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Effect of Heat Stress on Phytochemical Composition of Peanut Seedlings

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Abstract: The present work was carried out to study the effect of heat stress (25°C, 35°C, 40°C) on the early growth of one of the most important oil crops, peanut. Results of the present work showed that heat stress increased the polyunsaturated fatty acids than saturated. But the ratio between oleic acid and linoleic acid was increased under heat stress. Regarding iodine and peroxide values present were increased with increasing temperature. Phenolic contents of the peanut seedlings were reduced at the low temperature, but increased at the highest temperature (40°C). γ-tocopherol was the most prevalent tocopherol than α- and δ- tocopherol. While, β- Sitosterol was the most abundant sterol under heat stress. Campesterol and stigmasterol were also present in significant concentrations. Some metabolites increase and other decrease which alleviated negative effects of heat stress on plant growth, metabolites and protects against the adverse effects of heat stress.

Key words: Heat stress, peanut, fatty acids, phenolic compounds, tocopherol-phytosterol

INTRODUCTION

The consumption of nuts has been shown to be beneficial to health. This is primarily due to their desirable lipid profile, which is higher in unsaturated fatty acids than saturated fatty acids. Although classified as legume, peanuts have a high lipid content (ca. 46%) that is rich in monounsaturated fatty acids, and they don`t contain cholesterol[20]. Epidemiological and intervention studies have shown that the frequent consumption of peanuts promotes cardiovascular health by lowing serum low denisty lipoprotein (LDL)-cholesterol levels and reduces the risk of development of type II diabetes[25]. It has also been shown to promote weight management when consumed as part of a moderate fat diet as a result of its satiating effect[21]. In addition to their nutrient composition, peanuts contain certain bioactive compounds that may also play a role in the reduction of the risk for the development of chronic diseases such as cancer, diabetes and coronary heart diseases[20]. These compounds, such as the isoflavones and *trans*-resveratrol, have been previously identified and quantified in peanuts by[31]. Legumes are inexpensive sources of proteins; however, they contain antinutritional factors such as tannins, phytates and trypsin inhibitors, which if ingested can reduce the nutritional quality of the food and led to undesirable physiological effects[23]. Therefore, they need to be processed prior to consumption to reduce the levels of these antinutritional factors. Peanuts, like other members of the legume family, are consumed mostly as processed products. Maguire *et al*.,[34] found that nuts are high in fats but have a fatty acid profile that may be beneficial in relation to risk of coronary heart disease. Nuts also contain other potentially cardioprotective constituents including phytosterols, tocopherols and squalene. Also, Johnston *et al*.,[26] gas chomatographic analysis of ER membrane phospholipids indicated that the fatty acids of 40°C samples were more saturated than their heat-shocked counterparts. These data indicate that heat-induced increase in aleurone ER membrane phospholipid fatty acid saturation may be important in maintaining secretory protein expression at normally nonpermissive heat-shock temperatures. The properties that we have documented in heat-stressed barley aleurone layers characterize a classic example of acquired thermotolerance, a condition where normally deleterious temperature can be tolerated by the organism. Typically thermotolerance is established by a brief, sublethel heat shock, followed by a recovery period and then lethel heat shock[40]. However,[22] observed that, thermotolerance can also be established by slow heating to heat-shock temperatures. In contrast to most of the previous studies on the establishment of thermotolerance, we have examined discrete cellular functions and structures rather than the overall survival of the organism.

From such a perspective it is easier to appreciate the physiological concerns an organism must address at the cellular level in order meet the challenge of surviving lethel heat shock. Nuts also played a role in cuisines throughout the world. South America, Asia and the Mediterranean region have long used tree and

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peanuts as ingredient in savory souces, stuffings, entrees, snacks, appetizers and deserts[36]. Plant phenolic compounds such as phenolic acids, flavonoids, tannins and lignans that possess substantial anticarcinogenic and antimutagenic effects[51], antioxidant and antiradical activities and antiproliferative potential[55], these phenolics provided protection against harmful effects of free radials and are known to reduce the risk of certain types of cancer, coronary heart disease and cardiovascular disease. Moreover, plant phenolic compounds are known to have multifuntional properties, such as acting as reducing agents (free radical terminator / scavenger), metal chelators, singlet oxygen and free radical quenchers[41].

Some epidemiologic studies have shown that consuming diets rich in plant derived foods that are high in phenolic compounds, even while consuming high amounts of saturated fatty acids, are associated with a reduced incidence of cardiovascular mortality[29]. Nuts contain a wide variety of phenolic acids and flavonoids that are predominatly conjugated with sugars other polyols via o-glycosidic bonds or ester bonds[9]. The composition and distribution of these conjugated compounds (quercetin, kaempferol, isorhamnetin, (+)-catechin and (-)- epicatechin) substantially underly the differences observed in the culinary, physiological and medicinal properties of these plant foods[39]. Phytosterols are found in plant foods and are structurally and functionally analogous to cholesterol in vertebrate animals. B-sitosterol, campesterol and stigmasterol are the most commonly occurring phytosterols and constitute 95% of total phytosterols in the diet. In addition to nuts, phytosterols are found in a range of seeds, legumes, vegetables and unrefined vegetable oils[54]. The effects of dietary supplementation with phytosterols on serum cholesterol levels in humans have been reviewed comprehensively[38]. In general, phytosterol supplementation tends to decrease serum levels of total and LDL cholesterol, and has little effect on serum levels of HDL cholesterol and triglycerides.

Tocopherols are powerful antioxidants and in high doses have been shown to lower the risk of CHD[42]. This cardioprotective effect is thought to be due to inhibition of LDL cholesterol oxidation, a key step in the atherogenic process.

Nuts also supply important vitamins, such as vitamin E, folic acid, vitamin B-6 and niacin and minerals. In addition, they may be a source of healthful biologically active components called phytochemicals. Examples of these are ellagic acid, flavonoids, phenolic components and isoflavones. Phenolic compounds concentration of peanuts is much lower than in soy and soy products. It is therefore important to determine how different peanut processing methods affect the concentration of these and other bioactive compounds, such as *trans*-resveratrol in peanuts, as this would consequently affect the potential health benefits derived from the consumption of peanuts and peanut products[57]. Therefore, the objective of this study was to evaluate the effect of heat stress on biochemical composition such as total fatty acid, iodine value, peroxide value, tocopherol and phytosterol content of peanut seedlings.

MATERIAL AND METHODS

Material: Peanut pods (Arachis hypogaea L.), variety Giza-5, samples were obtained from the Field Crops Research Institute, Ministry of Agriculture. The study was conducted at the physiology laboratory, Botany Department, Faculty of Science, Zagazig University during the growing season 2004

Methods: Peanut plants were subjected to different temperature stress (control 22ºC, 25ºC, 35ºC and 40ºC) for 2 weeks. The seeds were sown in 500ml plastic pots, filled with a mixture of the clay and the fine sand about 100g and each pot was watered daily with water. After 2 weeks the seedlings were collected and dried to constant weight at room temperature and use for analysis.

Chemical Analysis: A transmethylation technique by GC-FID determination was used. Fatty acid methyl esters (FAME) were prepared pouring 0.5g of oil and 250ml of methanolic 2 M KOH into a 15ml vial mixed in a vibration mixer for 60min. After 15min rest, 6ml of n-hexane were poured into the vial and the mixture was shaken in a rotatory shaker for 10min. The layers were allowed to separate and the hexane fraction was injected into GLC for analysis. Preparation of fatty acid methyl esters: Fatty acid methyl esters (FAME) were prepared from extracted oil by the method of Slover and Lanza[48].

Iodine and peroxide values: The peroxide value was determined using standard methods[35] the International Dairy Federation Standard Method 74A. Freshly extracted oil (0.1 g) was weighed into a glass test-tube and 9.8 ml chloroform / methanol (70: 30 v/v) was added and mixed. Following this, one drop of ammonium thiocyanate solution (30 g / 100 ml) and one drop of ferrous chloride were added and the tubes were vortexed. The mixed solution was allowed to stand in subdued light for 5 min and the absorbance was mesured at 505 nm against a reagent blank (9.9 ml chloroform/methanol mix, one drop of ferrous chloride and one drop of ammonium thiocyanate solution). The peroxide value of the oil was expressed as meq of oxygen/kg fat. Iodine value was determined according to the method described by AOAC[1].

Phenolic content was estimated in the ethanolic K-hydroxyde extract. Paper chromatography carried out on Whatman No. 1 paper using solvent systems: H_2O: HoAc (47: 3 v/v) and BAW (4: 1: 5 v/v). Samples analysed using HPLC[16].

Saponification for sterol and tocopherol analysis: Briefly, 40 mg oil was mixed thoroughly with 300 µl of 50% KOH (w/v) and 2 ml of 1% ethanolic pyrogallol (w/v) in screw-top tubes and kept for 30min. at 70°C in a water bath. The tubes were cooled on ice and 1 ml water and 4 ml hexane were added. The tubes were shaken vigorously and then centrifuged at 2000 r p m for 10 min. The hexane layer was removed and the extraction was repeated with a further, addition of 2 ml hexane. The combined hexane extracts were dried under nitrogen. The extract was redissolved in 200 µl ethanol, transferred to a plastic insert in a HPLC vial and stored at –20°C for later analysis according to a standard procedure[35].

RESULTS AND DISSUSION

Table (1) Illustrates that, fatty acids of peanut seedlings are characterized by the high amounts of oleic acid (C18:1) and linoleic acid (C18:2) (8.13% and 8.57% respectively) at heat stress (25ºC), but sharply decrease at temperature 40°C. Also, saturated fatty acid (myristic acid C_{14}, palmitic acid C_{16} and stearic acid C_{18}) increased after 25°C and 35°C, but decrease one at 40°C with traces of arachidic acid (C_{20}) and erucic acid (C_{22}) as compared with the control. The results also indicated that saturated fatty acid of peanut seedlings after 2 weeks inceased, which increased in a shortening of the chain length toward 14- and 16 carbons of fatty acids (71.0% and 32.73% respectively) at heat stress 35°C. On the other hand, total polyunsaturated and saturated fatty acids ratio (3.25) were appearent suppressed at heat shock 40°C and the absence of the erucic acid (unusual acid C_{22}) are typical characteristics of an oil that is suitable for human consumption. Erucic acid alleged toxic properties[33]. In this respect, Shaw and Brodl,[47] the correlation between increase in fatty acid saturation in ER membrane phospholipids and the continued expression of secretory proteins in barley aleurone layers at the normally nonpermissive heat shock temperature of 40°C. This change is consistent with the principle of homeoviscous adaptation and in the case of secretory protein synthesis in barley aleurone layers the adaptation may specifically affect integral membrane proteins of the ER that are essential to the translation and translocation of intergral membrane proteins. Who have hypothesized that homeoviscous adaptation helps to minimize the need for HSPs proteins would be reduced following homeoviscous adaptation. In addition, the adapted membrane would potentially influence the heat-sensing signaling cascade, leading to the reduced HSP expression. This increase in fatty acid saturation is maintained during extened exposure to heat-shock temperature, when, after 18 hours at 40°C, aleurone layers recover the synthesis and secretion of α-amylase and α-amylase mRNA levels recover to ~75% of non-heat-shocked controls[5]. These changes in ER membrane biochemistry would increase membrane viscosity at high temperature, working toward the maintenance of integral membrane protein function[26]. The ratio of oleic and linoleic acids (O/L) important features in determining peanut seed shelf life and oil stability. However, polyunsaturated fatty acids like oleic acid, linoleic and linolenic acids are fundamental in the human diet as they can`t be produced by animal metabolism[46]. Linoleic acid is a component of ceramides and it is precursor of arachidonic acid that can produce prostaglandins, thromboxanes, prostacyclin and leukotrienes[4]. Delplanque[12] suggests that the total daily energy intake should contain 11-16% oleic oil, 4-6% linoleic acid and 1% α-linoleic acid for best beneficial effects on lipemia and atherothrombotic parameters. Moreover, it should be better to prefer seed oils with low amount of saturated fats like, lauric, myristic and beheric acids because the presence of such compounds can negatively affect human lipid concentrations[3]. On the other hand, relationships were determined between O / L, tocopherol and sugar contents and variation in temperature and rainfall during the grain filling period of Florman INTA peanuts and it has been used as a means of predicting shelf life and oil better stability[6]. On the other side, Fasino *et al*.,[14] revealed that viscosities of the plant oil decreased exponentially with temperature (5-90 °C) which, the amount of monounsaturated FA or polyunsaturated fatty acid (PUFA) highly correlated (R_2 > 0.82) whereas poor correlations (R_2 < 0.17) were obtained between viscosities and the amounts of saturated and unsaturated FA. It is worthy to point that, fatty acids of ground nut exhibited significant differences for oil, palmatic, oleic and linoleic acids content under temperature and total sunshine hours[18]. Table (1) also revealed that iodine and peroxide values varied in small range by increasing heat stress when compared with the control. Also, peanut seedlings had higher value at 40°C than other treatment. The iodine value was (5.24%) significanlly higher than the control at 40°C. These results are in agreement with those reported by[7] However, the peroxide value was (19.3%) higher at heat stress (40°C) than the control. Meanwhile, the results proved that heat stress at 40°C had considerable peroxidation than other control. This observation is in accordance with the results obtained in this reported by[13]. The iodine value which

Table 1: Influence of the heat stress on the total fatty acid, iodine value and peroxide value contents of (2 weeks old) peanut seedlings (lot%).

Content / Treatment	Saturated										Unsaturated										
	6:0	8:0	10:0	12:0	14:0	16:0	18:0	20:0	22:0	Total	16:1	18:1	18:2	18:3	20:3	20:5	22:6	Total	Unsat. / Sat.	Iodine Value %	Peroxide value meq O_2/kg^{-1}
Control																					
22 °C	0.08	0.04	0.055	0.9	3.49	11.12	0.93	0.26	0.23	17.11	0.24	49.91	44.9	0.79	0.05	0.04	0.91	96.84	5.66	88.7	4.87
25 °C	0.15	0.08	0.09	1.31	4.63	12.83	0.98	0.31	0.27	20.65	0.68	53.97	48.75	1.3	0.09	0.1	1.3	106.19	5.14	89.53	4.95
35 °C	0.19	0.23	0.31	1.38	5.97	14.76	1.67	0.41	0.11	25.03	0.15	47.83	42.12	0.9	0.07	0.03	0.95	92.05	3.68	92.74	5.56
40 °C	0.2	0.34	0.39	1.6	6.38	8.31	2.68	0.47	0.05	27.02	0.21	45.53	40.9	0.63	0.041	0.03	0.61	87.95	3.25	93.35	5.81

Table 2: Influence of heat stress on the phenolic contents of (2 weeks old) peanut seedlings (µg / ml).

Treatment	Control			
Phenolic contents	22 °C	25 °C	35 °C	40 °C
Vanillic acid	0.195	0.2	0.25	0.39
p-coumaric	0.21	0.25	0.26	0.32
Ferulic acid	0.21	0.24	0.27	0.31
Naringinin-7-o-glucoside	0.17	0.19	0.25	0.32
Isorhamnetin-3-o-rutinoside	0.16	0.18	0.26	0.295
Quercetin-3-o-gentiobiside	0.19	0.21	0.29	0.33
Quercetin-3-o-glucoside	0.17	0.19	0.3	0.38
Quercetin-3-o-galactoside	0.175	0.185	0.295	0.36
Quercetin-3-o-glucosyl galactoside	0.18	0.195	0.287	0.35
1,3,6 tri-o-gallayl-β-glucopyranose	0.93	1.11	1.19	1.49
Total	2.59	2.95	3.65	4.54

is an important parameter could be used as an indicator for the stability and shelf-life of oils. Data presented in table (1) showed that iodine value provide value oleic to linoleic (O / L) ratio for peanuts heat stress up to 40°C were close to acceptable limits of industry point of view. The obtained results confirmed that the heat shock process contribution to the instability of peanuts may be due to both severe chemical changes such as destruction of natural antioxidants and physical changes such as disruption of cellular compartmentalization[37].

Table (2), showed that the total polyphenols contents were higher than those of the control under heat stress. The highest phenols content of peanut seedlings at heat stress can be attributed to the presence of proanthocyanidins in the peanut skin. Previous studies on the polyphenolic content of peanut skin show that it is rich in proanthocyanidins[32]. Meanwhile, Karchesy and Hemingway,[28] estimated the procyanidin content of peanut skins to be 17% by weight, 50% of which were low molecular weight oligomers. It was also observed that processing did not affect the total flavonoid content of the peanuts. However, this was not the case for the total polyphenols. Heat stressed peanuts had significantly ($p \leq 0.05$) higher total polyphenol content (36.4-38.6 mg GAE/g) than raw and roasted peanuts (20.1-28.8 mg GAE/g). These values are much higher than those reported earlier by Talcott *et al*.,[52], who observed that changes in total soluble phenolics were initially similar among cultivars of peanut, but antioxidant capacity was found to decrease by 62% on average during storage at 35ºC, independently of rates of lipid oxidation. Free p-coumaric acid, three esterified derivatives of p-coumaric and two esterified derivatives of hydroxybenzoic acid were found in peanut. This may be due to interference by other UV absorbing compounds, such as amino acids and sugars present in the extracts, that were not corrected for in the Folin-Ciocalteau assay. Several studies have shown that peanut hulls are rich in polyphenolic compounds that increase with peanut maturity giving rise to the high antioxidant capacity of peanut hull extracts Yen *et al*.,[56]. Also, Chukwumah *et al.,*[8] reported that polyphenols occur in nature in free or bound froms; thus some processing methods such as heating have been shown to increase the polyphenolic content of foods, free and hydrozable polyphenols in 10 oilseeds. However, Dabrowski and Sosulki,[10] demonstrated that defatted peanut flour contained *p*-coumaric, ferulic and caffeic acids in esterified forms. Also, the presence of

Table 3: Influence of heat stress on total tocopherol contents of oil extract of (2 weeks old) peanut seedlings (mg / g oil).

Contents / Treatment	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total tocopherol
Control				
22 °C	0.073	0.312	0.013	0.400
25 °C	0.098	0.510	0.019	0.627
35 °C	0.150	0.580	0.025	0.755
40 °C	0.051	0.320	0.010	0.361

Table 4: Influence of heat stress on the phytosterol contents of (2 weeks old) peanut seedlings (µg / g oil).

Contents / Treatment	Campesterol	Stigmasterol	B-Sitosterol
Control			
22 °C	188.30	160.80	1357.30
25 °C	180.70	168.90	1360.90
35 °C	167.81	171.50	1366.80
40 °C	150.87	175.80	1370.54

vanillin in peanut hulls and kernels of heat stress peanuts formed by the hydrolysis of lignin, a major constituent of the peanut hull, was established by Sobolev,[49], who suggests that during the heat stress process, as the peanut kernel absorbs the water that has permeated the hull, water soluble polyphenols from the hulls are also absorbed by the kernels[2] during heating, compounds with free amino groups, such as lysine, undergo a sequence of complex reactions with carbohydrates to produce tetrahydrofuran, melanoidins, pyrazines and their derivatives that impart color, flavor and aroma. While peanuts were heat stressed in-shell with their skin intact, it is possible that these ions correspond to the monomeric and oligomeric proanthocyanidins that have been reported to be present in peanut skin Lazarus *et al*.,[30]. Also, the peanut hull (shell) has been reported to contain vanillin (a hydrolytic product of lignin) and luteolin Sobolev,[49]. Although the total isoflavone content remains unchanged during thermal processing, several studies on the thermal stability of isoflavones in soy have shown that the thermal conversion of glycoside conjugates and degradation of isoflavone aglycones occurred at temperatures above 70°C and was pH dependent[50]. Heat stress had a significant effect on the phytochemical composition of peanuts compared to oil- and peanuts at 40°C had higher total isoflavone content with a two- and fourfold increase in biochanin A and genistein content, respectively[8]. On the other hand, Hashim *et al*.,[17] revealed that olives and extra virgin olive oil, rich in minor compounds like phenols are supposed to be effective in cancer prevention and they can reduce the inflammation process in tissues[11]. The molecular structure of phenols is important for their antioxidant activity, as this activity is enhanced by a second hydroxyl or a methoxy group in the ortho- or para- position[53]. Phenolic compounds, such as ρ-coumaric, ferulic acid and other hydroxy and methoxyl-substituted benzoic acids, are not highly regarded as effective antioxidants in a lipophilic environment[24], but their possible existence in esterified or protein-bound froms may augment their radical-scavenging properties *in vivo*.[19] observed that other compounds potentially present in roasted peanuts, such as Maillard-derived compounds, were shown to be effecftive suppressants of rancidity in model and food system, while proteins and protein hydrolysates were also reported to be antioxidants in model systems.

Regarding table (3), all treatments of heat stress show a preponderance of γ- tocopherol than α- and δ-tocopherol. But, peanut seedlings under 40°C revealed a low concentration of total tocopherol contents. Usually high amounts of TOCs are associated with PUFA contents[4,45]. Tocopherols act as antioxidants by trapping the hydroperoxide intermediates and stopping the autoxidation chain reaction. Differences in relative amounts of tocopherol-roles are important. α-TOC affects human nutrition and health aspects, while γ-TOC shows a strong activity in the seed protecting compounds like fatty acids. In this respect, Tuberoso *et al*.,[53] found that the amount of tocopherols is very high in maize and soybean seed oils. Some reportes suggested that γ-tocopherol has a higher antioxidant capacity in model systems than α-tocopherol[43]. Peroxide values have been used to assess rancidity in nuts[15]. In this study the peroxide value ranged between 4.87 to 5.81 meq O_2 / kg oil for peanut seedling under heat stress. These levels

indicated that the nuts were of good quality from the perspective of oxidative stability. Also, Savage *et al*.,[44] observed a positive relationship between the peroxide value and total tocopherol contents.

Table (4) showed that the level of β-sitosterol was the most abundant sterol, ranged from 1357.3 to 1370.54 µg / g oil and was present in much higher concentration than campesterol and stigmasterol under heat stress. Campesterol and stigmasterol were present at similar concentration (Tab. 4). Similar findings were reported by Maguire *et al*.,[34]. In comparison with the other nuts, peanuts had a significantly higher campesterol and stigmasterol content than the other nuts[27].

In conclusion, the present study illustrates some differences in total fatty acid composition, tocopherol and phytosterol contents at different temperature levels. In general, peanut seedlings had a favourable polyunsatyrated / saturated fatty acid ratio. However, peanuts contained considerably higher levels of γ-tocopherol. Phytosterol levels were higher in concentration of β-sitosterol than other sterols.

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