controlled at ~6.5 by manual addition of diluted NaOH or HCl solutions. The solution temperature was checked at regular intervals and was maintained between 2 and 4 °C lower than air temperature (~22 °C). Plants were harvested at 97 days after planting, and seeds were air-dried in a greenhouse environment for 2 weeks.

Proximate Composition. Percentages of moisture, crude fat, total nitrogen, and ash of skinless peanut seeds were determined in triplicate according to AOAC Methods 950.46, 960.39, 928.08, and 923.03 (AOAC, 1995), respectively. Percentage of crude protein was estimated by multiplying total nitrogen by a factor of 6.25. The percentage carbohydrate was estimated by subtracting the sum of percentage of moisture, crude fat, crude protein, and ash from 100%.

Amino Acid Profile. Approximately 7.5 mg of peanut seeds was hydrolyzed in 6 N HCl/0.05% mercaptoethanol/0.02% phenol at 115 °C for 20 h and analyzed using a Beckman 7300 amino acid analyzer (Fullerton, CA) in duplicate. The amount of amino acid was calculated using amino acid molecular weight. Half-cystine was determined by performic acid oxidization prior to acid hydrolysis and calculated from cysteic/alanine ratio. A 48 h alkaline hydrolysis at 135 °C was performed for the determination of tryptophan.

Fatty Acid Composition. Peanut oil was extracted in duplicate from 250 mg of ground peanut seeds with 5 mL of methanol/chloroform/water (2:1:0.8, v/v/v) and subsequently methylated by BF_3/methanol (Sigma Chemical Co., St. Louis, MO) for GC analysis (Wu et al., 1991). Fatty acid methyl esters (FAME) of peanut oil were separated using a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector and a DB-225 fused-silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) (J&W Scientific, Folsom, CA) operated isothermally at 205 °C. FAME were identified by comparing retention times with pure standards and quantitated by an on-line computer. Carrier gas was helium flowing at 2 mL/min. The injector and detector temperatures were 250 and 260 °C, respectively. Heneicosanoic acid (21:0) was used as the internal standard for quantitation.

Mineral Content. The contents of 12 selected mineral elements in peanut seeds were determined in duplicate according to the method described by Jones (1977) using a Jarrell-Ash ICAP 9000 inductively coupled plasma spectrometer (Waltham, MA) for elemental analysis of plant tissue ash.

Statistical Analysis. The statistical analysis was carried out using the General Linear Model (GLM) procedures of the Statistical Analysis System (SAS, 1991) to perform analysis of variance (ANOVA) and Tukey's Studentized Range [Honestly Significant Difference (HSD)] test to examine the effects of different levels of CO_2 on the nutritive content of peanut seeds.

RESULTS AND DISCUSSION

Peanut is well recognized as an important source of protein and oil. Crude protein content of peanut seed ranges from 22 to 30% (Pancholy et al., 1978), and average oil content may reach 50% (Derise et al., 1974; Pancholy et al., 1978). The percentage protein levels of hydroponically grown peanut seeds (Table 1) are comparable with previously reported values for conventionally grown peanuts (Derise et al., 1974; Lusas, 1979). However, levels of peanut oil (Table 1, percentage crude fat) found in this study were approximately 30% and relatively low. The lower oil content may be cultivar-related and also possibly due to environmental conditions such as the hydroponic study's cooler growth temperature and high humidity, which might have affected the maturity of the seeds. The estimated percentage carbohydrate found in this study was between 30 and 32%, which included crude fiber, starch, pentosans, and sugars (Hoffpauir and Guthrie, 1945; Hoffpauir, 1953). In general, CO_2 concentration in hydroponics did not appear to affect the proximate nutritive composition of peanut seeds, which is in agreement with findings for other NASA candidate crops such as soybean and potato (Wheeler et al., 1994).

It has been reported that aspartic acid, glutamic acid, and arginine may account for about 45% of the total amino acid in peanut seeds, and lysine, methionine, and threonine are considered the limiting amino acids in peanuts (Young et al., 1973; Pancholy et al., 1978). Additionally, the content of amino acids in peanut seeds varies according to genotype, variety, growing season, environmental factors, and maturity (Young et al., 1973, 1974a; Hovis et al., 1982). Our results (Table 2) also indicate that arginine (119−139 mg/g of protein), aspartic acid (99−114 mg/g of protein), and glutamic acid (168−193 mg/g of protein) make up approximately 45% of the total amino acids in Georgia Red peanut seeds grown in hydroponics. The limiting amino acid, methionine, is about 1.2%; that is higher than the previously reported average of 0.79% from 77 peanut lines and cultivars (Pancholy et al., 1980) but close to the findings of Young et al. (1973) based on the study of 16 varieties of peanuts. The CO_2-enriched environment at 700 or 1400 ppm did not appear to significantly affect the amino acid profile of peanut seeds except for methionine (Table 2). However, that difference is only 2−3 mg/g of protein at 700 ppm and may not be meaningful.

The carbon number of fatty acids in peanut seed oil ranges from 16 to 24. Oleic acid (18:1) usually is the most abundant fatty acid in peanut oil followed by linoleic acid (18:2). The fatty acid composition of peanut oil may be affected by factors such as genotypes, geographic location, environmental condition such as temperature, and maturity stage (Worthington et al., 1972; Young et al., 1972, 1974a,b; Brown et al., 1975). In this study, contrary to the majority of fatty acid

Table 2. Amino Acid Profile of Peanut Seeds (Cv. Georgia Red) Grown Hydroponically at Elevated CO_2 Levels[a]

amino acid (mg/g of crude protein)	ambient	700 ppm	1400 ppm
alanine	33.80 ± 3.09[a]	38.16 ± 3.13[a]	36.75 ± 3.57[a]
arginine	132.90 ± 12.16[a]	142.60 ± 11.70[a]	146.05 ± 14.18[a]
aspartic acid	107.95 ± 9.52[a]	117.05 ± 9.60[a]	116.96 ± 11.35[a]
cystine/2	15.12 ± 1.39[a]	15.78 ± 1.30[a]	14.57 ± 1.42[a]
glutamic acid	188.35 ± 17.23[a]	193.04 ± 15.83[a]	200.05 ± 19.42[a]
glycine	51.85 ± 4.74[a]	50.76 ± 4.16[a]	54.01 ± 5.25[a]
histidine	20.47 ± 1.88[a]	22.41 ± 1.84[a]	21.96 ± 2.13[a]
isoleucine	28.88 ± 2.65[a]	32.01 ± 2.63[a]	31.64 ± 3.07[a]
leucine	56.43 ± 5.02[a]	61.44 ± 5.04[a]	61.68 ± 5.99[a]
lysine	32.03 ± 2.93[a]	34.27 ± 2.81[a]	33.88 ± 3.29[a]
methionine	12.01 ± 1.10[ab]	9.57 ± 0.78[b]	12.49 ± 1.22[a]
phenylalanine	45.61 ± 4.17[a]	51.09 ± 4.19[a]	51.13 ± 4.96[a]
proline	48.89 ± 4.47[a]	47.53 ± 3.90[a]	50.49 ± 4.90[a]
serine	48.56 ± 4.44[a]	51.09 ± 4.19[a]	52.09 ± 5.06[a]
threonine	25.66 ± 2.35[a]	28.65 ± 2.35[a]	27.64 ± 2.69[a]
tryptophan	11.95 ± 1.09[a]	12.16 ± 1.00[a]	11.89 ± 1.15[a]
tyrosine	35.11 ± 3.21[a]	38.80 ± 3.18[a]	38.55 ± 3.73[a]
valine	34.78 ± 3.18[a]	38.16 ± 3.13[a]	37.71 ± 3.66[a]

[a] Means in the same row with no superscripts in common differ ($p < 0.05$). Values are reported as means ± standard deviation ($n = 3$).

Table 3. Fatty Acid Profile of Peanut Oil from Peanut Seeds (Cv. Georgia Red) Grown Hydroponically at Elevated CO_2 Levels[a]

fatty acid[b] (mg/g of peanut seed)	ambient	700 ppm	1400 ppm
16:0	37.59 ± 3.10[b]	32.78 ± 0.60[b]	31.94 ± 0.10[b]
16:1 *n*−7	0.22 ± 0.05[b]	0.17 ± 0.01[b]	0.18 ± 0.00[b]
18:0	11.49 ± 1.09[b]	9.82 ± 0.12[b]	10.19 ± 0.01[b]
18:1 *n*−9	107.39 ± 10.60[b]	100.70 ± 0.77[b]	98.80 ± 0.50[b]
18:2 *n*−6	119.01 ± 10.29[b]	108.46 ± 1.10[b]	114.08 ± 0.31[b]
18:3 *n*−3	0.41 ± 0.10[b]	0.25 ± 0.01[b]	0.25 ± 0.00[b]
20:0	4.79 ± 0.48[b]	4.35 ± 0.04[b]	4.43 ± 0.01[b]
20:1 *n*−9	2.43 ± 0.23[c]	2.83 ± 0.01[bc]	3.05 ± 0.01[b]
22:0	8.92 ± 0.91[b]	9.18 ± 0.18[b]	9.68 ± 0.02[b]
24:0	3.80 ± 0.46[b]	3.82 ± 0.18[b]	4.06 ± 0.01[b]

[a] Means in the same row with no superscripts in common differ ($p < 0.05$). Values are reported as means ± standard deviation ($n = 3$). [b] Estimated by corresponding methyl esters.

profiles for peanut oil, which reported oleic acid as the predominant fatty acid (Worthington et al., 1972; Young et al., 1972, 1974a,b; Brown et al., 1975), we found that regardless of CO_2 concentration, linoleic acid was the most dominant fatty acid in oil extracted from peanut seeds grown hydroponically (Table 3). The observation apparently was related to growing the peanuts hydroponically and could be related to maturity of the seeds. Although the hydroponic seeds were harvested at 97 days after planting to avoid germination of the seeds resulting from the extremely high moisture in the hydroponic environment, these plants were 111 days old since an additional 14 days should be included from seeds to transplants. However, the cooler environmental temperature (22−28 °C) in hydroponics might still make these hydroponic seeds less mature than field-grown peanut seeds that would be harvested around 110−160 days (Young et al., 1972) from a much warmer outdoor climate. Young et al. (1972) stated that oleic acid increased with maturity, while linoleic acid decreased with maturity. Additionally, it has been demonstrated that environmental factors, including temperature and moisture, could significantly affect the ratio of oleic acid to linoleic acid in peanut oil. An increase in linoleic acid content and a decrease in oleic acid content were reported for dry land grown peanuts in Oklahoma and Georgia when supplemental irrigation was used during the 1968 and 1969 growing seasons by Young et al. (1974b). The effect of environmental temperature on peanut oil composition has been discussed extensively by Brown et al. (1975). They reported that monounsaturates increased and polyunsaturates decreased with increasing temperature. Quite often, air and topsoil temperatures could exceed 35 °C in the field. The environmental temperature inside hydroponic chambers where the peanut plants grew was generally lower (22−28 °C), which might also have contributed to the higher level of linoleic acid found in this study. However, other environmental factors such as photoperiod might also be involved and may be worth further investigation. Compared to peanuts grown at the ambient level, the fatty acid profile of peanut oil was not significantly affected by CO_2 enrichment (Table 3).

Table 4. Composition of Selected Mineral Elements in Peanut Seeds (Cv. Georgia Red) Grown Hydroponically at Elevated CO_2 Levels[a]

mineral element (mg/100 g of peanut)	ambient	700 ppm	1400 ppm
Ca	51.59 ± 0.32[c]	56.28 ± 1.71[b]	71.26 ± 0.42[a]
K	867.52 ± 21.10[a]	914.14 ± 24.80[a]	836.28 ± 17.78[a]
Mg	227.97 ± 2.69[a]	225.32 ± 6.02[a]	210.64 ± 8.38[a]
P	568.16 ± 7.97[a]	595.82 ± 19.33[a]	584.75 ± 17.24[a]
Al	0.11 ± 0.04[a]	0.23 ± 0.19[a]	0.16 ± 0.07[a]
B	2.50 ± 0.01[a]	2.54 ± 0.07[a]	2.29 ± 0.02[b]
Cu	0.05 ± 0.01[a]	0.08 ± 0.05[a]	0.07 ± 0.01[a]
Fe	1.17 ± 0.01[b]	1.85 ± 0.06[a]	1.28 ± 0.02[b]
Mn	1.86 ± 0.04[a]	1.59 ± 0.02[b]	1.72 ± 0.07[ab]
Mo	2.01 ± 0.02[ab]	2.20 ± 0.12[a]	1.75 ± 0.05[b]
Na	10.26 ± 2.40[a]	16.66 ± 9.88[a]	8.06 ± 1.44[a]
Zn	2.99 ± 0.03[a]	3.05 ± 0.10[a]	3.03 ± 0.01[a]

[a] Means in the same row with no superscripts in common differ ($p < 0.05$). Values are reported as means ± standard deviation ($n = 3$).

The mineral profiles of peanut seeds generally reflect the elemental nutrient supplements provided to the plant in its growing environment, which was solely supplied by the nutrient solution in this case. Potassium, phosphorus, magnesium, and calcium were the predominant mineral elements in these peanut seeds grown hydroponically. Certain trace elements in common practice may not be supplied in the field, and the plants must scavenge for these elements, which is very different from hydroponics, in which almost all of the elements are readily available. For instance, the absence of copper in the nutrient solution directly resulted in the nearly undetectable level of copper in these hydroponic seeds (Table 4). The content of selected minerals in peanut seeds grown hydroponically at elevated CO_2 levels compared to those grown under ambient CO_2 (control) was not significantly different, as was expected since all of the treatments were provided the same nutrient solution. Nevertheless, it would be interesting to examine the performance of peanut plants grown using different nutrient solutions since previous research has indicated that the omission of manganese and magnesium from the podding environment increased pod and seed weight, while omission of zinc reduced pod and seed weight (Zharare et al., 1993).

Our results demonstrate that certain peanut cultivars can be grown hydroponically using NFT and produce edible seeds under our experimental conditions. Ad-

ditionally, the relatively minor changes in nutritive composition of peanut seeds grown hydroponically compared to composition of conventionally grown peanut seeds confirm the potential of peanut as a suitable human food source in a CELSS as NASA originally intended. However, with its relatively lower oil content, the cultivar Georgia Red may not be the best choice of peanut cultivar since peanut was selected by NASA primarily for its oil. Additionally, the higher level of linoleic acid may result in a decrease in the shelf life of products containing peanut oil due to the higher degree of unsaturation. Therefore, other cultivars that express similar growth habits, with a fatty acid profile less sensitive to such environmental factors as moisture and temperature, may be incorporated in future studies. Nutritive analyses of peanut seeds grown hydroponically suggested that CO_2 enrichment appeared to have had little effect on proximate composition, amino acid profile, fatty acid profile, and mineral element content of peanut seeds compared to ambient CO_2 condition, although other portions of the biomass, such as the greens, may have been affected more significantly.

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