Lipid Oxidation of Edible Peanut Pastes during Storage with Variation of Environmental and Processing Factors [1,2]

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ABSTRACT

Storage studies were performed for a period of 127 days to evaluate factors of processing and storage environments affecting rancidity development of peanut paste. Factors of milling (stone slab or plate-type attrition mill), ethylenediaminetetraacetic acid (none or with 100 ppm EDTA additions), oxygen (vacuum or atmospheric packaging), light (dark or fluorescent illumination) and temperature (4C or 45C) were incorporated into a $2^{(5-1)}$ fractional-factorial design. Rancidity developments were monitored using thiobarbituric acid reactive substances (TBARS) number and peroxide values. Initially dominant, the positive effect of oxygen on peroxidation was subsequently outpaced by the effect of temperature. Throughout the study, the effect of temperature on peroxidation levels was consistently positive and statistically significant. Significant positive interactions of the two effects continued from the second week to the end of the first month and a late appearance of negative interactions between light and temperature were noted. Elevated levels of TBARS were shown after 8 weeks without consistent association to the particular design factors.

Key Words: Peroxide value, thiobarbituric acid reactive substances, half-fractional factorial, milling, package barrier.

Among grain legumes, peanuts (*Arachis hypogaea* L.) serve as an important source of fat and protein. Peanuts contain 47.5% fat and about 80% of the total fat is composed of unsaturated fatty acids which may undergo oxidation during storage. As with other food products, oxidative rancidity of peanut products, including peanut paste, poses a serious problem. St. Angelo and Ory (12, 13) examined the effects of enzymatic and non-enzymatic catalyzed peroxidation of raw and roasted peanuts during storage. They noticed that since roasting destroyed the enzymes present in raw peanuts, the greater rate of peroxidation for roasted peanuts was probably due to lipid oxidation catalyzed by metalloproteins in roasted peanuts. Effects of added salts and metalloproteins on lipid oxidation in peanut and other fat-containing food products have been studied (2, 8, 15, 16, 20). Hydroperoxide decompositions promoted by particular transition metals (especially metals possessing two potential oxidation-reduction states with prime concern noted for the lower valency value) have been postulated for the mechanisms of lipid oxidation (4, 19). Peroxide value (PV) and thiobarbituric acid reactive substances (TBARS), which are taken as indices of oxidative rancidity, are influenced by several factors (9, 14).

In many developing countries, particularly in the semi-arid and tropical (SAT) regions of Africa, peanut food products are consumed. A considerable amount of peanuts in SAT-Africa are consumed in the form of spreadable peanut paste prepared traditionally (17). The process involves a light roasting step followed by milling with a rolling action between a stationary, flat stone and a manually manipulated cylindrical object (e.g., stone or glass bottle). These manual efforts produce small quantities of paste by substantial labor inputs.

The proposed introduction of plate-type attrition mills as a more expeditious method of milling edible peanut pastes in the SAT-Africa region has raised a concern over any effects of metals from the cast and steel alloy surfaces of the mill on the rates of quality deterioration of peanut pastes obtained from such mills. The metal contents (copper, iron and zinc) of the peanut pastes may be used to estimate the fraction of metals introduced into the peanut paste preparations from the surfaces of the mill. However, further efforts to evaluate the effects of other storage conditions on metal catalysis of lipid oxidation are considered since interaction is the rule rather than the exception in real food systems such as this peanut product. This study focuses on examination of the effects of traditional milling and alternate processing and storage methods on lipid oxidation of peanut paste indicated in measures of peroxide value and thiobarbituric acid reactive substances.

Materials and Methods

US grade #1 peanuts, cv. Florunner, were obtained from GoldKist, Inc., Huntsville, AL and stored at room temperature until used.

Experimental setup

The experiment was performed using a $2^{(5-1)}$ fractional factorial design consisting of five factors (milling method, chelator, air, light, and temperature) each designated at "+" and "-" levels. The alternative processes of milling were designated negatively (-) for stone slab and positively (+) for the plate-type attrition device. Similarly, the conditions of storage including modified presence of air in the package (air-evacuated or open to atmospheric air), illumination (total darkness or fluorescent lighting), temperature (4C and 45C and sequestrant (with or without EDTA addition) were designated positively or negatively, respectively, according to whether the condition was the higher (+) or the lower or absent (-), respectively. Permutations of the five factors at two levels produce 2^5 combinations but half that number were used (i.e., $2^{(5-1)}=16$) in which all combinations of the first four factors were generated and the fifth was designated (plus or minus) by the algebraic product of the signs of the four (i.e., E=ABCD). Additionally, two partial replications and three control preparations were performed. Selected combinations of these five factors at their corresponding levels were assigned to the preparation of peanut paste in a randomly ordered process.

Peanut roasting

Peanuts were roasted for all preparations in a wok pan using an electric hot plate as the heat source. Ceramic beads, 1.6 mm in diameter (obtained from Coors Porcelain Co., Golden, Co), were used as a heat transfer medium in this particle-bed roaster. Ceramic beads were heated and stabilized at 210 ± 5C before the peanuts were added. The ratio of the ceramic beads to peanuts was 3:1 (w/w). Roasting lasted an estimated time of 15 min (starting from the time the temperature of the peanuts and the ceramic beads returned to 210 ± 5C) until nuts were separated from the heating medium. The testa of the roasted peanuts were loosened by hand rubbing and the loosened testa were winnowed free from the peanuts.

Milling and sequestrant treatments

In the preparations where the effects of metal chelators were tested,

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0.04 g EDTA was added to 400 g of roasted peanuts before milling. Milling alternatives included a traditional approach employing a flat marble slab and a smooth glass bottle as earlier described and a plate-milling option using a model 4-B Quaker City Mill (Philadelphia, PA). The spreadable peanut pastes prepared by these methods were placed into glass vials and stored in the alternate environments for further analyses as described below.

Temperature, light, and vacuum treatments

Peanut paste products designated for air-evacuated storage were placed into thick-film, clear, plastic pouches (approx. 6 vials per pouch) and sealed after evacuation using a Turbovac model SB-320 vacuumizing pouch sealer (Hantover, Kansas City, MO). Additional heat sealings between each of the individual vials permitted serial opening without affecting the vacuum treatment of the remaining samples. Other portions of the peanut paste materials were stored completely open to the air within the environmental chambers. Products designated for light treatment were stored in 704-series environmental chambers (Lab-Line Instruments, Inc., Melrose Park, IL) with a single 30W fluorescent tube illumination through the entire period of study, while those designated for dark were placed in 700-series chambers with no internal lighting and the windows occluded. The products designated for storage at refrigerated temperatures were kept, respectively, in cabinets 700-A and 704-A with temperatures set at 4C while those designated for above ambient storage were kept, respectively, in cabinets 700-SDH and 704-DH at 45C. Approximately half, except for partial replications (random), of the experimental pastes were prepared and stored at either the high or low level of each factor. Control products were stone milled without EDTA, without evacuation and stored in the dark at ambient temperature (approx. 25C).

Metal assays

Raw peanuts and roasted peanuts, milled alternately by the stone and plate methods, were each analyzed for iron, copper and zinc to estimate the fraction of metals introduced into the peanut samples from the surfaces of the attrition mill. Metals were determined using the atomic absorption spectrophotometric method of Persmark and Toregard (10) with the following modifications. Glassware was immersed in EDTA (0.5%) for 24 hrs and thoroughly rinsed with de-ionized glass-distilled water to prevent contamination by extraneous metals.

Measurements of rancidity

The developed rancidities of all the samples were monitored by measuring peroxide values on a weekly basis for eight weeks, biweekly for six additional weeks, and finally once more following a four-week extension. Thiobarbituric acid reactive substances (TBARS) were determined at one and two weeks and thereafter on an every other week basis until the 14th week and finally again after four more weeks.

Peroxide values were determined using the official method of the American Oil Chemists' Society (1) modified to permit determinations on 0.5 g of peanut oil. Approximately 5 g of each peanut paste sample was extracted with 30.0 mL of hexane. Hexane was evaporated under nitrogen using a Meyer N-Evap analytical evaporator to obtain the oil for peroxide values. The distillation method of Tarladgis *et al.* (18) as modified by Ke *et al.* (8) was used in distilling thiobarbituric acid reactive substances (TBARS) except that propylgallate and EDTA were added during the blending process as recommended by Rhee (11). The TBARS values were expressed as micromoles malonaldehyde per gram of peanut sample.

Statistical analyses

Three replications of each milling treatment with two parametric determinations of the metal levels as dependent variables were performed. Duncan's Multiple Range Test analyses were conducted with the measurements of the metal levels to determine differences of the milling treatments at $p < 0.05$ significance criteria level.

All of the independent variables in the fractional factorial scheme were designated as qualitative factors (not evaluated at more than two levels) rather than quantitative variables with continuous variation and magnitude. Therefore, the experimental set-up was designed to illustrate trends and relative magnitude of effects such that some projections towards optimization might be realized. Results of the various quality variables were displayed employing the CADE DISCOVERY Module (International Qual-Tech, Ltd. Plymouth, MN) in order to find the main effects and their interactions (5, 6). The sixteen (2^{5-1}) responses make up the orthogonal data sets for each measure and storage duration with the two partial replications only used to estimate a residual mean square employed to initially judge the significance of any of the five single-factor effects and the ten combination interaction effects. Because the experiment represented a half-fractional design four-factor interactions confound with the single-term factors (e.g., ABCD=E) and three-factor interactions with the two-factor terms (e.g., ABC=DE). This means that all two-factor interactions were not distinguishable from three-factor interaction effects and little can be offered to suggest meaning of the four-factor interactions.

The experimental design is still considered valuable because it permits discovery of significant effects without extending the size of the experiment to full factorial dimensions ($2^5 = 32$). Furthermore, judgements of factor and interaction significances can be particularly improved by selecting a reduced-factor model regression by eliminating single factors and two-factor (or their paired three-factor) interactions that are shown by their preliminary assessments to be not significant. Then an analysis of variance including the sums of squares associated with the discarded terms together with sums of squares from the replication can be pooled to form a better estimate of the residual mean square (error term).

Furthermore, distinctions between the confounded two-factor and three factor interactions can frequently be offered by interactive modeling provided by the CADE routine in which alternate regression model expressions composed of the terms of either the two- or three-component interactions can be analyzed. Selection of the best alternate of the confounded pair will be based upon the analysis of variance that shows the greatest degree of variance accounted for by either of the alternate regression models and appropriate significance.

Results and Discussion

Metal content

The levels of copper, iron and zinc of raw peanuts and peanut pastes prepared by stone milling and plate milling are presented in Table 1. Except for copper the levels of metal detected in the stone-ground product was not significantly increased compared to the raw peanuts suggesting only a minor change with this milling process. Peanut pastes prepared by plate milling contained higher amounts of copper, iron and zinc compared to the stone ground product and raw peanuts. Of the three, iron content was especially high in plate milled peanuts. Tay *et al.* (20) implicated a dominant role of iron in oxidative rancidity of meat. The catalytic effect of copper was judged approximately the same as that of iron in oxidation of meat lipids (7). Since iron and copper possess alternate oxidation states and may catalyze lipid oxidation by enhancing the decomposition of hydroperoxides, reductions of activity for these contaminants should be favored in order to maintain peanut paste quality. Inclusion of suitable chelators may help reduce the activity of any metal contaminants.

Peroxide value

Assessments of developed lipid oxidation as measured by peroxide values are shown in Table 2. The changes of the overall factorial experiment means (16 determinations per time) during the 127 day period indicate that the peroxide titers increased to a maximum of 15.43 meq/kg at day 85 and subsequently declined. Because the experimental factors

Table 1. Concentrations of copper, iron and zinc in raw peanuts and pastes of roasted peanuts prepared by stone-milling and plate-milling methods.

	ppm[1]		
Treatment	Cu	Fe	Zn
Raw peanuts	11.4[c2]	31.1[b]	71.7[b]
Roasted & stone-milled	13.0[b]	42.8[b]	83.5[b]
Roasted & plate-milled	14.5[a]	456.1[a]	102.8[a]

[1] Means of 3 replications with duplicate analyses for each parameter assayed by atomic absorption spectrophotometer.

[2] Means not followed by same letter within the respective columns are significantly different by Duncan's Multiple Range Test ($p < 0.05$).

Table 2. Peroxide value mean effects and significant main factor and interaction effects for peanut pastes prepared with alternate processing methods and stored with contrasting environments for varying lengths of time.

Peroxide Values (meq/kg)

Day of assay = d	d8	d15	d22	d29	d36	d43	d50	d57	d71	d85	d99	d127
Overall Means	**.14**	**1.96**	**3.64**	**4.69**	**4.66**	**5.47**	**6.18**	**7.07**	**8.80**	**15.43**	**9.50**	**13.00**
A=Milling Method												
B=Chelator												
C=Air (Oxygen)	.39*	2.96***	3.10*	5.72***	+NS	+NS		3.11*	5.30*	+NS		
D=Light							(-)		(-)		(+)	+NS
E=Temperature	.36*	2.76***	**6.48*****	**8.78*****	**8.70*****	**10.06***	**10.76*****	**12.06*****	**13.03*****	**24.70*****	**13.18*****	**14.55****
Interactions:												
CE=abd		2.26***	2.95*	5.42***								
DE=abc							-NS		-5.00*		-2.92*	
RMS (error est.)	.1062	.5038	1.992	1.817	4.914	25.07	10.37	8.025	15.52	38.95	7.487	61.56
Coef. determination (R^2)	.492	.942	.908	.962	.799	.525	.721	.842	.813	.823	.881	.446

BOLD print identifies the mean effect and the largest factor effect on any day of assaying.
*** indicates significance at $p < 0.001$
** indicates significance at $p < 0.01$
* indicates judged significant at $p < 0.05$
All judgments of significance for the single-factor and interaction effects were made with orthogonal data sets (no partial replications) employing the Model 1 LSMLMW least squares application of Harvey (3).
When present "NS" indicates the second ranked effect but not significant.
Where present, entry in parentheses (+/-) indicate non-significant linear effect (positive or negative) in model necessary to consider interaction term composed of the same term.

bracketed the conditions of the three controls completed concurrently with the factorial experiment no significant differences were found between the means of either group as anticipated.

Table 2 shows each single-factor term determined significant and two of the two-factor-interaction effects that were sometimes judged significant in interactive modeling. A single-factor effect indicates the difference between the average of the results with a particular factor (e.g., C for air level) when (by design) the independent factor is designated high versus the average results when the factor is designated low or absent. As early as day 8 in this assessment, the peroxidation effect associated with presence of air (C) was judged clearly significant (0.39* meq/kg, $p < 0.05$). Temperature (E) was shown with nearly as much effect as air (0.36*, $p < 0.05$, i. e., peroxidation averaging 0.36 meq/kg higher when storage at the higher temperature was contrasted to the refrigerated). Additional factor terms and interactions of these terms which were preliminarily judged significant for the day 8 assays were eliminated by interactive modeling as the clearly non-significant terms were pooled into the residual mean square estimate of error for the analysis of variance.

Two-factor interactions displaying significance such as DE) imply that greater than random differences occur in the average effects of one of the factors as the other factor changes than would be expected without some manner of mutual augmentation by the factors. Consider a comparison made between the averages of all responses when both factors (e.g., D & E) were designated high (light *on* and 45°C) and also when both low (dark and 4°C) in contrast to the average of all responses when one factor was designated low (e.g., D: low = dark) and the other high (e.g., E: high = 45°C) or the reversed combinations (high and low). Thus, light and temperature interaction (DE) implied that when both were represented at their high value (or possibly both low) much greater lipid oxidation occurred than when either one of these design factors was designated high by itself.

Over the long term, presence of air and temperature effects were evident with the effect of air stronger for the first two weeks and the temperature effect surpassing it for the remaining time. Interactions (CE) for these two design factors (C & E) remained from the second to fourth week and then diminished at about the same time that the effect of air was judged no longer significant. While a resumption of significance was noted for the presence of air as a single-factor effect, the interaction effect of air with temperature (temperature continued as the most significant effect) did not return. Total or perpetual exclusion of air from the vacuumized pouches was not expected. Rather the design was to consider a downward modification of the level of air and the oxygen it contains. A mechanism of oxygen permeating into the pouch is suggested, since the air (oxygen) factor effect was not maintained in the most dominant place as was seen from the beginning.

The emergence of a negative interaction effect (DE=abc) was shadowed in the 7th week and clearly indicated at -5.00* on day 71 and -2.92* on day 99. This DE interaction appearing positive in the earliest weeks (but failing to prove significant) subsequently became negative might mean that peroxides were degraded into secondary products by the combined influence of light and elevated temperature. The data show declines of the peroxide titer with light *on* versus *off* at the high temperature but still some increases in titer with light on at the lower temperature. The high overall mean peroxide value at day 85 followed by decline to values in line with the trend previously seen gives credence to a decomposition hypothesis. This hypothesis of decomposition mediated by light and temperature (DE) was validated by considerations of the confounding three-factor interaction ABC. Each regression expression including the terms of the ABC interaction proved to be inferior in terms of the coefficients of determination offered for these alternate models.

Table 3. Thiobarbituric acid reactive substance (TBARS) mean effects and selected main factor and interaction effects for peanut pastes prepared with alternate processing methods and stored with contrasting environments for varying lengths of time.

TBARS (micrograms/g peanut paste)

Day of assay = d	d8	d15	d22	d29	d36	d43	d50	d57	d71	d85	d99	d127
Overall Means	**1.16**	**1.54**	**ND**	**2.16**	**ND**	**2.11**	**ND**	**7.35**	**6.08**	**9.04**	**7.96**	**7.97**
A=Milling Method				(-)		**-0.52***		(+)			(-)	
B=Chelator				(+)		(+)		(-)		(+)		
C=Air (Oxygen)	(+)	(+)						(-)			(+)	
D=Light		(+)							**1.28***		(+)	(-)
E=Temperature	(-)	(+)		(-)		(-)		(+)		(-)		
Interactions:												
BE=ACD‡										**-BE**	**-ACD**	
CD=ABE‡		**0.50*CD**		**+ABE**		**0.50*ABE**		**-0.97*ABE**				
CE=acd†	**0.16***	0.48*CE										
de=ABC†								**1.20*ABC**				
RMS (error est.)	.0234	.1642	ND	.3853	ND	.2052	ND	.8184	.9865	2.750	1.013	1.804
Coef. determination (R^2)	.227	.340		.373		.510		.605	.242	.355	.343	.158

BOLD print identifies the mean effect and the largest factor effect on any day of assaying.
* Indicates signifincance at $p < 0.05$
Parentheses with sign (+/-) identifies a non-significant effect included in the interaction models with either positive or negative indication of linear effect.
‡ Alternate two- or three-factor interaction effects identified in this row on the basis of best fit analysis described in the text.
† Consistently distinguished term of confounded pair is shown in CAPITAL LETTERS.
ND identifies assay not determined in the time indicated.

Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances are defined as the carbonyl secondary reaction products of lipid oxidation produced during the later stages of peroxidation of lipids. The trends of the mean effects of all the variables on the thiobarbituric acid reactive substances (TBARS) measured of the peanut pastes throughout the storage period are shown in Table 3. Significance was not evident between the three controls and the overall means of the sixteen factorial experimental determinations. During the initial storage period, there were limited increases in the levels of TBARS. However, during the later storage period (week eight and later), high levels of TBARS were observed. In contrast, Table 2 showed the mean effects of all the variables on the formation of the peroxides, the primary products of lipid oxidation. There were steady increases in peroxides formed and then some leveling off of the early rates of accumulation of peroxidation products that coincided approximately with the marked increases observed in the titer of the secondary products of lipid oxidation. Degradation was suspected with the peroxides and possibly best attributable to the interaction of temperature and light factors. Little or no consistent emergence of candidate factors are seen to sustain any basis for changes in the malonaldehyde levels from the factorial indicators of Table 3 since no significant factors were continued across the time series.

Conclusion

The influences of air (oxygen) and temperature on promotion of lipid peroxidation measured by peroxide values were recognized as the strongest effects portrayed by this study. Selection of non-corroded metal surfaces and other adaptations that would reduce the shedding of reactive metals into the peanut pastes during the processing of the peanut pastes with a plate mill are recommended. After each of these factors are adequately controlled, some marginal benefits might be seen by addition of metal chelator and/or elimination of light on the product. Assuming a 10 meq/kg peroxide value quality threshold and a goal of extending storage beyond the third or fourth week, elimination of oxygen contact with peanut pastes and promotion of low-temperature storage offer the best strategies to control peroxidation of peanut paste.

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