Effects of Cold and Heat Stress on the Chemistry and Cell Structure of Peanut Seeds[1]

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ABSTRACT

Frost damage and/or cold stress to crops in the United States runs more than $1 billion per year. In North Carolina during 1983, about 30,000 t of peanuts valued at $16 million were diverted from the edible market due to freeze damage. It is difficult to distinguish if a lot of peanut seed has been exposed to cold or heat stress because in both cases anaerobic respiration predominates. This research was undertaken to distinguish between cold and heat stresses at the cellular level on peanut seed by using dynamic headspace analysis, specific conductivity of the seed leachate, ion chromatography for cations and organic acids, ninhydrin detection for amino acids, and light microscopic techniques. Acetaldehyde, ethanol, and ethyl acetate are produced by both stress conditions. Two isomers of 2,3-butanediol and short-chain acids were identified from cold-stressed seed. These isomers were not found in heat-stressed peanut tissues and can be used to differentiate between the two environmental stresses. Specific conductivity of the leachate was higher from cold-stressed seed than from normal or heat-stressed seed, due to a higher efflux of potassium and acetic acid from the cells. Plasmolysis of the cold-stressed seed cells was consistently observed. Aleurone grains appeared to be larger, more dispersed, and tended to migrate towards the cell wall in the heat-treated samples, whereas the aleurone grains in the cold-stressed samples were smaller and more concentrated at the center of the cell. Irregular cell shape was common to both stresses.

Key Words: Cell structure, cold stress, heat stress, volatiles.

Plants of subtropical origin such as peanut are highly susceptible to chilling injury and/or freeze damage (Benedict and Ketring, 1972; Levitt, 1972a; Lyons, 1973). Biological stress to plants can be defined as any environmental factor capable of inducing injury. Although plants are incapable of regulating their temperature, they can erect barriers between the environment and the living matter within the cells (Levitt, 1972b). The effects of near freezing (less than 10 C) on plants of subtropical origin can be classified as chilling injury. On the other hand, freezing stress occurs only when temperatures drop below 0 C. Heat stress on highly hydrated resting tissue generally occurs at temperatures between 35 and 50 C (Levitt, 1972a,b,c). Singleton *et al.* (1971) found that peanut seed cured at 45 C had a higher concentration of off-flavor components than peanuts cured at 35 C or lower. Membrane damage occurs in cells exposed to either cold or heat stress and results in leakage of cellular constituents which disrupts metabolic processes.

Peanuts are harvested in late September to early October in North Carolina and Virginia. When peanut seed are removed from the ground, they have a moisture content ranging from 35 to 55% (wet basis) (Woodroof, 1973). The peanut vines are inverted and left in windrows for 3 to 4 d to reduce the moisture content to about 25% prior to combining. If the temperature drops below freezing, peanuts in the windrow are exposed to repeated freeze-thaw cycles which can produce extracellular ice and subsequent cellular dehydration. The economic loss to peanut farmers caused by freeze damage can be enormous. For example, in North Carolina during 1983, about 30,000 t of peanuts valued at $16 million were diverted from the edible market due to freeze damage.

Ashworth *et al.* (1985) found that factors effecting ice nucleation in soybeans, corn, and cotton plants include mass of sample, temperature, length of exposure, cooling rate, and the presence or absence of ice-nucleating bacteria. Moisture content, seed size, maturity, and cultivar were also important factors. Mass of sample and maturity in peanuts are related since at any harvest date there are some immature peanuts. Wampler (1983) found that peanut seeds weighing 0.8 g or less have a higher moisture content and are more affected by freezing temperatures than larger seeds. Singleton and Pattee (1989) determined that small seed (5.9 to 6.4 mm long) were more susceptible to chilling injury than larger seed, had higher concentrations of acetaldehyde and ethanol, and increased concentrations of organic and inorganic carbon in their leachate.

Both heat and cold stresses have the same effect on respiration in peanut seed. Dickens (1957) found that high-moisture peanuts subjected to heat stress produced an off-flavor. Whitaker and Dickens (1964) attributed this off-flavor to products synthesized during anaerobic respiration. Since both hot and cold conditions result in anaerobic respiration, similar volatile seed components may be produced as a result of either stress and similar off-flavors would be expected. Therefore, this study was initiated to elucidate the effects of cold and heat stress on the chemistry and cell structure of peanut seed and to identify chemical and physical differences to identify each type of stress.

Materials and Methods

Materials. Peanuts (cv. NC 7) were grown at the Peanut Belt Research Station, Lewiston, NC using recommended

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cultural practices. Cold-stressed peanuts were dug just prior to a freeze warning and were exposed to night temperatures ranging from -4.4 to -2.2 C over a 4-d period. The moisture content was about 25% when the peanuts were combined. They were bagged, transported to the laboratory, placed in a drying bin, and dried to a moisture content of about 7% using forced air at room temperature (25 to 28 C). The control peanut sample for both treatments was harvested immediately after digging, transported to the laboratory, and dried to 7% moisture as described above. All peanut samples were obtained from the same field.

Heat-stressed samples were produced by subjecting freshly dug peanuts to a drying air temperature of 40 C with 70% relative humidity. Air flow was 0.42 m/sec and type T thermocouples were used to monitor temperature. The air humidity conditions were controlled by spraying water at a controlled temperature into a chamber through which all drying air was circulated. Drying was terminated when moisture content reached about 7%.

Isolation of Volatiles. Volatiles were removed from homogenized peanut samples using a dynamic headspace and porous polymer trapping technique (Singleton and Pattee, 1980). A 100-g sample was blended with 300 mL of distilled, deionized water in an Omni blender for 1 min. An internal standard (2-propanol) was added to the sample mixture prior to blending. The homogenate was placed in a 1-L round-bottom flask fitted with a porous polymer trap. Valves were closed and the sample equilibrated for 15 min with constant agitation using a magnetic stirring bar. Volatiles were removed by aspirating for 5 min under vacuum (760 mm) and adsorbed on a Chromosorb 102 (60-80 mesh) polymer trap at ambient temperature. The polymer trap was removed, placed in the injector port of a Varian 3700 gas liquid chromatograph, and volatiles thermally desorbed at 200 C. Volatiles were separated on a Chromosorb 102 (80-100 mesh, 182.9 × 0.31 cm) column with the oven programmed from 100 to 200 C at a rate of 2 C/min. Peaks were integrated, concentrations calculated using an internal standard method, and data stored using a Spectra-Physics Winner Data collection system. A standard mixture of acetaldehyde, ethanol, pentane, ethyl acetate, pentanal, hexanal, and 2-propanol was used for calibration and calculation of response factors. To isolate 2,3-butanediol the temperature of the reaction flask was raised to and held at 50 C.

Mass Spectrometry. Confirmatory identification of the volatiles was accomplished using GLC/MS. Volatiles were trapped as previously described and thermally desorbed onto a glass capillary Carbowax 20 M column (30 m × 0.25 mm) installed in a Hewlett Packard gas liquid chromatograph. The oven of the chromatograph was programmed from 60 to 200 C at a rate of 2 C/min. The effluent from the gas chromatograph was split with a portion of the effluent gas stream directed into the ion source of a Hewlett Packard Model 5985B electron impact mass spectrometer. Final identification of the separated volatiles was made by comparing molecular ions, fragmentation patterns, and mass spectra data with authentic standards.

Conductivity Measurements. A 50 g sample of whole peanuts from each treatment was placed in an Erlenmeyer flask with 250 mL of distilled, deionized water, and shaken for 2 hr at 25 C on a wrist-action shaker. The leachate was decanted and conductivity was measured using a conductivity meter (Amber Science, San Diego, CA) equipped with a dip cell. Triplicate analyses were run for both control and treated samples.

Cation Analysis. Cation composition of the above leachates was determined by ion chromatography using a DIONEX 4000i ion chromatograph equipped with a cation micromembrane suppressor and a conductivity detector. A 20-μL filtered (nylon 0.22 m, 25-μm syringe filter, Micron Separations Inc., Westboro, MA) sample of leachate without further concentration was injected onto a HIPC-CS3 column (4 × 250 mm) via an air-actuated valve. Cations were eluted isocratically with 27.5 mM HCL/2.25 mM DL-2,3-diaminoproponic acid monohydrochloride (DAP)/2.25 mM L-histine monohydrochloride monohydrate solution at a flow rate of 1 mL/min. The regenerate solution was 70 mM tetrabutylammoniumhydroxide (TBAOH) flowing through the micromembrane suppressor at 5 mL/min. Eluted cations were detected with a conductivity detector at 300 mS. A potassium standard curve was used to establish linearity, and concentrations of potassium in the samples were calculated with an external chromatographic calculation method via a Spectra-Physics Winner (San Jose, CA) data collection system.

Organic Acid Analysis. Acetic acid concentration in the leachate from peanut seed was determined by ion chromatography using a conductivity detector. A filtered 20-mL sample from the leachate was injected onto a Dionex AS1 (ion exclusion) column and eluted isocratically with a 0.001 M HCl solution at a flow rate of 0.8 mL/min. The regenerant was a 0.1 M solution of TBAOH flowing through a microsuppressor membrane at 5 mL/min to suppress background conductivity. Concentrations of acetic acid were calculated using an external calculation method.

Amino Acid Analysis. Free amino acids in the leachate were separated on an AminoPac PA1 column using post-column derivatization with ninhydrin and an absorbance detector equipped with a ninhydrin filter. The eluting solvents and the gradient program used are presented in Table 1. Concentrations of amino acids were calculated with an external chromatographic standard calculation method.

Tissue Sample Preparation. Peanut seeds of the same

Table 1. HPLC gradient program for fast amino acid analysis.

Time	Eluant valve select[a]			
	No. 1	No. 2	No. 3	No. 4
min	%	%	%	%
0.0	10	0	90	0
5.5	10	0	90	0
5.6	12	50	38	0
13.0	12	50	38	0
13.1	0	45	35	20
17.0	0	45	35	20
20.0	0	0	20	80
22.0	0	0	0	100
24.0	0	0	0	100
24.1	100	0	0	0
28.0	100	0	0	0
28.1	10	0	90	0
40.0	10	0	90	0

[a]No. 1 = 0.3 M NaOH/0.11 M $Na_2B_4O_7$; eluant. No. 2 = 0.1 M NaOH; eluant. No. 3 = Water; eluant. No. 4 = 0.4 M Na acetate/0.01 M NaOH; eluant.

size from both stress treatments were cut to about 7-mm cubes and fixed in FAA (70% ethanol:glacial acetic acid:formalin; 9:0.5:0.5) for 72 hr and then transferred to 70% ethanol. Paraffin-embedded samples were cut in cross sections at 10 µm thickness. One-half of the samples were stained with safranin-fast green which stained the aleurone grains red, and the other half were stained with potassium iodide to visualize the starch granules. The samples were then processed for light microscopy as described by Pattee *et al.* (1983).

Results and Discussion

Stress Production of Volatiles. Results show acetaldehyde and ethanol increased to very high levels in peanut seed exposed to either stress condition when compared to the control (Table 2). Both of these chemicals compounds are products of anaerobic respiration and have been found in small peanut seed and bananas exposed to chilling temperatures (Murata, 1969; Singleton and Pattee, 1989). Ethyl acetate also was produced under both stress conditions but was not found in the control. There was no apparent effect on the pentane concentration, whereas hexanal concentration decreased under both stress conditions. Pentane and hexanal are produced by the enzyme lipoxygenase when peanut seeds are crushed (Singleton *et al.*, 1975, 1976), and the decrease in hexanal concentration was due to some lipid peroxidation. The highly unsaturated phospholipids in the cellular membrane are oxidized to some extent under heat and cold stress conditions (Singleton and Stikeleather, 1995).

Table 2. Effect of cold and heat stress on headspace volatile concentration.

	Treatment[a]					
	Control		Cold-stressed		Heat-stressed	
Compound	Avg	SD	Avg	SD	Avg	SD
	ppm		ppm		ppm	
Acetaldehyde	2.22	0.88	45.47	7.42	26.52	3.57
Ethanol	1.07	0.19	134.56	16.72	73.68	7.45
Pentane	11.31	0.86	12.51	2.04	12.96	0.98
Ethylacetate	0.00	--	1.23	0.33	1.49	0.49
Pentanal	0.01	0.01	0.01	0.01	0.01	0.01
Hexanal	23.33	1.27	13.85	1.77	14.50	2.69

[a]Average of three replications. SD = standard deviation.

Qualitatively, the data presented in Table 2 do not differentiate between cold- or heat-stressed peanuts but, quantitatively acetaldehyde and ethanol are significantly different. Increasing the temperature to 50 C during the isolation procedure enabled us to detect a qualitative difference between cold-stressed and heat-stressed peanuts. Two isomers of 2,3-butanediol were isolated by capillary GLC and identified by electron impact mass spectrometry. These isomers are not highly volatile due to the two attached hydroxyl molecules. Mass spectral data for one of the isomers and an authentic standard are presented in Table 3. Glycols and polyols have been isolated from other plants exposed to low temperatures and even from some insects with the onset of cold temperatures (Baust and Lee, 1983; Block and Somme, 1983; Roser *et al.*, 1992). The mechanism for the production of 2,3-butanediol in cold-stressed peanut seed has not been elucidated, but the presence of 2,3-butanediol in peanut seed could be a chemical indicator to differentiate between peanuts exposed to cold stress from peanuts subjected to heat stress.

Table 3. Major mass ion fragments of 2,3-butanediol.

	Ion intensity	
M/Z[a]	Standard	Freeze-damaged
	%	%
31	0.49	1.79
43	14.08	11.82
45	100.00	100.00
57	10.49	9.29
90	1.64	1.12

[a]Mass/charge.

Membrane Leakage. When plants and/or seed are exposed to cold or heat stress, alteration occurs in the integrity of the cellular membrane. Conductivity of the leachate from peanut seed exposed to both stress conditions was measured and cations, organic acids, and amino acids quantitated. Peanut seed have a relatively high concentration of potassium (Derise *et al.*, 1974). An increased efflux of both potassium and acetic acid occurred when peanut seed are exposed to cold or heat stress (Table 4). Conductivity of the leachate in the cold-stressed peanut seed was more than 3× greater than the control. In heat-stressed peanut seed, conductivity was about 1.6× greater than the control. Acetic acid concentration in cold-stressed seeds was about 8× greater than the control, whereas acetic acid in heat-stressed seeds was about 19× greater than the control. Although a trace amount of calcium also was found in the leachate, the increase in the conductivity of the leachate from stressed seed can be primarily attributed to the efflux of potassium and acetic acid. Ethyl acetate, which was present in the volatile profile (Table 2), may be due to an esterification reaction between acetic acid and ethanol which

Table 4. Conductivity, acetic acid, and potassium concentration of leachate from cold-and heat-stressed peanut seed.[a]

Sample	Conductivity		Potassium		Acetic acid	
	µmhos	SD	ppm	SD	ppm	SD
Control	127	32	24.67	2.70	1.69	0.52
Heat-stressed	213	20	33.03	7.20	20.68	2.5
Cold-stressed[b]	392	21.5	63.14	5.46	8.61	3.08

[a]Average of three replications. SD = standard deviation.
[b]Freeze-damaged in the windrow.

was found in the volatile profile of both stress conditions. Arora and Palta (1986) demonstrated that secondary freezing injury could be induced by placing onion bulb cells in a hypotonic solution of KCl, and leakage of potassium ions from cells has been associated with freezing injury (Palta *et al.*, 1977). Conceivably the secondary freezing injury mechanism may be the same in other plant tissue subjected to stress conditions. Regardless of the mechanism, membrane damage was caused by both stress conditions in this experiment.

Effect of Cold Stress on Concentrations of Free Amino Acid Found in the Leachate. Free amino acids increased in the leachate from cold-stressed peanut seed with the exception of arginine and phenylalanine. The greatest amino acid concentration increases in stressed seed, as compared to the controls, were a 4× increase in alanine and a 3× increase in serine. Kozukue *et al.* (1984) also found a 3× increase in alanine and a 2× increase in the serine fraction from the extract of eggplant (*Solanum melongena* L.) stored at 1 C for 13 d. They also found a 7× increase in alanine from the extract of cucumbers stored for 14 d at 1 C. The specific accumulation of alanine and serine in chilling-sensitive plants cannot readily be explained; however, it is well established that keto acids accumulate due to freezing injury (Hulme *et al.*, 1964; Murata, 1969) and that alanine is related to the enzyme glutamate-pyruvate transaminase reaction (Wrightman and Forest, 1978). Membrane leakage results in the leaching of cellular constituents, leading to a metabolic imbalance, and would render these seeds unusable for the edible seed market. The heat-stressed sample was not analyzed due to extended equipment breakdown. However, similar leaching would be expected because heat stress also causes membrane damage.

Visual Characteristics. Peanut seed lots suspected of being exposed to freezing temperatures are diverted from the edible market after visual inspection. If the percentage of freeze-damage seeds exceeds 0.5% for segregation no. 1 farmer stock peanuts, the lot is placed in Commodity Credit Corporation loan and is diverted from the edible market (Anon, 1991). When seed coats are removed from freeze-damaged and control peanut seed samples, the most evident characteristic of freeze damage is the shining-glassy appearance on the inner and outer surfaces of the cotyledons as opposed to nonglassy cotyledons in healthy tissue (Fig. 1). Freeze-damaged seed also have pitted surface areas (Fig. 1), darkened embryos, seed coats with a lighter color, and a rubbery texture.

Effects on Cellular Structure and Subcellular Particulate Bodies. Both heat (Fig. 2A) and cold (Fig. 2C) stress conditions produced cells of irregular shape as compared to the regular shape and symmetry of the cells in the control sample (Fig. 2B). Protoplasmic swelling occurred in some of the cells in the cold-stressed sample. Freeze damage is transient and, at any given time, only a proportion of the cells will exhibit this cytological aberration (Arora and Palta, 1986).

Subcellular particulate bodies visible in the photomicrographs are protein bodies called aleurone grains. In the most severely damaged cells of the cold-stressed

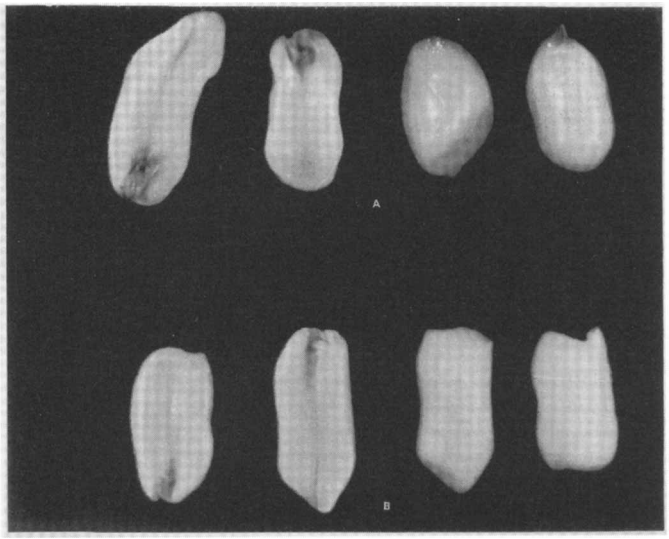

Fig. 1. Comparison of freeze damaged and undamaged cotyledons: (A) cold-stressed, (B) control.

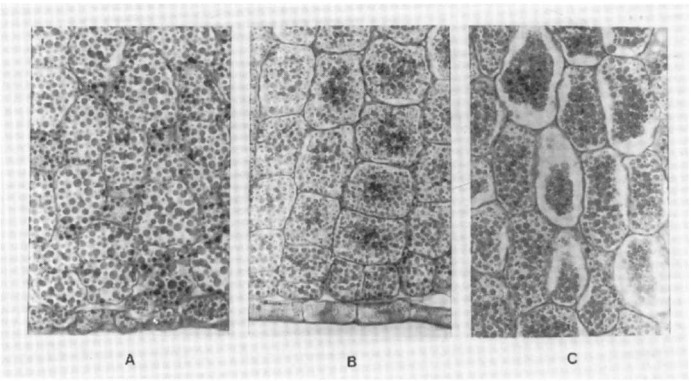

Fig. 2. Effect of cold and heat stress on aleurone grains in peanut tissue: (A) heat-stressed seed, (B) control seed, (C) cold-stressed seed. Aleurone grains in peanut tissue were identified by safranin-fast green staining of cross-sections which stained the aleurone grains red.

peanut seed tissue the aleurone grains are concentrated in the center of the cell and are not as well defined or dispersed as the aleurone grains in the control. Aleurone grains in the heat-stressed tissue appeared to be larger than those in the cold-stressed and control samples. This was probably due to cellular expansion which occurs under heat stress (Levitt, 1972c). Aleurone grains are the major depositories for potassium and magnesium as well as phytic, oxalic, and citric acids (Dieckert *et al.*, 1962). Membrane damage occurring under stress conditions results in leakage of cellular constituents, which corresponds to increased levels of potassium found in the leachate from peanut seed exposed to both cold and heat (Table 4).

Starch granules in the heat-stressed sample (Fig. 3A) were considerable smaller, fewer in number, and migrated to the outer edges of the cell as compared to the starch granules in the control (Fig. 3 B). Injury due to heat stress increases the rate of respiration and decreases the reserves of starch and protein (Levitt, 1972c). Differ-

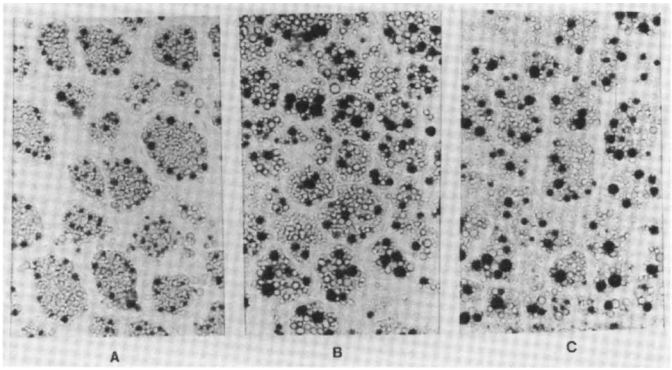

Fig. 3. Effect of cold and heat stress on starch granules in peanut tissue: (A) heat-stressed seed, (B) control seed, (C) cold-stressed seed. Starch granules in peanut tissue were identified by potassium iodide staining of cross-sections.

Table 5. Effect of freeze damage on the concentration of amino acids found in the leachate from peanuts.[a]

Amino acid	Control		Freeze damage	
	Avg	SD	Avg	SD
	nM		nM	
ARG	120.54	10.55	117.19	1.82
LYS	45.50	2.64	56.41	2.48
THR	16.75	3.32	150.53	0.74
ALA	43.57	7.75	180.88	12.72
GLY	10.24	1.64	78.80	4.30
SER	42.00	5.88	149.42	5.41
VAL	35.16	0.97	53.81	2.74
PRO	18.79	0.91	30.64	7.34
ILE	19.79	0.35	34.78	2.58
LEU	17.06	0.09	31.71	4.01
MET	3.03	0.26	5.81	0.75
HIS	8.85	0.32	13.30	1.11
PHE	40.40	13.56	35.98	2.72
GLU	92.05	3.84	125.02	1.80
ASP	45.89	1.25	88.23	0.54
CYS	9.62	1.60	24.88	5.07
TYR	27.52	5.24	33.85	4.27

[a]Average of three replications. SD = standard deviation.

ences in the starch granules between the cold-stressed and the control sample were not readily apparent; however, starch granules in the control appeared to be darker. Starch granules have been shown to decrease in size in apple cortical cells when exposed to low temperatures (Kuroda and Sagisaka, 1993). Distinct differences were observed in the size and placement of starch granules in the heat- and cold-stressed peanut tissue samples (Fig. 3A and 3C, respectively). Extracellular freezing induces cell contraction (Levitt, 1972), whereas heat stress induces cell expansion. This would explain the differences in the placement of starch granules relative to the cell wall in the cold- and heat-stressed peanut seed.

Conclusions

Cold and heat stresses induce anaerobic respiration in peanut seed resulting in relatively high concentrations of acetaldehyde and ethanol in the volatile profile. Ethyl acetate also was produced under both stress conditions. 2,3-Butanediol production was induced in cold-stressed peanut seed, whereas this compound was not found in heat-stressed peanut seed. Membrane damage was caused by both cold and heat stresses, resulting in leakage of cations, amino acids, and organic acids into the leachate. Photomicrographs of tissue slices from stressed peanut seed showed that cellular damage had occurred, as evidenced by irregular cell shape, contraction of cellular constituents in cold-stressed seed, and expansion of cellular constituents in heat-stressed seed.

Literature Cited

Anon. 1991. Farmer Stock Peanuts Inspection Instructions, USDA, Agric. Marketing Service, Fruit and Vegetable Div., Washington, DC. p. 35.

Arora, R., and J. P. Palta. 1986. Protoplasmic swelling as a symptom of freezing injury in onion bulb cells. Plant Physiol. 82:625-629.

Ashworth, E. N., G. A. Davis, and J. A. Anderson. 1985. Factors affecting ice nucleation in plant tissues. Plant Physiol. 79:1033-1037.

Baust, J. G., and R. E. Lee, Jr. 1983. Population differences in antifreeze/cryoprotectant accumulation in Antarctica insects. Oikos 40:120-124.

Benedict, C. R., and D. L. Ketring. 1972. Nuclear gene affecting greening in virescent peanut leaves. Plant Physiol. 49:972-976.

Block, W., and L. Somme. 1983. Low temperature adaptations in beetles from the sub-Antarctica island of South Georgia. Polar Biol. 2:109-114.

Derise, N. L., H. A. Lau, S. J. Richety, and E. W. Murphy. 1974. Yield, proximate composition and mineral element content of three cultivars of raw and roasted peanuts. J. Food Sci. 39:264-266.

Dickens, J. W. 1957. Observations related to the flavor of bulk cured peanuts. Assoc. South. Agric. Workers Proc. 54:37-38.

Dieckert, J. W., J. E. Snowden, A. T. Moore, D. E. Heinzelman, and A. M. Altschul. 1962. Composition of some subcellular fractions from seeds *Arachis hypogaea*. J. Food Sci. 27:321-325.

Hulme, A. C., W. H. Smith, and L. S. C. Wooltorton. 1964. Biochemical changes associated with the development of low temperature breakdown in apples. J. Sci. Food Agr. 15:303-307.

Kozukue, N., E. Kozukue, T. Hirose, and S. Mizuno. 1984. Accumulation of alanine in chilling-sensitive crops. Hort. Sci. 19:498-501.

Kuroda, H., and S. Sagisaka. 1993. Ultrastructural changes in cortical cells of apple (*Malus pumila* Mill) associated with cold hardiness. Plant Cell Physiol. 34:357-365.

Levitt, J. 1972a. Low-temperature stresses—The freezing process, pp. 44-60. *In* T. T. Kozlowski (ed.) Responses of Plants to Environmental Stresses. Academic Press, New York and London.

Levitt, J. 1972b. Stress and strain terminology, pp. 2-8. *In* T. T. Kozlowski (ed.) Responses of Plants to Environmental Stresses. Academic Press, New York and London.

Levitt, J. 1972c. High-temperature or heat stress, pp. 229-271. *In* T. T. Kozlowski (ed.) Responses of Plants to Environmental Stresses. Academic Press, New York and London.

Lyons, J. M. 1973. Chilling injury in plants. Ann. Rev. Plant Physiol. 24:445-446.

Murata, T. 1969. Physiological and biochemical studies of chilling injury in bananas. Physiol. Plant. 22:401-411.

Palta, J. P., J. Levitt, and E. J. Stadelmann. 1977. Freezing injury in onion bulb cells. I. Evaluation of the conductivity method and analysis of ion and sugar efflux from injured cells. Plant Physiol. 60:393-397.

Pattee, H. E., S. C. Mohapatra, and E. K. Angello. 1983. Specimen preparation techniques for scanning electron microscopy of developing peanut pegs. Peanut Sci: 10:93-97.

Roser, D. J., D. R. Melick, H. U. Ling, and R. D. Seppelt. 1992. Polyol and sugar content of terrestrial plants from continental Antarctica. Antarctic Sci. 4:413-420.

Singleton, J. A., and H. E. Pattee. 1980. A preconcentration and subsequent GLC analysis method for trace volatiles. J. Amer. Oil Chemists Soc. 57:405-408.

Singleton, J. A., and H. E. Pattee. 1989. Effect of chilling injury on windrowed peanuts. Peanut Sci. 16:51-54.

Singleton, J. A., H. E. Pattee, and E. B. Johns. 1971. Influence of curing temperature on the volatile components of peanuts. J. Agr. Food Chem. 19:130-133.

Singleton, J. A., H. E. Pattee, and T. H. Sanders. 1975. Some parameters affecting volatile production in peanut homogenates. J. Food Sci. 40:386-389.

Singleton, J. A., H. E. Pattee, and T. H. Sanders. 1976. Production of flavor volatiles in enzyme and substrate enriched peanut homogenates. J. Food Sci. 41:148-151.

Singleton, J. A., and L. F. Stikeleather. 1995. High-performance liquid chromatography analysis of peanut phospholipids. II. Effect of postharvest stress on phospholipid composition. J. Amer. Oil Chem. Soc. 72:485-488.

Wampler, J. D. 1983. Detection of freeze damage in Virginia peanuts (*Arachis hypogaea* L.) by conductivity, tetrazolium, and visual test. M.S. Thesis. Virginia Polytech. Inst., Blacksburg, VA. p. 54.

Whitaker, T. B., and J. W. Dickens. 1964. The effects of curing on respiration and off-flavor in peanuts, pp. 71-80. Proc. Third Nat. Peanut Conf. Res. Div., Virginia Polytech. Insit., Blacksburg, VA.

Woodroof, J. G. 1973. Peanuts: Production, Processing, Products. AVI Publ. Co., Westport, CT.

Wrightman, F., and J. C. Forest. 1978. Properties of plant aminotransferase. Phytochem. 17:1455-1471.

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