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# INFLUENCE OF SOME PROCESSING CONDITIONS ON THE QUALITY OF COFFEE BREW

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*ABSTRACT*

*The influence of some processing conditions on the organoleptic quality of coffee beverage (grinding, proportion of coffee to water, temperature of water and pressure during brewing) has been investigated by panel sensory analysis and physico-chemical measurements (soluble contents, pH, acidity, viscosity, optical density, electrical conductivity and caffeine). The study has been performed on a standard ARABICA roasted coffee and with an experimental extraction machine (expresso type) permitting control of the processing conditions.*

*A linear multiple regression analysis demonstrated that the brewing temperature and the grind-water ratio were the main parameters influencing the soluble content of the extract. They also influenced qualitatively the extract as shown by sensory analysis and physico-chemical properties. An attempt was made to optimize the brewing conditions in order to obtain a coffee beverage with definite organoleptic properties.*

## INTRODUCTION

Coffee-drink preparation and its study are very complex due to the numerous factors involved: (1) quality of green coffee bean, its fermentation and roasting, (2) freshness of roasted coffee, water characteristics, (3) materials in contact with water, grind and coffee-drink, (4) water temperature, coffee-water proportion, brewing time and possibly, depending on the principle used, injection pressure of water into the grind, and (5) granulometry and grind pressing, grind vessel shape, are important parameters to process coffee-drink and for its organoleptic properties.

  

The present study is limited to the influence of operating conditions for the extraction process.

For a better analysis of each extraction parameter effect, an experimental extractor was built, allowing each to be varied independently and over ranges covering largely the present conditions used in household appliances. Following a first set of experiments, some parameters were noted as important and were retained for a physico-chemical and organoleptic study.

Several authors looked for correlations between physico-chemical and organoleptic properties for various food products (Carter and Cornell 1977, Powers *et al.* 1977, Aishima and Nobuhara 1977). Coffee was investigated by Biggers *et al.* (1969), Tassan and Russell (1974) and Pictet and Vuataz (1977). Studies about organoleptic qualities of coffee-drink were investigated by Punnett (1962), Reese and Stevens (1960), Pangborn *et al.* (1971-1977). One of the aims of this work is to find chemical or physical characteristics, allowing the organoleptic properties of coffee beverage to be assessed.

## EXPERIMENTAL CONDITIONS

### Coffee Beverage Preparation

A sufficient amount of green coffee (100% ARABICA) was roasted for a full series of experiments, then put in 250 g-packets and preserved at $-45°C$. Prior to each experiment, an adequate amount of coffee was ground in a mill (Malkonig). Mineral water (Volvic) was used. The experimental extractor consisted of three components: mechanical, electronic and pneumatic.

The working principle is as follows: water is admitted from the boiler into the dosing cell whose volume has been fixed before. The water is made to come into contact with the grind by opening the dosing cell-grind vessel connection. The water is pressed through the grind by means of a piston. The coffee beverage is collected. The collector, as well as the boiler is kept at constant pressure (5 bars) to allow extraction to be carried out at a temperature above $100°C$. "Injection pressure" is the difference between the pressure exerted above the grind and "artificial pressure" i.e. that maintained in the collector. Pick-offs allow to control (and record):

(1) the temperature of water in the boiler and dosing cell, the grind, and coffee beverage in the collector, and

(2) the pressure at the upper face of the grind.

The grind is kept between two metallic sieves and two paper filters (Whatman 113). The walls in contact with water are of stainless steel.

**Experimental**

A preliminary study of the influence of operating conditions on the solid content of coffee-drink allowed the main parameters to be determined.

Three extraction parameters were retained: water temperature (70-95-105°C); grind-water proportion (6-10-25/100), the grind amount was fixed to avoid modifying the grind bed and the water volume was caused to vary; and grind granulometry (coarse and fine).

All experiments were made with the same injection pressure, except two trials which were made with a higher one. All other parameters were fixed. All combinations were considered.

Filtration time could not be fixed independently. It varied (1 to 50 s) with the water volume in contact with the grind (linear correlation coefficient = 0.67), the grind characteristics (granulometry and pressing), water temperature and injection pressure. Regression analysis shows that more than 60% of the sum of squares of filtration time differences is explained by three parameters water volume, temperature and grind granulometry.

Elementary relations between properties were analyzed using the linear correlation coefficient. More complex relations between a given property (e.g. S) and other parameters (e.g. temperature, grind granulometry, grind-water proportion) were analyzed using the multiple, linear correlation coefficient square (or determination coefficient) given by a linear, multiple regression analysis. In both cases, the validity conditions of the analyses (normal variables, homogeneous variances) were assumed fulfilled.

Physico-chemical measurements are solid content, soluble yield, pH, acidity, conductance, optical density at 430 nm, caffeine and viscosity:
(1) Solid content (S): It is the concentration of the extract in soluble elements expressed in grams per 100 ml of coffee drink.
(2) Solubles Yield (SY): It is defined in % by the ratio of solid weight to the weight of coffee-grind:

$$SY\ (\%) = \frac{S \times \text{volume of coffee-drink}}{\text{weight of coffee-grind}} \times 100$$

(3) Optical density, measured at 430 nm on coffee-drink diluted at 1/20. (OD)
(4) Viscosity. As reported by Sivetz and Foote (1963), coffee drink is a Newtonian fluid. The viscosimeter used was a Rheomat 15 (Contraves — Zürich) with coaxial cylinders (Epprecht type).
(5) Electrical conductivity. According to Heiss *et al.* (1969) and Czechoskva *et al.* (1976) conductance is closely related to the solid

Table 1. Solid content (S) of coffee drink versus extraction parameters

| Grind—Water Proportion | Granulometry[1] | Temperatures (°C) 70 | 95 | 105 |
|---|---|---|---|---|
| | | S(g/100ml) | S(g/100ml) | S(g/100ml) |
| 6/100 | fine | 0.95 | 1.02 | 1.12 |
| | coarse | 1.10 | 1.11 | 1.67 |
| 10/100 | fine | 2.26 | 2.53 | 2.89 |
| | coarse | 1.96 | 2.18 | 2.71 |
| 25/100 | fine | 4.07 | 5.26 | 4.29 |
| | coarse | 3.67 | 4.05 | 4.87 |

[1] Granulometry

Granulometric Distribution %

| Size ($\mu$m) | Fine | Coarse |
|---|---|---|
| 800—630 | 7.3 | 27.6 |
| 630—500 | 39.2 | 35.4 |
| 500—315 | 47.5 | 30.7 |
| 315—200 | 5.7 | 5.6 |
| <200 | 0.5 | 0.8 |

content of the extract. Measurements were made using a Metrohm (EA 608—08) cell with a constant equal to 0.82 $cm^{-1}$, connected to a Universal Bridge B 221 conductivimeter (Wayne-Kerr). Measurement sensitivity was about 0.1%.

(6) Acidity. It was measured by neutralizing a coffee-drink sample with NaOH (N/100). It is expressed in $H_2SO_4$ milliequivalents per 100 ml, and

(7) Caffeine. It was dosed by gas phase chromatography following the Vitzthum *et al.* technique (1974).

## RESULTS AND DISCUSSION

### Physico-Chemical Properties of Extract

The extraction conditions and the experimental results are listed in Table 1. If we study correlation, we observe (Table 2) that there is a

Table 2. Linear correlation coefficient between extraction conditions and physico-chemical properties

| Operating Conditions | Physical—Chemical Property | Correlation Coefficient (r) |
|---|---|---|
| Grind—water proportion | Solid content | 0.91[c] |
| | Soluble yield | −0.49[a] |
| | Acidity | 0.87[c] |
| | Conductance | 0.85[c] |
| | Optical density (430 nm) | 0.75[c] |
| | Caffeine | 0.87[c] |
| | Viscosity | 0.76[c] |
| Temperature | Solid content | 0.11 |
| | Soluble yield | 0.40 |
| Granulometry (Pressure) | No significant correlation | |

Significance a : 5%
b : 1%
c : 0.1%

significant, positive relation between the grind-water proportion and the solid content values. The relation between temperature and both properties (S an SY) is also positive but not significant.

Alternatively, (S) and (SY) seem to be independent of granulometry and pressure. This phenomenon is confirmed by multiple regression analysis. Taking into account the four extraction parameters, explains 85% of the sum of the squares of (S) differences; taking into account the sole grind-water parameter, the proportion is 81%.

Filtration time is strongly, negatively correlated with solid, content ($r = 0.66^c$). This is logical, account taken of the extraction process used. The smaller the water volume, the larger the solid content, but the shorter the filtration time.

Among extraction conditions (Table 2), the grind-water proportion is observed to play the major part on the physico-chemical properties.

Optical density at 430 nm, conductance, viscosity, caffeine content and acidity vary as solid content (Table 3). In addition, the physico-chemical properties statistically correlated with (S) are also correlated between themselves. That means that they have the same behavior. We observed also that conductance, optical density at 430 nm acidity or by caffeine content are linear functions of solid content in different operating conditions. For example the correlation coefficient between (S) and conductance or acidity is equal to 0.95.

Table 3. Linear correlation coefficient between solid content (S) and other physico-chemical characteristics of extract

| Physico-Chemical Properties | Correlation Coefficient |
|---|---|
| pH | 0.15 |
| Soluble yield | −0.11 |
| Acidity | 0.95[c] |
| Conductance | 0.95[c] |
| Optical density at 430 nm | 0.92[c] |
| Caffeine | 0.91[c] |
| Viscosity | 0.76[c] |

Significance a : 5%
b : 1%
c : 0.1%

**Organoleptic Properties**

Sensory evaluation technique: The aim was to do an objective sensory evaluation of coffee-drink. The tasters (13 people trained to evaluate coffee quality) were characterized and selected by a study of recognition and differential threshold for various substances in aqueous solution or in coffee.

The characterization techniques (Voilley *et al.* 1977) were as follows: (1) for aqueous solutions: determination of the recognition threshold by a 5/2 test of acid (tartric acid 0.5 g/l), bitterness (caffeine 0.2 g/l) and natural aroma of coffee (1 ml/l aromatic concentrate), and determination of the differential threshold by a classifying test of 7 glasses with respect to their concentration in acid, bitterness and coffee for acid solutions between 0.5 and 1.49 g/l, for caffeine solutions between 0.25 and 1.20 g/l, and for aromatic solutions between 1 and 11.4 ml/l and (2) for coffee-drink, only the differential threshold was determined for acid, bitterness and coffee aroma. Four various coffee-drinks were prepared taking into account their initial concentration, as organoleptically quantitatively evaluated for acidity, bitterness and aroma.

Pair tests were used for coffee-drink testing. A sample was always compared with a reference and the taster indicated whether there was a difference. The reference had been submitted to a prior study. It was made by mixing two coffee-drinks made with the same apparatus and the same conditions. It was verified to be organoleptically similar from one experiment to the other and to be in the middle of the range of the samples to be tasted, If the sample is "the more" with respect to the reference, it is given one point and inversely. The points obtained are summed and the ratio brought back to 100. This result is used for statistical computation.

Table 4. Linear correlation coefficient between extraction conditions and organoleptic properties

| | | Temperature | Grind-Water Properties | Granulometry |
|---|---|---|---|---|
| Odor | Quality | 0.08 | 0.62[b] | +0.06 |
| | Intensity | 0.02 | 0.56[a] | +0.15 |
| Aroma | Quality | 0.41 | 0.43 | −0.06 |
| | Intensity | 0.08 | 0.65[b] | 0.12 |
| Bitterness | | −0.01 | 0.58[b] | 0.36 |
| Acidity | | 0.16 | 0.82[c] | −0.04 |

Significance a : 5%
b : 1%
c :0.1%

Table 5. Linear correlation coefficient between organoleptic and physicochemical properties

| | Organoleptic Properties | | | | | |
|---|---|---|---|---|---|---|
| | Odor | | Aroma | | Bitterness | Acidity |
| | Quality | Intensity | Quality | Intensity | | |
| S | 0.70[b] | 0.70[b] | 0.53[b] | 0.82[b] | 0.70[b] | 0.89[b] |
| SY | 0.03 | 0.18 | 0.20 | 0.16 | 0.18 | 0.01 |
| pH | 0.10 | 0.13 | −0.22 | −0.00 | −0.05 | 0.01 |
| Acidity | 0.62[b] | 0.61[b] | 0.67[b] | 0.84[c] | 0.74[c] | 0.96[c] |
| OD 430nm | 0.59[a] | 0.64[b] | 0.74[b] | 0.83[c] | 0.74[c] | 0.88[c] |
| Viscosity | 0.30 | 0.27 | 0.26 | 0.36 | 0.35 | 0.56 |
| Conductance | 0.65[b] | 0.64[b] | 0.69[b] | 0.83[c] | 0.70[b] | 0.89[c] |
| Caffeine | 0.72[b] | 0.69[b] | 0.62[a] | 0.85[c] | 0.77[c] | 0.88[c] |

Significance a : 5%
b : 1%
c :0.1%

### Influence of Extraction Conditions on Organoleptic Properties

All organoleptic properties increase with grind-water proportion, except quality of aroma. Bitterness is influenced by grind granulometry slightly: The finer the grind the larger the amount of bitter substances extracted (Table 4). The temperature of the water between 70 and 105°C does not influence the organoleptic properties of coffee-drink.

### Relation Between Organoleptic and Physico-Chemical Characteristics

Solid content is positively and significantly related to each organoleptic parameter since the correlation coefficient value is close to 0.7. This value means that half of the variations observed for each organoleptic property (Table 5) is explained by the variations of solid content.

It is noticed, as expected, that the sensory properties are well correlated with the physico-chemical properties which are also well correlated with (S). However, the sensory approach remains essential. The relation is strongest between sensory acidity and acidity evaluated chemically: $r = 0.956$ and $r_2 = 0.91$. Yet, the differences between experimental and theoretical values remain important. Taking into account all other physico-chemical properties does not cause the determination coefficient ($r^2$) to increase more than 1% as it reaches only 0.92.

### Relation Between the Various Organoleptic Characteristics

The intensity of odor and aroma are well correlated with each other (r is about 0.80). The lower correlations are between quality of flavor and bitter or acid tastes. But we observed a good correlation between intensity of aroma and acidity. That suggests the higher the acidity, more important the exaltation of aroma.

## CONCLUSION

On the whole, there are important relations between physico-chemical choosen and organoleptic properties (e.g. between sensory acidity and acidity evaluated chemically) but these relations are still too far from value 1: to evaluate the organoleptic quality of a coffee beverage, a sensory evaluation is still required. For example, we showed that caffeine represents only 10% of bitterness of coffee (Voilley *et al.* 1977) but at present the substances responsible of this property are not identified.

With the experimental set-up used, the most important operating parameter to prepare a strong and aromatic coffee-drink was to use a high grind-water proportion.

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## REFERENCES

AISHIMA T. and NOBUHARA A. 1977. Flavour evaluation by multiple regression analysis of gas chromatographic patterns in soy sauce. Food Chem. *2*, 161—175.

BIGGERS R. E., HILTON J. J. and GIANTURCO M. A. 1969. Differentiation between coffea Arabica and coffea Robusta by computer evaluation of gas chromatographic profiles. Comparison of numerically derived quality predictions with organoleptic evaluation. J. Chromat. Sci. *7*, 453—472.

CARTER R. D. and CORNELL J. A. 1977. Use of Regressions Models in Objective Flavor Evaluation of Processed Orange Juice During Four Seasons. Flavor Quality Objective Measurement, (R. E. Scanlan, ed.) ACS Symposium series 51—70.

CZECHOSKVA M., GOLEBIOWSKI T., PIECZONTA W. and SOLARZ A. 1976. Brauchbarbeit der refraktometrischen and conduktometrischen Methode zur Bestimmung des Extrakts von Bohnen Kaffee aufgüssen. Kaffee und Tee pp. 258—260.

HEISS R., RADTKE R. and SCHUTZ B. 1969. Untersuchungen über die Herstellung von Bohnen Kaffee aufgüssen durch filtrieren — Kaffee und Tee Markt *19*, 8, 35—43.

PANGBORN R. M., TRABUE I. M. and LITTLE A. C. 1971. Analysis of coffee, tea and artificially flavored drinks prepared from mineralized water. J. Food Sci. *36*, 355—362.

PANGBORN R. M. 1977. Criteria for sensory evaluation of coffee beverage. 8ème Colloque Scientifique International sur le café. Abidjan C. I.

PICTET G. and VUATAZ L. 1977. Etude des techniques d'infusion. 8ème Colloque Scientifique International sur le café. Abidjan C. I.

POWERS J. J., BARGMAN R. E. and GODWIN D. R. 1977. Relation between Sensory and Objective Measurements for Quality Evaluation of Green Beans. Flavor Quality Objective Measurement. Ed. ACS Symposium series 51—70.

PUNNETT P. W. 1962. What constitutes good coffee brew? Tea and Coffee trade J. 26—30.

REESE T. S. and STEVENS S. S. 1960. Subjective intensity of coffee odor. *73*, 424—428.

SIVETZ M. and FOOTE H. E. 1963. *Coffee Processing Technology*, *2*, The Avi Publishing Co., Inc., Westport, Conn.

TASSAN C. G. and RUSSELL G. F. 1974. Sensory and gas chromatographic profiles of coffee beverage headspace volatiles entrained on porous polymers. J. Fd. Sci. *39*, 64—68.

VITZTHUM Q. G., BARTHELS M. and KWASNY H. 1974. Lebensm. z. Unters. Forsch., *154*, 135—140.

VOILLEY A., SAUVAGEOT F. and DURAND D. Influence sur l'amertume d'un café boisson de quelques paramètres d'extraction. 8ème Colloque Scientifique International sur le café. Abidjan C. I. Novembre 1977.

# PROTEIN AGGREGATION IN FOOD MODELS: EFFECT OF γ-IRRADIATION AND LIPID OXIDATION

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*ABSTRACT*

*Myoglobin and serum albumin have been irradiated in aqueous solution in the presence of varying amounts of carbohydrates and lipids, and the yield of protein aggregates has been determined by gel filtration. With myoglobin the formation of aggregates evolving from the reaction with oxidizing lipids was observed, which was not found for serum albumin. The production of protein-lipid complexes, in which lipid material was occluded in the high-molecular aggregates by physical forces was demonstrated. Gel filtration and gel electrophoresis, both in the presence of SDS, and thin-layer isoelectric focusing revealed distinct structural differences between the protein aggregates induced by irradiation and the aggregates formed by interaction with oxidizing lipids.*

## INTRODUCTION

Studies of the radiation chemistry in model systems of food compounds establish a rational basis for extrapolating the results obtained, to the actual complex situation which exists in food. By first studying isolated molecular components and subsequently their mutual interactions, the complexity of the reactions occurring in foods preserved by ionizing radiation can be better unraveled. Knowledge of these radiation-induced reactions will help to predict radiolysis products and explain their implications to the wholesomeness of irradiated foods (Diehl and Scherz 1975).

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In our study protein aggregation, i. e. the formation of high molecular weight products from the monomeric proteins, which is one of the main reactions on irradiation of proteins, has been chosen as an index of radiation-induced changes. With the aid of thin-layer gel filtration protein aggregates can be rather easily estimated. Previous experiments have shown that the aggregation varies for different proteins and strongly depends on protein concentration (Delincée and Jakubick 1977). The addition of carbohydrates greatly reduced the amount of radiation-induced protein aggregates, whereas the addition of sunflower oil had little effect on aggregate formation. Conflicting results were obtained when carbohydrates and lipids together were added to the protein solutions, and it seemed that the addition of lipids partly cancelled the decrease of aggregation which was caused by added carbohydrates (Delincée and Jakubick 1977; Diehl *et al.* 1978).

Preliminary experiments indicated, however, that the increase in protein aggregates in the presence of lipids might be attributed to the action of intermediates arising from lipid oxidation (Diehl *et al.* 1979). In another study about the nutritive value of dry casein, irradiated and stored in the presence of lipids, the formation of protein aggregates was observed, the amount of which increased during storage in air (Harmuth-Hoene and Delincée 1978). Protein aggregation seem thus to be caused both by the reactive intermediates induced by irradiation and by the reactive intermediates of autoxidizing lipids. In a comprehensive review Schaich (1980) pointed out the similarities of reactions involving proteins, which were initiated by ionizing radiation, light or oxidizing lipids, and the need of a more thorough understanding of these processes. The present investigation was carried out to clarify some of the conflicting results and to compare different proteins in their behaviour towards irradiation and autoxidizing lipids. In particular, we searched for differences between aggregates induced by irradiation and those induced by autoxidizing lipids.

## MATERIALS AND METHODS

Sperm whale myoglobin and bovine serum albumin came from SERVA (Heidelberg, FRG). The carbohydrates employed were trehalose (SERVA, Heidelberg, FRG) and soluble starch (MERCK, Darmstadt, FRG). The lipids used were either commercial sunflower oil (THOMY, Karlsruhe, FRG) or well-defined triglycerides: tristearin, triolein and trilinolein from SIGMA (München, FRG). Samples of these three triglycerides were also obtained from UNILEVER (Hamburg, FRG). D, L-$\alpha$-tocopherol was from MERCK, Darmstadt, FRG.

Sample preparation and irradiation in a $^{60}$Co-Gammacell 220 were carried out as described previously (Delincée and Jakubick 1977). Storage of the samples ensued under aerobic conditions at refrigerator temperature (4 — 6°C). Thin-layer gel filtration was performed on Sephadex Superfine G-75 or G-200 using 0.02 M phosphate buffer pH 7.2 containing 0.5 M NaCl as a solvent (Delincée and Jakubick 1977). Thin-layer gel filtration in the presence of sodium dodecylsulfate (SDS) was carried out with Bio-Gel P-200 (—400 mesh) using 0.02 M sodium phosphate buffer pH 7.2 containing 0.1% SDS as solvent. Samples (~1% protein) for separations in SDS-containing buffers were denaturated for 2 min at 100°C in the presence of 3% SDS and 3% dithioerythritol.

Densitometry of the stained paper prints was carried out in remission and the peak areas quantified by the MOP integrator (KONTRON, München, FRG). Preparative gel filtration in Sephadex layers has already been described (Delincée and Radola 1975a). Electrophoresis in polyacrylamide slabs (12.5%) with TRIS buffer in the presence of SDS was performed according to the procedure of Weber and Osborn (1975). Thin-layer isoelectric focusing in Sephadex was carried out using Ampholine (LKB, Bromma, Sweden) carrier ampholytes (Radola 1973). The formation of carbonyl compounds in oxidized lipids was determined using the thiobarbituric acid (TBA) test as described by Tarladgis *et al.* (1960). The commercial sunflower oil employed, showed a TBA-value less than 0.2 mg malondialdehyde/kg.

## RESULTS

### Protein Aggregates Induced by Irradiation or by Lipid Oxidation

The food model proposed by Diehl and Scherz (1975), consisting of 80% water and equal parts of protein, fat and carbohydrate forming the other 20%, served as a basis for our experiments. In Table 1 the results of the influence of storage on the formation of aggregates from myoglobin on irradiation, and the effect of added carbohydrates, lipids and vitamin E are listed. Those samples containing sunflower oil showed increasing amounts of aggregates with time. However, immediately after irradiation a slight decrease in the amounts of aggregates was noted on addition of lipids, indicating some radical scavenging by the lipids. The addition of antioxidant — commercial sunflower oil containing about 500 ppm vitamin E — did not inhibit the increased aggregate formation, not even when the vitamin E content was raised to 0.1%.

When bovine serum albumin was employed as the protein component, in contrast to myoglobin, no increasing amounts of aggregates were

Table 1. Amounts of aggregates (% of total protein) in myoglobin solutions: effect of added carbohydrates, lipids, antioxidant and subsequent storage. Radiation dose 2 Mrad

| Myoglobin Solution + Additives | Treatment | Storage Time at 4 — 6°C in Air (Days) | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 7 | 14 |
| 6.6 % myoglobin | control | 13 | 14 | 14 | 13 |
| | irradiated | 43 | 44 | 43 | 43 |
| 6.6 % myoglobin<br>2 % trehalose<br>4 % starch | control | 14 | 13 | 14 | 13 |
| | irradiated | 25 | 25 | 26 | 26 |
| 6.6 % myoglobin<br>6 % sunflower oil | control | 18 | 19 | 31 | 47 |
| | irradiated | 37 | 39 | 41 | 44 |
| 6.6 % myoglobin<br>2 % trehalose<br>4 % starch<br>6 % sunflower oil | control | 15 | 18 | 32 | 47 |
| | irradiated | 23 | 23 | 34 | 48 |
| 6.6 % myoglobin<br>6 % sunflower oil added 500 ppm α-tocopherol | control | 17 | 23 | 35 | 47 |
| | irradiated | 33 | 36 | 46 | 48 |

The variation coefficient of the amounts of aggregates estimated by our procedure amounted to about 10%

noted on storage, indicating a different response of different proteins. On irradiation of pure serum albumin 29% of the protein formed aggregates. The addition of carbohydrates reduced aggregation to about 10%, the addition of lipids yielded 27% aggregates and with both carbohydrates and lipids added 12% aggregates were formed (Delincée and Jakubick 1977).

When sunflower oil was replaced by defined triglycerides, namely the saturated tristearin, mono-unsaturated triolein and di-unsaturated trilinolein, only in the myoglobin solutions with trilinolein did aggregate formation increase with storage time (Table 2). The addition of carbohydrates decreased radiation-induced aggregation, but did not influence aggregate formation during storage. Neither did the addition of 0.05% vitamin E to trilinolein reduce aggregation. However, exclusion of oxygen (i.e. preparation of the lipid emulsion, irradiation and subsequent storage under an argon atmosphere), from the trilinolein-containing samples led to no further increase of aggregates during

Table 2. Amounts of aggregates (% of total protein) in myoglobin solutions: effect of the degree of unsaturation of triglycerides, exclusion of oxygen, added carbohydrates, antioxidant and subsequent storage. Radiation dose 2 Mrad

| Myoglobin Solution + Additives | Treatment | Atmosphere | Storage Time at 4 — 6°C (Days) 1 | 2 | 7 | 14 |
|---|---|---|---|---|---|---|
| 6.6 % myoglobin | control | air | 14 | 13 | 16 | 13 |
| 6 % tristearin | irradiated | | 32 | 30 | 33 | 35 |
| 6.6 % myoglobin | control | air | 15 | 13 | 15 | 13 |
| 6 % triolein | irradiated | | 34 | 32 | 36 | 34 |
| 6.6 % myoglobin | control | air | 21 | 26 | 42 | 52 |
| 6 % trilinolein | irradiated | | 38 | 42 | 62 | 68 |
| 6.6 % myoglobin | control | argon | 18 | 19 | 18 | 19 |
| 6 % trilinolein | irradiated | | 35 | 33 | 37 | 34 |
| 6.6 % myoglobin | | | | | | |
| 6 % trilinolein | control | air | 21 | 25 | 39 | 52 |
| 2 % trehalose | irradiated | | 26 | 30 | 51 | 59 |
| 4 % starch | | | | | | |
| 6.6 % myoglobin | | | | | | |
| 6 % trilinolein | control | air | 18 | 23 | 41 | 52 |
| added 500 ppm α-tocopherol | irradiated | | 35 | 39 | 63 | 67 |

The variation coefficient of the amounts of aggregates estimated by our procedure amounted to about 10%

storage. Similarly, no increase in aggregate formation during storage was observed in myoglobin solutions containing sunflower oil when oxygen was excluded, confirming the influence of oxidizing lipids.

Increasing levels of oil (2%, 4% and 6% sunflower oil) led to increased aggregation after storage, independent of added carbohydrates. This could not be observed immediately after irradiation. When the protein concentration was decreased to 1%, a higher amount of aggregates was formed either by irradiation or by interaction with oxidizing lipids. Irradiation of a 1% myoglobin solution with 0.75 Mrad yielded about 56% aggregates. The presence of oil (1% or 6%) reduced the amount of aggregates to 34% immediately after irradiation, thus a considerable protective effect was exerted by the oil. Storage of the myoglobin solutions containing 1% or 6% lipids led to autoxidation-catalyzed aggregates, which after 14 days reached levels of 70% and 80% aggregation, respectively. Similar levels of aggregation were also found after a period of 14 days in the unirradiated myoglobin solutions containing 1% or 6% oil.

**Nature of Aggregates**

In another study (Harmuth-Hoene and Delincée 1978) the formation of high-molecular aggregates in an irradiated dry casein-lipid mixture has been revealed by thin-layer gel filtration. The amount of aggregates was found to increase during storage in air. When this stored dry mixture was extracted with acetone and diethylether to remove the lipids, the amount of high-molecular aggregates was greatly diminished in the gel chromatographic pattern, indicating noncovalent protein-lipid complexes. In this study solutions of myoglobin, which were irradiated in the presence or absence of lipids, were stored, extracted with diethylether and the amounts of aggregates estimated by thin-layer gel filtration before and after extraction. The amounts of aggregates were not influenced by the liquid-liquid extraction either in irradiated or in unirradiated samples. In another attempt, the myoglobin samples were freeze-dried, extracted with acetone and ether, redissolved in buffer and chromatographed. Here a different behaviour was observed for the aggregates induced by irradiation compared to those formed during storage by oxidizing lipids. In the myoglobin samples without lipids, the radiation-induced aggregates were only slightly affected by extraction, whereas in the stored samples containing lipids the amount of the high-molecular aggregates was reduced. Particularly, in the unirradiated myoglobin solutions containing lipids this effect was noticeable, although not all aggregates disappeared.

Further experiments served to identify the difference between protein aggregates induced by irradiation and those induced by lipid oxidation. In two samples of myoglobin, similar amounts of aggregates were produced, one being irradiated (2 Mrad) and the other unirradiated but stored in the presence of sunflower oil. These samples were fractionated by preparative gel filtration on a 40 cm- long plate into monomers, dimers and higher aggregates. This fractionation was checked by analytical thin-layer gel filtration. Here a spontaneous dissociation of the isolated radiation-induced aggregates was observed (Fig. 1) — in agreement with previous results on irradiated proteins (Radola 1974; Delincée and Radola 1974 a, b, 1975b). When thin-layer gel filtration of the separated fractions was carried out in the presence of SDS, dissimilar patterns for the higher aggregates formed by irradiation or by lipid oxidation were detected (Fig. 1). The polymers induced by irradiation were dissociated by SDS into a series of aggregates with molecular weights in between the monomer and the original high-molecular weight polymer. Even some fragments smaller than the monomer were detected. In the case of the polymer formed through oxidizing lipids, only a very small percentage of the high-molecular weight fraction dissociated and

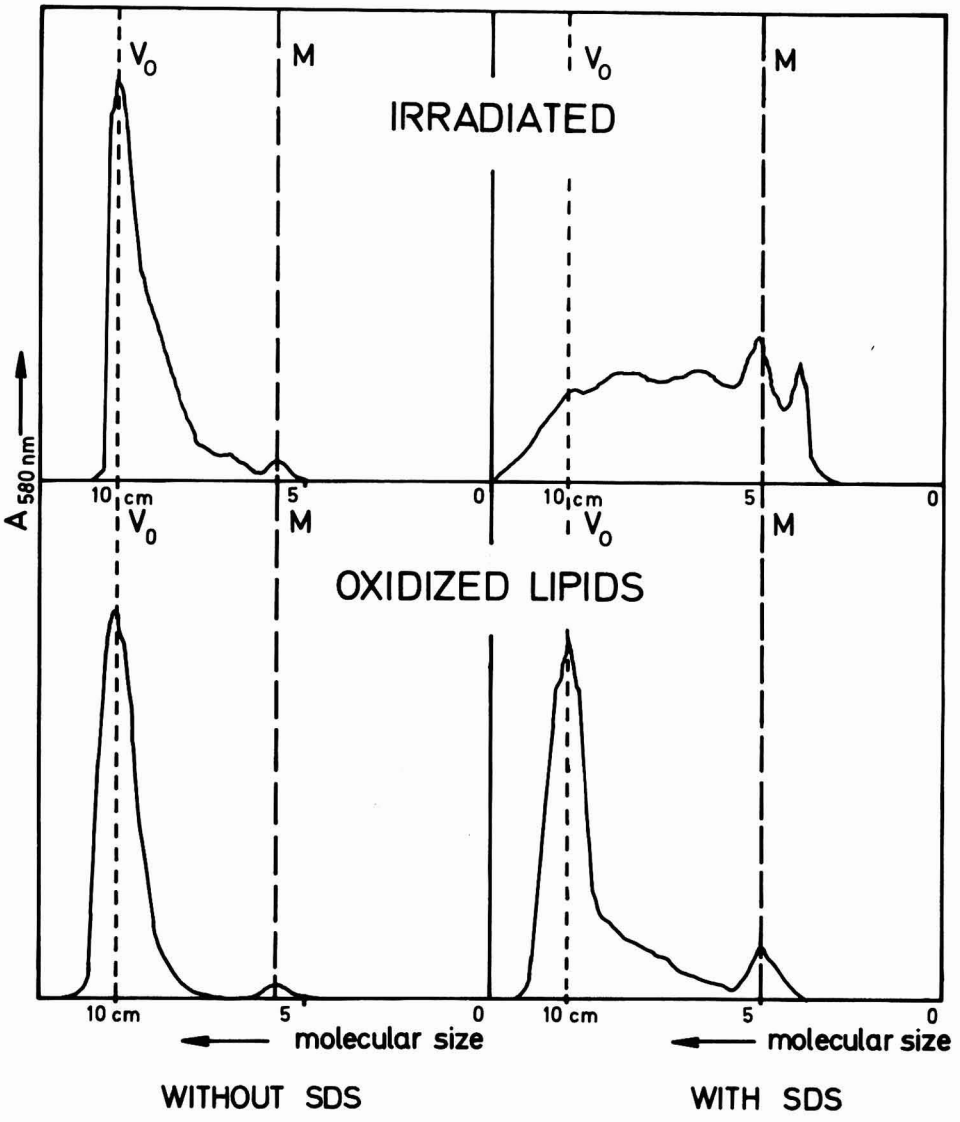


FIG. 1. THIN-LAYER GEL FILTRATION OF THE ISOLATED HIGHER AGGREGATES OF MYOGLOBIN, EITHER FROM IRRADIATED SAMPLES (UPPER PART) OR SAMPLES REACTED WITH OXIDIZING LIPIDS (LOWER PART)

Both tracings to the right represent gel filtration in the presence of SDS. $V_o$ denotes the void volume and M the monomeric myoglobin. (Myoglobin at a concentration of 6.6% was either irradiated with 2 Mrad or exposed to aerobic storage in the presence of 6% sunflower oil for 14 days. Gel filtration on Sephadox G-75 Superfine; to the right the application site, thus the molecular size increases from right to left in the diagrams; protein staining with Amidoblack 10 B; densitometric scans (in remission) at 580 nm).

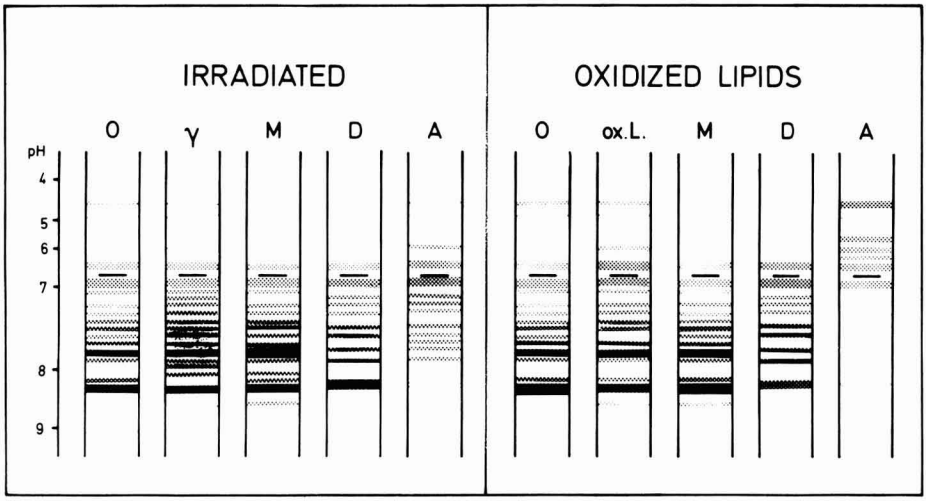


FIG. 2. THIN-LAYER ISOELECTRIC FOCUSING OF AGGREGATED MYOGLOBIN, EITHER IRRADIATED (γ) OR REACTED WITH OXIDIZING LIPIDS (ox. L.)

M-monomeric, D-dimeric and A-higher aggregates. On the left the untreated-O and the exposed unfractionated, γ or ox. L., myoglobin. (Focusing in 1% Ampholine (pH 3.5 — 10/pH 7— 9 mixed 1 : 1) at 250 V for 2 h followed by 800 V for 2 h. Separation distance 15 cm, carrier gel Sephadex G-75 Superfine, protein staining with Coomassie Brilliant Blue G-250).

the major peak remained at the void volume. Similar results were found with polyacrylamide gel electrophoresis in the presence of SDS: A diffuse stained track with peaks indicated at the dimeric, trimeric and tetrameric position was obtained for the radiation-induced polymer, whereas the lipid oxidation-induced polymer was mainly retained at the application site of the gel, being unable to penetrate the gel.

Thin-layer isoelectric focusing of the separated fractions, monomers, dimers and higher aggregates again revealed differences between the irradiated samples and those stored in the presence of oxidizing lipids (Fig. 2) which were barely discerned by analysis of the unfractionated samples. The pattern of the irradiated monomeric myoglobin was modified in comparison to the control, mainly through the diffuse staining - "smearing" - between the native zones, and the appearance of some new zones. The isoelectric pattern of the dimer and the higher aggregates consisted of multiple zones with "smearing" in between, and an increasing displacement of the components to lower isoelectric points. The myoglobin exposed to oxidizing lipids also showed for the monomer the appearance of a new zone with higher pI, but not the radiation-characteristic "smearing" between the zones. The dimer fraction showed multiple zones with a displacement to lower isoelectric points, very

similar to the radiation-induced dimer. The main difference compared to irradiation, however, was found for the higher aggregate-fraction, a number of zones being observed with clearly lower isoelectric points - below pH 6.

Since the formation of noncovalent protein-lipid complexes is very probable, and the presence of lipids might be responsible for the observed differences in the isoelectric patterns in Fig. 2, the separated fractions of myoglobin stored in the presence of lipids were freeze-dried, extracted with acetone and ether, and the amount of extracted lipid determined. Only the higher aggregates contained a substantial level of lipid material, which amounted to about 15%. The defatted fractions were again subjected to gel filtration, polyacrylamide gel electrophoresis and isoelectric focusing, and patterns similar to those observed before defatting were obtained, e.g. for focusing as shown in Fig. 2.

Another reason for the differences in the isoelectric patterns of the higher aggregates induced either by irradiation or by lipid oxidation could be the larger percentage of the higher aggregates (of total aggregates) in the myoglobin-oxidizing lipid samples. An increase in radiation-induced higher aggregates were produced by irradiation of myoglobin with increasing doses, 2, 4, 6, 8 and 10 Mrad. The higher aggregates, which for all doses were migrating with the void volume on Sephadex Superfine G-75, were isolated by preparative gel filtration and subsequently analyzed by thin-layer isoelectric focusing. The patterns of the higher aggregates showed multiple zones in the pH range 6 - 8 as demonstrated for 2 Mrad in Fig. 2. With increasing dose the zones accumulated in the pH 6 - 7 range. The patterns were, however, distinctly different from the isoelectric pattern shown in Fig. 2. for the higher aggregates formed through oxidizing lipids.

## DISCUSSION

Interactions of proteins with lipids are of great importance in food chemistry, especially the reactions with autoxidizing lipids, which may lead to considerable deterioration of foodstuffs. Among the many possibilities of reactions may be mentioned the formation of protein-lipid complexes that are bound through physical forces, covalent crosslinking of proteins either by pure protein radical recombination or by bridging with degradation products from the oxidizing lipids such as malondialdehyde, protein scission, and blocking of essential amino acids (Kummerow 1966; Karel 1975; Pokorny and Janicek 1975; Matsushita 1975; Gardner 1979; Schaich 1980). Feeding trials of proteins reacted with

oxidizing lipids revealed a decreased nutritive value of these proteins (Horigome *et al.* 1974; Horigome and Miura 1974; Yanagita and Sugano 1975; Sugano and Yanagita 1978; Pokorny 1977). Since lipids treated with ionizing radiation in the presence of air undergo similar reactions to autoxidized lipids (Nawar 1977), the interaction of lipids with proteins on irradiation may seriously affect the nutritive value of the protein (Takigawa *et al.* 1976; Harmuth-Hoene and Delincée 1978; Yousri and Harmuth-Hoene 1979). Therefore, investigations with different proteins were initiated in order to characterize the structural changes which occur in proteins upon irradiation and lipid oxidation.

Our results show that if myoglobin in the presence of polyunsaturated lipids is irradiated and stored under aerobic conditions, aggregates will arise due to interaction with oxidizing lipids, in addition to those induced by irradiation (Table 1, 2). Carbohydrates appear not to influence the protein aggregation induced by oxidizing lipids, since similar levels of aggregation were found with and without the addition of trehalose and starch. On the other hand, radiation-induced aggregation was strongly reduced by the addition of carbohydrates, probably by the scavenging of $OH^{\bullet}$ radicals. Non-oxidizing lipids also exert a protective effect on radiation-induced aggregation. Certainly the concentration of both protein and lipid influence aggregate formation. On irradiation with constant dose, the amount of radiation-induced aggregates increased with decreasing protein concentration (Delincée and Jakubick 1977). In the case of aggregates induced by oxidizing lipids, their amount was somewhat higher when the myoglobin concentration was decreased to 1%. With increasing amounts of polyunsaturated lipids, aggregation increased after storage. Although irradiation accelerates lipid oxidation, under our mild conditions the amount of myoglobin aggregates induced by oxidizing lipids was not affected, since similar levels of aggregation were found in unirradiated samples and in samples in which the lipid emulsion was irradiated with 2 Mrad before addition of the myoglobin.

A difference noted between myoglobin aggregation induced by irradiation or by lipid oxidation was the course of polymerization. When myoglobin is irradiated with increasing doses, aggregation proceeds stepwise through a dimer, trimer, etc. to higher aggregates (Delincée and Jakubick 1977). In contrast, in myoglobin solutions containing oxidizing lipids higher aggregates are already observed at commencement of storage together with the intermediate dimer, trimer, etc., which then gradually increase during storage.

Myoglobin but not serum albumin formed aggregates by interaction with the oxidizing lipids, most probably due to the catalyzing effect of the heme group on lipid oxidation. In unirradiated myoglobin-sunflower oil mixture the TBA number (mg malondialdehyde/kg lipid) in-

creased from 49.8 to 84.8 and 158.8 after 1, 7 and 14 days' storage, respectively. With bovine serum albumin or without any protein added, the TBA number remained constant at a level of 1.5.

The similarity of radiation effects and the reactions of oxidizing lipids on proteins has been pointed out by Tappel (Roubal and Tappel 1966a, b; Chio and Tappel 1969) and Karel (Zirlin and Karel 1969; Schaich and Karel 1975; Karel *et al.* 1975; Kanner and Karel 1976). Protein aggregation occurs in both cases, and since at least part of the aggregation in the presence of oxidizing lipids seems to proceed via a free radical mechanism (Gamage *et al.* 1973; Roubal and Tappel 1966b; Karel *et al.* 1975; Kanner and Karel 1976), similar free radicals being measured both on irradiation of proteins and interaction of proteins with oxidizing lipids (Schaich and Karel 1975; Karel *et al.* 1975; Schaich 1980), the formed aggregates are probably similar, mainly crosslinked by covalent protein-protein bonds (Roubal and Tappel 1966b; Kanner and Karel 1976; Funes *et al.* 1980). The second major mechanism of lipid oxidation-induced aggregation may be the crosslinking of proteins with small break-down products from the oxidizing lipids (Roubal and Tappel 1966b; Chio and Tappel 1969; Gamage *et al.* 1973; Matsushita 1975; Kanner and Karel 1976). Both mechanisms are in accordance with the finding that higher amounts of covalently bonded lipids are not observed in the purified polymerized products (Roubal and Tappel 1966b; Gamage *et al.* 1973). In addition to these nearly pure protein aggregates, also noncovalently bonded protein-lipid complexes are formed.

Our results with myoglobin clearly demonstrate structural differences between aggregates induced by irradiation and by lipid oxidation. Protein-lipid complexes are formed by reaction with oxidizing lipids, and practically only the higher aggregates contained lipid material. A certain denaturation of the protein thus seems to be a necessary prerequisite for the complex formation with lipids as already stated by Narayan and Kummerow (1963). Since the lipids - at least part of them - could be extracted after freeze-drying, they could only have been occluded in the protein aggregates by physical forces. The remaining aggregates after treatment with organic solvents are unequivocally shown to be different from radiation-induced aggregates by gel filtration and gel electrophoresis in the presence of SDS and by thin-layer isoelectric focusing (Fig. 1, 2). These observations were only possible by analyzing isolated aggregates, since in the unfractionated samples differences were barely discernable. The isoelectric patterns of the different fractions, isolated by preparative gel filtration of irradiated myoglobin, namely the modification of the monomer, the accumulation of the multiple zones and the displacement to somewhat lower isoelectric points for

the dimer and higher aggregates, accompanied by the radiation-characteristic "smearing" between the zones, are in accordance with previous results on irradiated proteins (Radola 1974; Delincée and Radola 1974a, b, 1975b). The isoelectric pattern of the higher aggregates of myoglobin induced by oxidizing lipids with a displacement to even lower isoelectric points (Fig. 2) indicates the preferential damage to basic amino acids on lipid oxidation (Matsushita 1975; Pokorny and Janicek 1975; Pokorny 1977; Gardner 1979).

The nature of the bonds involved in protein aggregation is still not completely resolved; upon irradiation covalent and hydrophobic, ionic and hydrogen bonding may occur (Yamamoto 1977; Jakubick and Delincée 1978). The results after gel filtration and gel electrophoresis in the presence of SDS of the higher aggregates of irradiated myoglobin show that both covalent and noncovalent bonding plays a role, since partial dissociation was observed (Fig. 2). The existence of hydrophobic bonds in irradiated proteins, which could be cleaved by SDS, was also observed by Schüssler *et al.* (1975, 1980). In the case of protein aggregates formed by reaction with oxidizing lipids, the question still remains, whether they contain fragments derived from the lipids such as carbonyls or peroxides. Experiments with uniformly radioactively-labeled lipids followed by an estimation of the yield of radioactive material in the aggregates may help to answer this question (Roubal and Tappel 1966b; Zirlin and Karel 1969; Gamage *et al.* 1973). Further experiments employing peptide fingerprints, sequence determinations and amino acid analysis to identify the sites of crosslinking seem to be necessary for a better understanding of protein aggregation.

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## REFERENCES

CHIO, K. S. and TAPPEL, A. L. 1969. Inactivation of ribonuclease and other enzymes by peroxidizing lipids and by malondialdehyde. Biochemistry *8*, 2827–2832.

DELINCÉE, H. and JAKUBICK, V. 1977. The effects of carbohydrates and lipids on the radiation-induced aggregation of proteins. Int. J. Appl. Radiat. Isotop. *28*, 939–945.

DELINCÉE, H. and RADOLA, B. J. 1974a. The effect of γ-irradiation on the charge and size properties of horseradish peroxidase. Radiat. Res. *58*, 9–24.

DELINCÉE, H. and RADOLA, B. J. 1974b. The effect of γ-irradiation on the charge and size properties of horseradish peroxidase: Individual isoenzymes. Radiat. Res. *59*, 572–584.

DELINCÉE, H. and RADOLA, B. J. 1975a. Fractionation of horseradish peroxidase by preparative isoelectric focusing, gel chromatography and ion-exchange chromatography. Eur. J. Biochem. *52*, 321–330.

DELINCÉE, H. and RADOLA, B. J. 1975b. Structural damage of gamma-irradiated ribonuclease revealed by thin-layer isoelectric focusing. Int. J. Radiat. Biol. *28*, 565–579.

DIEHL, J. F., ADAM, S., DELINCÉE, H. and JAKUBICK, V. 1978. Radiolysis of carbohydrates and of carbohydrate-containing foodstuffs. J. Agric. Food Chem. *26*, 15–20.

DIEHL, J. F. DELINCÉE, H. and PAUL, P. 1979. Radiation-induced changes in food models: Protein aggregation. Lebensm. Wiss. Technol. *12*, 121–122.

DIEHL, J. F. and SCHERZ, H. 1975. Estimation of radiolytic products as a basis for evaluating the wholesomeness of irradiated foods. Int. J. Appl. Radiat. Isotop. *26*, 499–501.

FUNES, J., YONG, S. and KAREL, M. 1980. Changes in lysozyme due to reactions with volatile products of peroxidizing methyl linoleate. J. Agric. Food Chem. *28*, 794–798.

GAMAGE, P. T., MORI, T. and MATSUSHITA, S. 1973. Mechanism of polymerization of proteins by autoxidized products of linoleic acid. J. Nutr. Sci. Vitaminol. *19*, 173–182.

GARDNER, H. W. 1979. Lipid hydroperoxide reactivity with proteins and amino acids: A review. J. Agric. Food Chem. *27*, 220–229.

HARMUTH-HOENE, A.-E. and DELINCÉE, H. 1978. Changes in the nutritive value of casein, irradiated and stored in the presence of lipids. Int. J. Vit. Nutr. Res. *48*, 62–74.

HORIGOME, T. and MIURA, M. 1974. Interaction of protein and oxidized ethyl linoleate in the dry state and the nutritive value of the reacted protein. J. Agric. Soc. Jap. *48*, 437–444.

HORIGOME, T., YANAGITA, T. and MIURA, M. 1974. Nutritive value of proteins prepared by the reaction with oxidized ethyl linoleate in aqueous medium. J. Agric. Soc. Jap. *48*, 195–199.

JAKUBICK, V. and DELINCÉE, H. 1978. Strahleninduzierte Aggregatbildung von Proteinen: Bindung von Aminosäuren an Myoglobin. Z. Naturforsch. *33c*, 203–209.

KANNER, J. and KAREL, M. 1976. Changes in lysozyme due to reactions with peroxidizing methyl linoleate in a dehydrated model system. J. Agric. Food Chem. *24*, 468–472.

KAREL, M. 1973. Protein-lipid interactions. J. Food Sci. *38*, 756–763.

KAREL, M., SCHAICH, K. and ROY, R. B. 1975. Interaction of peroxidizing methyl linoleate with some proteins and amino acids. J. Agric. Food Chem. *23*, 159–163.

KUMMEROW, F. A. 1966. Formation of lipid-protein complexes. Nutr. Rev. *24*, 147–149.

MATSUSHITA, S. 1975. Specific interactions of linoleic acid hydroperoxides and their secondary degraded products with enzyme proteins. J. Agric. Food Chem. *23*, 150—154.

NARAYAN, K. A. and KUMMEROW, F. A. 1963. Factors influencing the formation of complexes between oxidized lipids and proteins. J. Am. Oil Chem. Soc. *40*, 339—342.

NAWAR, W. W. 1977. Radiation chemistry of lipids. In *Radiation Chemistry of Major Food Components*,(P. S. Elias and A. J. Cohen, eds.) pp. 21—61, Elsevier, Amsterdam.

POKORNY, J. 1977. Interactions of oxidized lipids with protein. Riv. Ital. Sost. Grasse *54*, 389—393.

POKORNY, J. and JANICEK, G. 1975. Wechselwirkung zwischen Proteinen and oxydierten Lipiden. Nahrung *19*, 911—920.

RADOLA, B. J. 1973. Isoelectric focusing in layers of granulated gels. I. Thin-layer isoelectric focusing of proteins. Biochim. Biophys. Acta *295*, 412—428.

RADOLA, B. J. 1974. Identifizierung von bestrahltem Fleisch mit Hilfe der Dünnschicht-Gelchromatographie und Dünnschichtisoelektrischen Fokussierung. In *Proceedings Int. Colloq. Identification of Irradiated Foodstuffs*, 24—25 Oct. 1973, Karlsruhe. EUR 5126, pp. 27—44, Commission of the European Communities Luxembourg.

ROUBAL, W. T. and TAPPEL, A. L. 1966a. Damage to proteins, enzymes and amino acids by peroxidizing lipids. Arch. Biochem. Biophys. *113*, 5—8.

ROUBAL, W. T. and TAPPEL, A. L. 1966b. Polymerization of proteins induced by free-radical lipid peroxidation. Arch. Biochem. Biophys. *113*, 150—155.

SCHAICH, K. M. 1980. Free radical initiation in proteins and amino acids by ionizing and ultraviolet radiations and lipid oxidation. Part I-III. CRC Crit. Rev. Food Sci. Nutr. *13*, 89—129, 131—159, 189—244.

SCHAICH, K. M. and KAREL, M. 1975. Free radicals in lysozyme reacted with peroxidizing methyl linoleate. J. Food Sci. *40*, 456—459.

SCHÜSSLER, H., NIEMCZYK, P., EICHHORN, M. and PAULY, H. 1975. On the radiation-induced aggregates of lactate dehydrogenase. Int. J. Rad. Biol. *28*, 401—408.

SCHÜSSLER, H. and HERGET, A. 1980. Oxygen effect in the radiolysis of proteins. I. Lactate dehydrogenase. Int. J. Rad. Biol. *37*, 71—80.

SUGANO, M. and YANAGITA, T. 1978. Nutritive deterioration of food proteins due to interaction with oxidized fats. In *Abstracts of the Vth Intern. Congr. Food Sci. Technol.*, 17—22 Sept. p. 278, Kyoto, Japan.

TAKIGAWA, A., DANBARA, H. and OHYAMA, Y. 1976. Gamma ray irradiation to semi-purified diet — peroxide formation and its effect on chicks. Japan. J. Zootechn. Sci. *47*, 292—302.

TARLADGIS, B. G., WATTS, B. M. and YOUNATHAN, M. T. 1960. A distillation method for the quantitative determination of malondialdehyde in rancid foods. J. Am. Oil Chem. Soc. *37*, 44—48.

WEBER, K. and OSBORN, M. 1975. Proteins and sodium dodecyl sulfate: Molecular weight determination on polyacrylamide gels and related procedures. In *The Proteins* (Third Ed. Vol. I, H. Neurath and R. L. Hill, eds.) pp. 179—223, Academic Press, New York.

YAMAMOTO, O. 1977. Ionizing radiation-induced crosslinking in proteins. In *Protein Crosslinking, Biochemical and Molecular Aspects* (M. Friedman, ed.) pp. 509—547. Plenum Press, New York.

YANAGITA, T. and SUGANO, M. 1975. Liver and plasma lipids in rats fed casein reacted with oxidized ethyl linoleate. Agr. Biol. Chem. *39*, 63—69.
YOUSRI, R. M. and HARMUTH-HOENE, A.-E. 1979. The nutritional value of irradiated casein-fat mixtures — effect of anaerobic conditions, storage time, added vitamin E and degree of unsaturation of lipids. Int. J. Vit. Nutr. Res. *49*, 171—181.
ZIRLIN, A. and KAREL, M. 1969. Oxidation effects in a freeze-dried gelatin-methyl linoleate system. J. Food Sci. *34*, 160—164.

# THE EFFECT OF HOT—PROCESSING, SEASONING AND VACUUM PACKAGING ON THE STORAGE STABILITY OF FROZEN PORK PATTIES

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*ABSTRACT*

*The storage stability of frozen pork patties was studied using a 2X2X2 factorial design comparing hot boned (H) versus chilled meat (C), seasoned (S) versus nonseasoned (N), vacuum (V) versus oxygen permeable (P) packaging and their interactions. Results showed that the effect of hot—processing is dependent on the packaging method and the presence of seasoning. In general, vacuum packaging is required to optimize the advantages of hot—processing in retarding lipid oxidation. The increased moisture cooking loss of hot processed product can be reduced significantly by the addition of seasoning.*

## INTRODUCTION

A major problem in frozen storage of pork products is the development of off-flavors due to oxidation of unsaturated fatty acids in the pork. Pork is more susceptible to oxidation than beef because of its higher content of polyunsaturated fatty acids (Urbain 1970). Ground pork is even more prone to lipid oxidation because disruption of muscle membranes exposes labile lipid components to oxygen and other catalysts (Sata *et al.* 1971).

Proper packaging retards lipid oxidation. At −18°C, vacuum packaged pork fat can be stored for 6—8 months without developing rancidity, but non-packaged fat develops a fishy flavor after storage for 4 months (Zlender *et al.* 1975). Anaerobic packaging without vacuum has also proven useful for retarding lipid oxidation. Its effectiveness is related to the reducing activity of the meat (Greene 1969; Pearson *et al.* 1977). In addition, hot-boned meat processing has some advantages over conventional processing methods (Mandigo 1968; Follett *et al.* 1974) and has been suggested as a means of reducing fat oxidation and microbial contamination (Henrickson 1968).

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The objective of this study was to evaluate the effect of a commonly used ground pork seasoning in combination with vacuum packaging on the frozen storage stability of chilled and hot—processed ground pork. No other studies have simultaneously examined all of these factors in a single experiment.

## MATERIALS AND METHODS

Four replicates of two carcasses each were used in a 2X2X2 factorial experiment. Half of each carcass was stored at 4°C for 2 days and then processed into pork patties. The other half was hot-boned and processed immediately without chilling. Boneless shoulder and ham were chopped in a Kramer-Grebe chopper, sampled for fat analysis (Hobart fat analyzer model F-101) and then chopped with additional fat to obtain approximately 25% fat in the final product. A spice mix, which contained approximately 80% salt, 6.7% monosodium glutamate, 5.5% hydrolyzed vegetable protein, 2% tricalcium phosphate and other ingredients (P & C Barbeque patty spice, Custom Foods, Chicago), was added at this stage where appropriate. The chopped meat was ground (3/16 in. plate) and stuffed into oxygen-permeable or air-impermeable packaging, chilled in air blast freezer (—20°C) and sliced into patties (Hobart model 1612 slicer). The time from slaughter to placing the product in the freezer was approximately 3 h for hot—processing. The temperature of the hot and chilled processed meat was above 28°C and below 14°C, respectively, through the processing procedures. Two packaging methods were used after slicing; vacuum packages were prepared using a Kenfield vacuum sealer (Model 141), with IKD Allvak-13 bags (International Kenfield Distributing Co.). Air permeable packages contained ten patties separated by waxed paper and were wrapped in waxed freezer paper. All packages were stored at —20°C until the time of assay.

At each sampling period (2, 4, 8, 16, 24 weeks storage), patties from various treatments were randomly selected for TBA value analysis, sensory evaluation, total plate count and moisture cooking loss determination. TBA value was measured by the procedure of Witte *et al.* (1970) as modified by Hunt (1978). A fifty-gram frozen sample was blended in a Waring Blender at high speed for 30 s with 75 ml of 10% perchloric acid and 100 ml of deionized, distilled water. The resulting slurry was transferred to a beaker, and the blender was rinsed with an additional 25 ml of deionized distilled water and added to the beaker, and the volume was brought to 250 ml and filtered through Whatman No. 2 filter. Five ml of filtrate was pipetted into a test tube and mixed with 5 ml of 0.02 M TBA reagent. The test tubes were then covered with

paraffin film, mixed, and stored in the dark for 15 h at room temperature. Absorbance was measured at 529.5 nm on a Beckman DU Spectrophotometer, model 2400. The TBA values (mg of malonaldehyde per 1000 g of meat) were then determined using 1, 1, 3, 3-tetraethoxypropane (TEP) standard solution and were adjusted based on an 84.04% recovery, as determined in a preliminary test.

AMSA recommended procedures (American Meat Science Association, 1977) were used as a guideline for organoleptic testing and cooking loss determination. Frozen pork patties were thawed at room temperature and cooked in a preheated rotary oven (165°C) for 30 min to an internal temperature of 75°C. Flavor was evaluated by six trained taste panelists. To avoid sensory fatigue, samples were prepared and tasted in two sessions in a day as shown below. This plan was based on a $2^3$ factorial design with partial confounding, each 2-factor interaction being confounded with sessions for two tasters.

| Taster | I & IV | II × V | III × VI |
|---|---|---|---|
| Session I | CNP, HNP, CSV, HSV, Control | CNP, CNV, HSP, HSV, Control | CNP, HNV, CSP, HSV, Control |
| Session II | CNV, CSP, HNV, HSP, Control | CSP, HNP, CSV, HNV, Control | HNP, CNV, HSP, CSV, Control |

C = chilled processing
S = seasoned during storage
P = air permeable packaging
H = hot—processing
N = nonseasoned during storage (seasoning added prior to cooking)
V = vacuum packaging

FDA bacteriological analytical procedure was followed for bacterial count (FDA 1976). All the samples were evaluated along with freshly prepared control samples processed by conventional methods. Data were analyzed by the method of least squares with SOUPAC statistical programs (SOUPAC1976).

## RESULTS AND DISCUSSION

One of the possible advantages of hot processing in combination with air-impermeable packaging is to utilize the high reducing activity of the hot-boned muscle to metabolize the entrapped oxygen in the ground meat. Hence, there would be less oxygen available for lipid oxidation

Table 1. Regression equations of TBA values

| Treatment | Equation |
|---|---|
| CSV | TBA Value = .623 + .145 X LOG W |
| HSV | TBA Value = .494 + .145 X LOG W |
| HSP & CSP | TBA Value = $10.022 - 60.533 \times \text{LOG W} + 127.627 \times (\text{LOG W})^2 - 102.611 \times (\text{LOG W})^3 + 28.532 \times (\text{LOG W})^4$ |
| HNV & CNV | TBA Value = $.246 + .525 \times \text{LOG W} - .233 \times (\text{LOG W})^2$ |
| HNP | TBA Value = .321 + .366 X LOG W |
| CNP | TBA Value = .248 X LOG W |

W = Frozen storage time in weeks
H = Hot processing
S = Inclusion of seasoning during storage
V = Vacuum packaging
C = Chilled processing
N = Nonseasoned during storage (Seasoning added prior to testing)
P = Air permeable packaging

during storage. Protein denaturation can occur during mechanical processing while the meat is at a relatively high temperature and may be a factor in accelerating lipid oxidation. Therefore, without air-impermeable packaging, the advantages of hot processing may not be as evident. Our results have shown a significant ($P<.01$) interaction between processing method and packaging method for TBA analysis.

The change of TBA value with various treatments and storage times can be expressed by regression equations (Table 1). Treatments which show significant effects are represented by different equations. However, treatments which are not significantly different are represented by a single equation. TBA values increased linearly with time for CSV, HSV, HNL and CNL. TBA values of other treatments increased in a nonlinear manner.

Table 2 shows the least square estimate of TBA values at various storage periods. Since the error variance was found independent of processing methods (H or C), the combined standard errors were given in this table. The results show that when seasoned samples were vacuum packaged, the chilled processed products (CSV) had significantly higher TBA values than the hot processed samples (HSV) at each testing time ($P<.01$). However, without seasoning and vacuum packaging, the hot processed sample (HNP) had significantly higher TBA values than the chilled processed samples (CNP) ($P<.05$). This shows the significant interaction between processing and packaging methods and demonstrates

Table 2. Least square estimate of TBA values

| Treatment | Storage Time 2 weeks | 4 weeks | 8 weeks | 16 weeks | 24 weeks | AVG Std Error |
|---|---|---|---|---|---|---|
| HSV | .54 | .19 | .62 | .67 | .69 | .03 |
| CSV | .67 | .71 | .75 | .80 | .82 | |
| HSP & CSP | .38 | 1.19 | 2.85 | 3.02 | 3.35 | .07 |
| HNV & CNV | .38 | .48 | .53 | .54 | .53 | .03 |
| HNP | .43 | .54 | .65 | .76 | .83 | .03 |
| CNP | .36 | .47 | .58 | .69 | .75 | |

H = Hot processing
S = Inclusion of seasoning during storage
V = Vacuum packaging
C = Chilled processing
N = Nonseasoned during storage (Seasoned prior to testing)
P = Air permeable packaging

the beneficial effect of hot processing when combined with vacuum packaging.

Taste panel evaluation further illustrates the storage stability of frozen pork patties (Fig. 1). As a whole, hot processing had little effect on taste panel rancidity evaluation. As expected seasoning added during frozen storage reduced the flavor scores significantly ($P<.01$). Vacuum packaging significantly improved flavor of the seasoned products ($P<.01$) to a level similar to nonseasoned patties stored in air-permeable packaging. The prooxidative effect of NaCl is well known, particularly when moisture is removed by freezing (Watts 1962). The commercial seasoning used in this experiment contained approximately 80% NaCl and definitely resulted in increased lipid oxidation. It must be remembered when interpreting these results however that the exact composition of the commercial spice blend was not known since this is proprietary information. Although salt is the major ingredient, other spices are also present and it is known that some spices have antioxidant properties (e.g. rosemary and black pepper). The purpose of the present research was to demonstrate the effect of a practical and commonly used ground pork seasoning and show its interaction with different packaging and processing methods.

Bacterial counts were not significantly different among the treatments and throughout the 24 weeks of frozen storage. This has ruled out the possible interference effect of microorganisms on the treatment effects.

The effect of hot processing on cooking loss was also measured. When meat is processed hot, protein denaturation may occur resulting in the

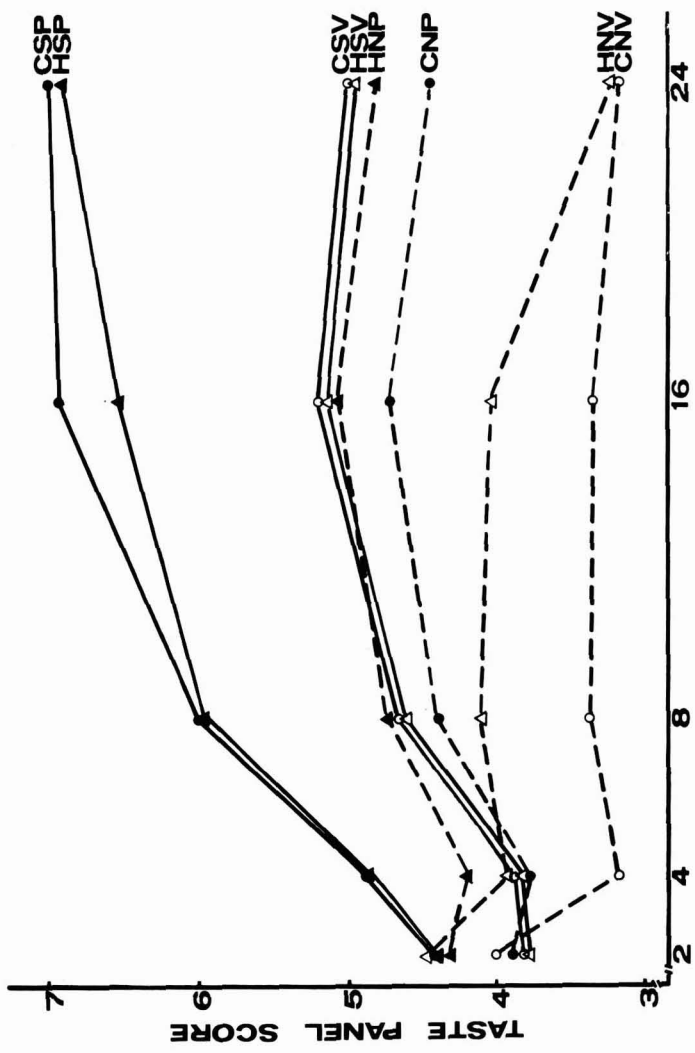


FIG. 1. CHANGE IN TASTE PANEL FLAVOR SCORE DURING FROZEN STORAGE (−20°C)

H=Hot processed; C=Chilled processed S=Inclusion of seasoning during storage; N=Nonseasoned during storage, but seasoned just prior to cooking; V=Vacuum packaging; P=Air permeable packaging. Sensory evaluation is based on a one to eight score. 1=extremely flavorful; 2=very flavorful; 3=moderately flavorful; 4=slightly flavorful; 5=slightly rancid; 6=moderately rancid; 7=very rancid; 8=extremely rancid.

Table 3. Least square estimate of mean moisture cooking loss for processing methods and seasoning

| | S | N | Significance Level |
|---|---|---|---|
| H | 24.6 | 29.4 | P<.01 |
| C | 23.6 | 26.8 | P<.01 |
| Significance Level | P<.01 | P<.01 | |

H = Hot processing
C = Chilled processing
S = Inclusion of seasoning during storage
N = Nonseasoned during storage (Seasoned prior to cooking)

reduction of water holding capacity (WHC). However, when meat is salted in the presence of ATP, it has a high WHC (Hamm 1960). Our results showed that chilled processed patties had significantly lower moisture cooking loss than did hot processed patties, particularly when no seasoning was added (Table 3). As expected, the addition of seasoning (contains 80% salt) decreased the moisture cooking loss.

In conclusion, the addition of a high salt seasoning, as expected, greatly enhanced lipid oxidation and this oxidation was markedly inhibited by the use of vacuum packaging. The results also demonstrated the interaction of processing method and packaging method and showed that with proper packaging methods, hot processing reduced lipid oxidation, particularly in ground pork seasoned with a commonly used spice mixture.

## ACKNOWLEDGMENT

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## REFERENCES

American Meat Science Association. 1977. Recommended Guidelines for Cookery and Sensory Evaluation of Meat.

F.D.A. 1976. Aerobic plate count. In *F.D.A. Bacteriological Analytical Manual*, Chapt. 4.

FOLLETT, M. J., NORMAN, G. A. and RATGLIFF, P. W. 1974. The ante-rigor excision and air cooling of beef semimemranosus muscles at temperatures between −5°C and 15°C. J. Food Technol. *9*, 509—523.

GREENE, B. E. 1969. Lipid oxidation and pigment changes in raw beef. J. Food Sci. *34*, 110—115.

HAMM, R. 1960. Biochemistry of meat hydration. In *Advances in Food Research.* Vol. 10, pp. 355—463. Academic Press, New York.
HENRICKSON, R. L. 1968. High temperature processing effect on physical, chemical, microbial and flavor properties of pork. Proc. of the MIRC, pp. 49—62.
HUNT, M. C. 1978. Personal Communication.
MANDIGO, R. W. 1968. High temperature pork processing, cutting, fabricating, handling and yield. Proc. of the MIRC, pp. 41—48.
PEARSON, A. M., LOVE, J. D. and F. B. SHORLAND. 1977. "Warmed-over" flavor in meat, poultry and fish. Adv. in Food Res. *23*, 1—74.
SATO, K. and HEGARTY, G. R. 1971. Warmed-over flavor in cooked meat. J. Food Sci. *36*, 1098—1111.
SOUPAC. 1976. SOUPAC Manual. Computing Service Office, University of Illinois at Urbana-Champaign.
URBAIN, W. M. 1970. Meat preservation. In *The Science of Meat and Meat Products*, (J.F. Price and W. H. Schweigert, eds.) p. 411. Freeman and Co., San Francisco.
WATTS, B. M. 1962. Lipids and their oxidation on meat products. In *Symposium on Foods-Lipids and Their Oxidation.* Chapter 11. pp. 202—217.
WITTE, V. C., KRAUSE, G. F. and BAILEY, M. E. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. J. Food Sci. *35*, 582—585.
ZLENDER, B. and BUCAR, F. 1975. The influence of packaging, storage time and thermal treatment on the oxidation and rancidity of pork fat. Hrana i Ishrana *15* (11/12), 541—546.

# PHYTATE CONTENT AND ITS EFFECT ON COOKING QUALITY OF BEANS

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*ABSTRACT*

*Storage of dry beans under conditions of relatively high moisture and temperature increased the cooking time of the beans about 5-fold. Among the changes that occur in beans stored in this way, the reduction (about 65%) in phytic acid content was the best indicator of increased cooking time. Cooking times of various legumes studied correlated well with the ratio of % phytic acid/% Ca present in the beans. Soaking high moisture beans in a solution of either phytic acid or EDTA reduced cooking time to that of control beans. Cooking time of control beans soaked under the same conditions was reduced by between 1/3 and 1/2, depending on the solution used.*

## INTRODUCTION

Dry beans, when stored under adverse conditions, especially elevated temperature and humidity, become hard to cook. Beans stored for about ten weeks at 25°C (77°F) and at relative humidities of 65% and above, will increase in moisture content to above 13.5%, depending on the relative humidity (Weston and Morris 1954). When those beans are stored for a total of about ten months, the time required for their cooking increases by about 5-to-6-fold as compared with control beans.

The conditions described above (25°C and 65% relative humidity) are common in many countries, especially in the tropics and subtropics, under normal conditions of commerce. This increase in cooking time causes increased loss of some nutritional qualities and increases usage of scarce fuel resources.

Some previous investigation as to the causes of this "hardening" ruled out any changes in fat (Takayama *et al.* 1965), or pectin (Kon 1968) as a cause for this increase in cooking time. Previous results in our laboratory (Kon 1979) indicated that changes in organic phosphate content

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might have an effect on the cooking time for beans. The present investigation was conducted for the purpose of determining the cause of this hardening and to devise a method to reduce the time that is required for cooking under those circumstances.

## MATERIALS AND METHODS

California small white (CSW) beans (*Phaseolus vulgaris*), obtained from the growing area in the Salinas valley, were treated to raise their moisture content to 16% and were stored at 32°C (90°F) for ten months and, subsequently, were kept frozen. The other legume varieties and species used in this report were: black beans, pinto beans, and kidney beans, all *Phaseolus vulgaris*, lima beans — *Phaseolus lunatus*, fava beans and ful beans (Egyptian fava beans) both *Vicia fava*, blackeyed pea — *Vigna unguiculata*, and green pea — *Pisum sativum*. All were obtained from a local store.

Cooking of the beans was done in the bean cooker as described by Burr *et al.* (1968). This cooker was composed of a frame that holds 100 saddles in each of which is placed a presoaked bean. A plunger terminating at its lower end in a stainless steel rod rests on each bean. The saddles were perforated vertically and when a bean becomes sufficiently tender, the rod penetrates the bean and drops a short distance through the hole in the saddle. During experimental runs the lower portion of the cooker holding the beans is lowered into a water bath maintained at about 99°C (210°F). The operator counts the number of plungers that have penetrated the beans and thus obtains the percentage of cooked beans at any given time. This cooker allows an objective determination of cooking rates of beans held and treated differently.

Phytic acid was determined as described by Wheeler and Ferrel (1971) and as modified by Chang *et al.* (1977) using 3% TCA to extract the phytate and determining phosphate after perchloric acid digestion. Ca and Mg were determined by atomic absorption. All other analytical determinations were done by AOAC methods.

Sodium phytate (inositol hexaphosphoric acid sodium salt) lot 84966 was obtained from General Biochemicals. Ethylenediamine tetraacetic acid (EDTA) was obtained from Sigma Chemicals. All other chemicals used were reagent grade.

## RESULTS AND DISCUSSION

As can be seen in Fig. 1, CSW beans treated to contain 16% mois-

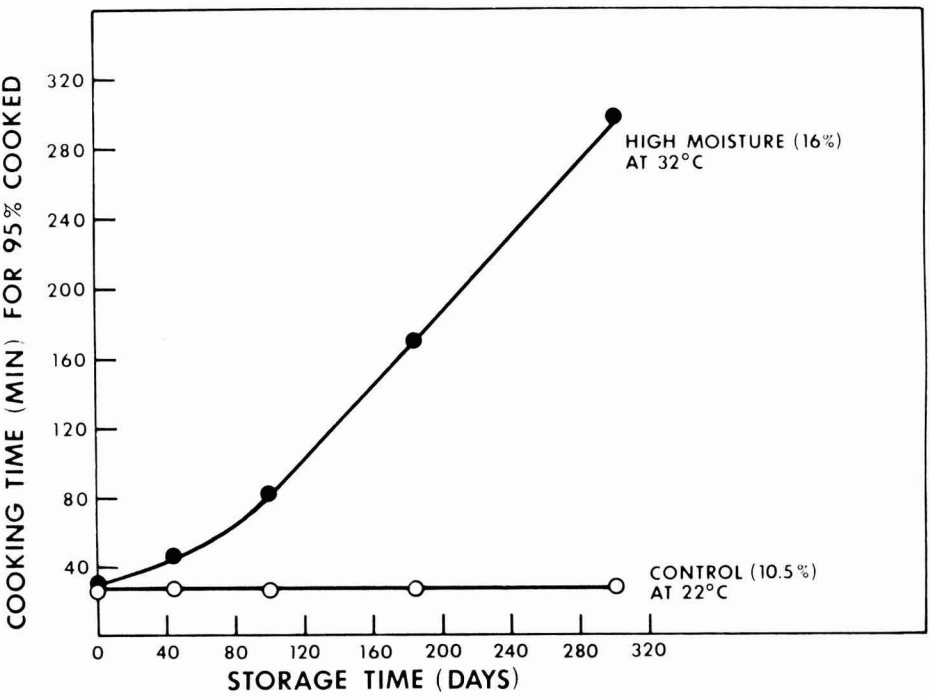


FIG. 1. COOKING RATE OF TEST BEANS STORED AT HIGH MOISTURE AND TEMPERATURE COMPARED TO CONTROL BEANS STORED AT LOW MOISTURE AND TEMPERATURE

ture and stored at 32°C (90°F) started deteriorating almost immediately as measured by cooking time and compared to control beans that contained 10.5% moisture and were stored at 22°C (70°F). At the end of ten months' storage it took 5 h to cook the high moisture beans to 95% cookability as compared with 30 min required to cook control beans to the same degree of tenderness. At the same time viability of the seeds was tested using 0.05%, 2, 3, 5-triphenyl-2H tetrazolium chloride solution and actual germination experiments. Viability was found to be 100% during the 10 month testing period for control beans but fell to only 70% after 3 months; to 50% after 6 months and to no viability at all after 10 months storage of test beans (16% moisture at 32°C). Similar loss of viability was observed for high moisture, high temperatue storage of wheat (Glass *et al.* 1959), rice (Houston *et al.* 1957), and other seeds (Gane 1947). As reported by Morris and Wood (1956) there was a reduction in both catalase (about 45%) and phosphatase (about 80%) activity and more than doubling (2.4 times) of the lipid acid value.

In determining the vitamin content of those beans using the method described by Miller *et al.* (1973) we found no difference in the amount

Table 1. Chemical composition and cooking time of some legumes

| Common Name | %$H_2O$ | % N | % Fiber (Crude) | % Ash | % Fat | % Ca | % Mg | % Sugar | % Phytic Acid | Time (min) for 50% Cook |
|---|---|---|---|---|---|---|---|---|---|---|
| CSW (High) | 14.87 | 3.32 | 4.75 | 3.65 | 2.12 | 0.240 | 0.155 | 6.0 | 0.260 | 134 |
| CSW (Control) | 12.11 | 3.45 | 4.88 | 3.20 | 1.73 | 0.210 | 0.133 | 6.1 | 0.710 | 36 |
| Black Beans | 11.64 | 3.66 | 4.10 | 3.94 | 1.63 | 0.150 | 0.168 | 6.0 | 1.040 | 30 |
| Pinto Beans | 11.70 | 3.74 | 3.83 | 3.53 | 1.27 | 0.140 | 0.163 | 7.0 | 0.994 | 31 |
| Kidney Beans | 11.63 | 3.80 | 4.36 | 3.32 | 1.75 | 0.175 | 0.149 | 6.9 | 0.946 | 35 |
| Lima Beans | 11.15 | 3.87 | 5.27 | 4.76 | 1.47 | 0.095 | 0.172 | 7.2 | 0.662 | 31 |
| Faba Beans | 12.11 | 3.90 | 7.58 | 3.22 | 0.97 | 0.175 | 0.122 | 6.6 | 0.887 | 40 |
| Ful Beans | 11.02 | 3.85 | 8.58 | 3.10 | 1.02 | 0.135 | 0.142 | 5.5 | 0.887 | 27 |
| Black-Eyed Pea | 11.15 | 3.80 | 2.84 | 3.54 | 1.13 | 0.105 | 0.190 | 7.4 | 0.946 | 23 |
| Green Pea | 10.93 | 3.54 | 4.47 | 2.55 | 1.09 | 0.175 | 0.131 | 6.6 | 0.710 | 30 |

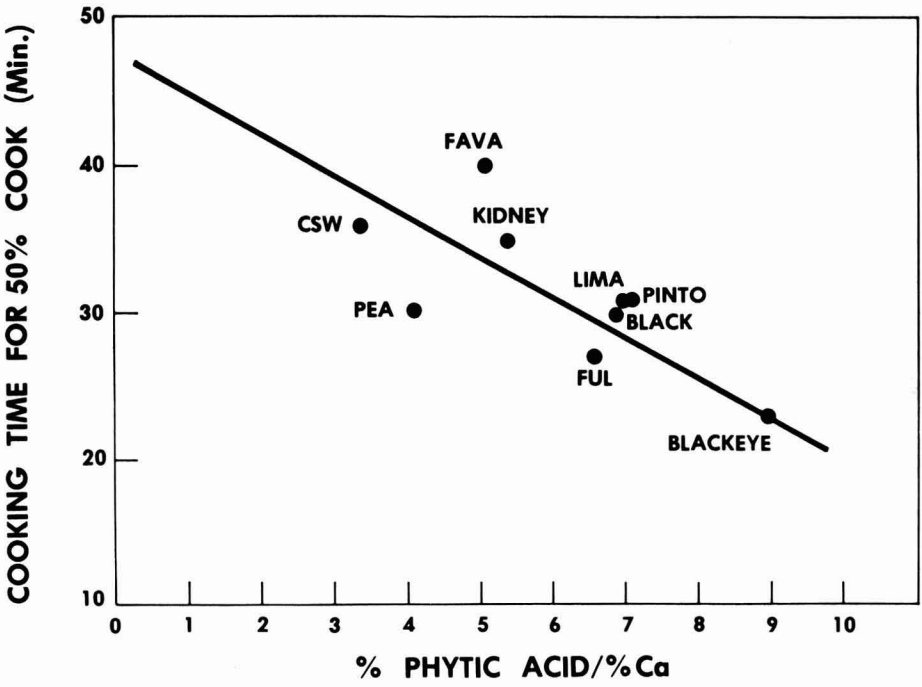


FIG. 2. COOKING RATE AS A FUNCTION OF THE RATIO OF PERCENT PHYTIC ACID TO PERCENT Ca IN CSW BEANS

of niacin, riboflavin and pyridoxine present in high moisture beans as compared to control beans. However, the concentration of thiamine in the high moisture beans was only 15% of its concentration in control beans.

PER (protein efficiency ratio) was found to be reduced by about 20% for cooked high moisture beans as compared to the cooked control (from 1.40 for the cooked control to 1.10 for the high moisture beans). This was done by feeding tests with rats as described by Derse (1965) for specially formulated diets in which 10% protein was supplied entirely by the respective bean samples. This is due to the longer cooking time required (300 min versus 30 min), because the same reduction in PER was found when control beans were cooked for the longer time. There does not seem to be any important difference in nitrogen or total digestibility under those conditions.

Previous results in our laboratory (Kon 1979) indicated that there is a good correlation between the amount of organic phosphate present in the beans and cooking time. As shown in Table 1, we tried to relate cooking time of ten different legumes with their chemical composition. A cursory examination of the results for the two CSW samples points

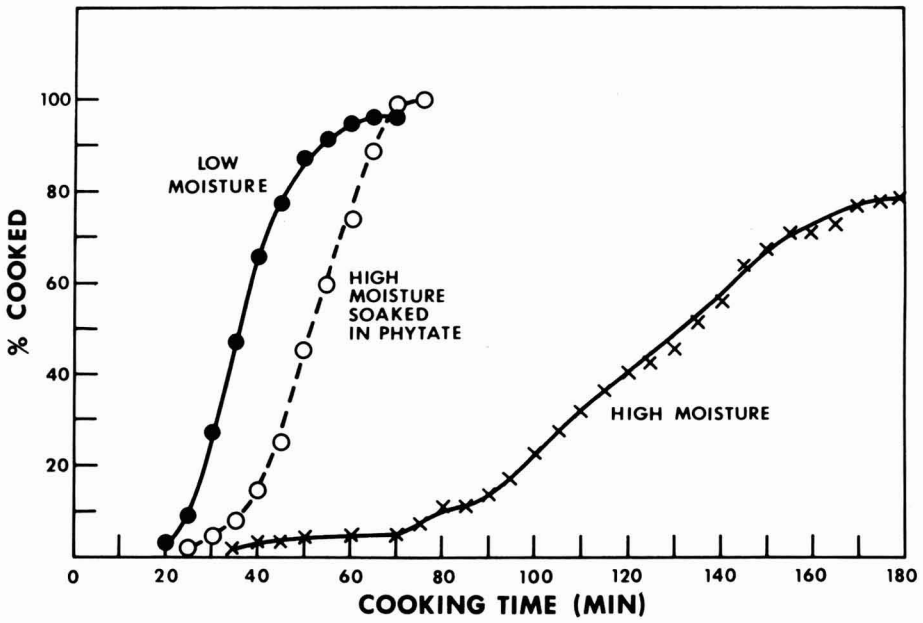


FIG. 3. COOKING RATE FOR HIGH AND LOW MOISTURE BEANS AND THE EFFECT OF PHYTATE ADDITION ON COOKING RATE OF HIGH MOISTURE BEANS

to a very good relationship between cooking time and phytic acid content. As can be seen in the table, the only important differences between control and high moisture CSW beans was in their phytic acid content—only about 1/3 as much in the high moisture beans as compared to regular; and in the cooking rate — about 4 times longer for the high moisture beans.

When looking at the results in Table 1, for the different varieties and species of fresh dry legumes that were analyzed, it appears that there is no relationship between cooking rate and phytic acid content. However, sometime ago we proposed a model for softening legumes during cooking, which involved an exchange of Ca (from the mostly insoluble Ca pectate in legumes) (Kon 1968) with Na and K (from the mostly soluble Na and K phytate) (Chang *et al.* 1977). Therefore, we tried to relate the ratio of % phytic acid to % Ca present in those legumes with their cooking time. This relationship, between the ratio % phytic acid/ % Ca and cooking time, is shown in Fig. 2 which is plotted using the

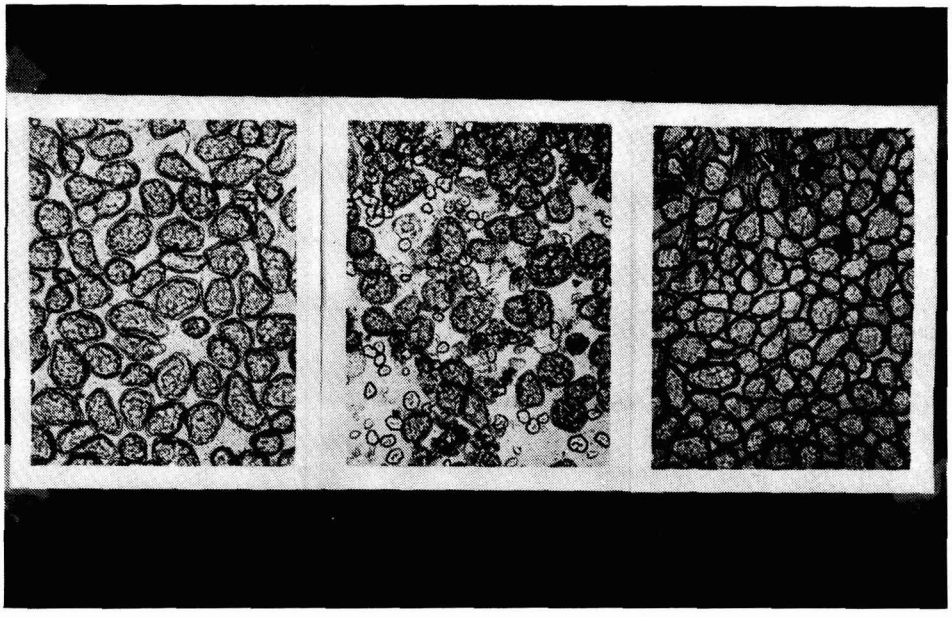

FIG. 4. PHOTOMICROGRAPHS OF (a) CONTROL BEANS SOAKED IN WATER AND COOKED FOR 1 h (b) HIGH MOISTURE BEANS SOAKED IN WATER AND COOKED FOR 1 h (c) HIGH MOISTURE BEANS SOAKED IN PHYTIC ACID AND COOKED FOR 1 h

least squares method with a calculated equation for the curve of $Y = 43.79 - 2.04 X$, and a correlation coefficient of 0.71.

This negative correlation between phytic acid and cooking rate suggested a method of reducing the cooking time for those slow cooking beans. If what caused them to be slow cooking was the destruction of phytic acid during storage, then infiltrating those slow cooking beans with phytic acid should make them quicker cooking. As can be seen in Fig. 3, the high moisture beans that originally required a very long time to cook became very similar in cooking characteristics to control beans. The results shown in Fig. 3 were for beans soaked overnight in either 6% or 1% sodium phytate which was adjusted to pH 7.0 and gave a soaking media of pH 6.5 at the end of the soak period. When the pH was not adjusted, the soaking media had a pH of 9.5 at the end of the soak period, and the cooking rate was even faster; about 25 min for 50% cooking. We tried to stay away from using alkaline conditions during the soaking period because of the possible formation of some unusual amino acids, such as lysinoalanine and lanthionine, which might reduce the availability of both lysine and cysteine (Robbins *et al.* 1980).

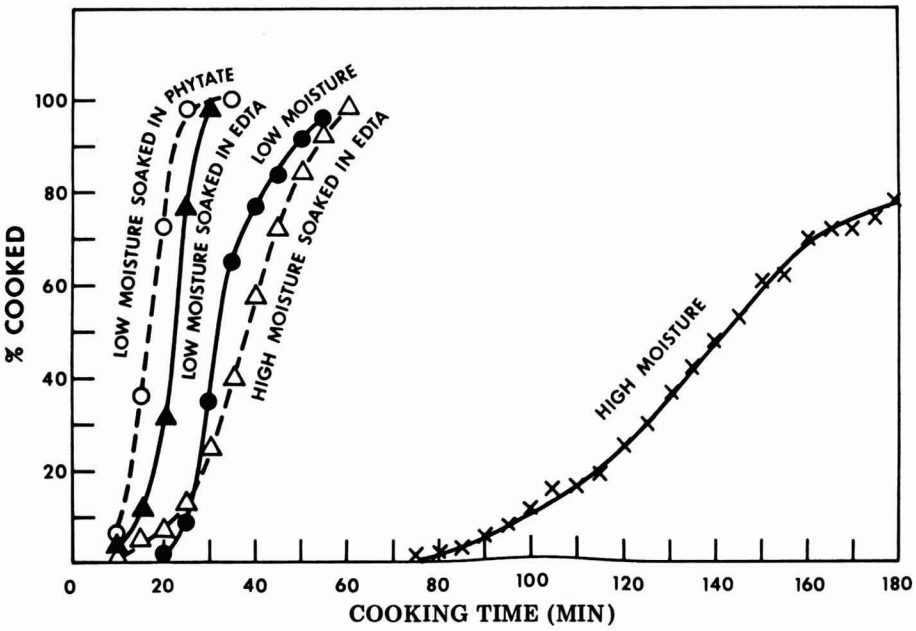


FIG. 5. COOKING RATE FOR HIGH AND LOW MOISTURE BEANS AND THE EFFECT OF PHYTATE AND EDTA ON THE COOKING RATES OF BOTH

As can be seen in Fig. 4, soaking the high moisture beans in phytic acid prior to cooking (Fig. 4c) enhances cell separation rather than breakage (Fig. 4c) in a similar manner to control beans (Fig. 4a). This might be another indication of changes in the middle lamella pectins during cooking when the ratio of phytic acid to Ca is adequate.

As can be seen in Fig. 5, soaking of the high moisture beans in EDTA (ethanol diaminetetra-acetic acid) will also reduce cooking time. Comparing results as shown in Fig. 3 with results shown in Fig. 5 it seems that, under the same conditions, EDTA is more effective in lessening cooking time than phytic acid. Soaking of control beans in a solution of either phytate or EDTA reduces the cooking time of those beans also, and again, it seems that under the same conditions EDTA is more effective than phytate. It would seem that any chelating agent capable of removing Ca from Ca pectate during the cooking process will reduce cooking time.

The choice of the chelating agent is important as some chelating

agents might reduce the physiological availability of some required metal ions like Fe, Ca, or Zn. Oberleas *et al.* (1966) found that phytate and EDTA behave differently with regard to the physiological availability of Zn for the rat. It seems from their work that EDTA has a stronger affinity to Zn (similar to our results regarding Ca) and that EDTA forms a soluble complex which allows absorption across membranes, as opposed to Zn phytate which precipitates out at intestinal pH.

## REFERENCES

BURR, H. K., KON, S. and MORRIS, H. J. 1968. Cooking rates of dry beans as influenced by moisture content and temperature and time of storage. Food Technol. *22*, 336–338.

CHANG, R., SCHWIMMER, S. and BURR, H. K. 1977. Phytates: removal from whole dry beans by enzymatic hydrolysis and diffusion. J. Food Sci. *42*, 1098–1101.

DERSE, P. H. 1965. Vitamins and other nutrients. Evaluation of protein quality (biological method). J. Assoc. Off. Anal. Chem. *48*, 847–850.

GANE, R. 1947. The effect of temperature, water content and composition of the atmosphere on the viability of carrot, onion and parsnip seeds in storage. J. Agricul. Sci. *38*, 84–89.

GLASS, R. L., PONTE, J. G., CHRISTENSEN, C. M. and GEDDES, W. F. 1959. The influence of temperature and moisture levels on the behavior of wheat stored in air or nitrogen. Cereal Chem. *36*, 341–356.

HOUSTON, D. F., STRAKA, R. P., HUNTER, I. R., ROBERTS, R. L. and KESTER, E. B. 1957. Changes in rough rice of different moisture content during storage at controlled temperatures. Cereal Chem. *34*, 444–456.

KON, S. 1968. Pectic substances of dry beans and their possible correlation with cooking time. J. Food Sci. *33*, 437–438.

KON, S. 1979. Effect of soaking temperatue on cooking and nutritional quality of beans. J. Food Sci. *44*, 1329–1335.

MILLER, C. F., GUADAGNI, D. G. and KON, S. 1973. Vitamin retention in bean products: cooked, canned and instant bean powders. J. Food Sci. *38*, 493–495.

MORRIS, H. J. and WOOD, E. R. 1956. Influence of moisture content on keeping quality of dry beans. Food Technol. *10*, 225–229.

OBERLEAS, D., MUHRER, M. E. and O'DELL, B. L. 1966. Dietary metal-complexing agents and Zn availability in the rat. J. Nutr. *90*, 56–62.

ROBBINS, K. R., BAKER, D. H. and FINLEY, J. W. 1980. Studies on the utilization of lysinoalanine and lanthionine. J. Nutr. *110*, 907–915.

TAKAYAMA, K. K., MUNETA, P. and WIESE, A. C. 1965. Lipid composition of dry beans and its correlation with cooking time. Agr. Food Chem. *13*, 269–272.

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Reference to a company and/or product named by the Department is only for purposes of information and does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

WESTON, W. J. and MORRIS, H. J. 1954. Hygroscopic equilibria of dry beans. Food Technol. *8*, 353–355.
WHEELER, E. L. and FERREL, R. E. 1971. A method for phytic acid determination in wheat and wheat fractions. Cereal Chem. *48*, 312–320.

# THE INTERACTION BETWEEN ACRYLONITRILE AND ACRYLONITRILE – STYRENE COPOLYMER BY INVERSE GAS CHROMATOGRAPHY

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*ABSTRACT*

*The interaction between acrylonitrile and an acrylonitrile-styrene copolymer was studied by the use of Inverse Gas Chromatography (IGC). The Gibb's free energy, the ethalpy and entropy were calculated from the chromatographic data. It was found that the negative value of the free energy increases with the decrease in temperature and that the negative value of the heat of adsorption increases with the decrease in the amount of ACN injected into the gas chromatograph. These results indicate that the polymer-monomer interaction increases with the decrease in temperature and monomer concentration pointing to the possibility that at very low monomer concentrations no migration may occur, at ambient temperatures, from a package made of the studied polymer.*

## INTRODUCTION

The migration of low molecular weight compounds, including monomers, from polymeric packaging materials has received a growing attention in recent years due to the possible effect of the migrants on the sensory quality and the safety aspects of the contained food. Many of the reported studies in this area deal with the migration of vinylchloride monomer (VCM) and different additives from polyvinylchloride (PVC), Figge (1972), Daniels and Proctor (1975), Gilbert (1975) Chudy and Crosby (1977), Morano *et al.* (1977). Only limited information is

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available on acrylonitrile (ACN) migration from its polymers, mainly at low levels, Federal Register (1977), US Courts of Appeals (1979). One way to investigate the propensity of a migrant entrapped in a polymeric package, to penetrate the food is by actual migration studies. Such studies have the advantage of providing direct measurements of the migrant concentration in the food at equilibrium. This technique is applicable, however, only at relatively high migrant concentrations in the polymer. Migration studies are also very time consuming.

Another way to estimate the propensity of a compound to migrate from a polymeric package and penetrate the contained food is to study the interaction between the migrant and the polymer. The stronger the interaction between the two the less is the chance that migration will occur.

Inverse Gas Chromatography (IGC) is a relatively new technique in monomer-polymer interaction studies. This technique is fast and has proven itself to provide reliable thermodynamic parameters, Stein *et al.* (1971), Braun and Guillet (1976), Coelho *et al.* 1979a,b), Llorente *et al.* (1979). The present study deals with the interaction between ACN and an acrylonitrile-styrene copolymer as calculated by IGC. In gas chromatography, the net retention volume, $V_n$, is given by the equation:

$$V_n = jF_m(t_r - t_o)\left(\frac{P_o - P_w}{P_o}\right)\frac{T_c}{T_a} \qquad (1)$$

where j is the James and Martin (1952) compressibility factor, $F_m$ is the flow rate measured at the outlet of the column, $t_r$ is the peak retention time, $t_o$ is the retention time of a noninteracting fluid, $P_o$ and $P_w$ are the atmospheric pressure and the vapor pressure at room temperature respectively and $T_c$ and $T_a$ are the column and ambient temperatures, respectively. The specific retention volume, $V_g^o$ (defined as the net retention volume per unit weight of polymer and corrected to O°C) is given by:

$$V_g^o = J\,F_m\,(t_r - t_o)\left((P_o - P_w)/P_o\right)\frac{273}{T_a}\frac{1}{W_s} \qquad (2)$$

where $W_s$ is the polymer weight.

The retention volume of the solute, $V_r$, can also be expressed by:

$$V_r = V_o + K_P \cdot V_s \qquad (3)$$

where $V_o$ is the retention volume of a noninteracting solute, $K_P$ is the partition coefficient (defined as the ratio between the solute concen-

tration in the polymer and in the mobile phase, respectively) and $V_s$ is the volume of the polymer. The net retention volume can therefore be expressed as:

$$V_n = K_p \cdot V_s \tag{4}$$

combining Eq. (1), (2) and (4) results in:

$$K_P = \frac{V_g^o \zeta_s T_c}{273} \tag{5}$$

where $\zeta_s$ is the density of the polymer. The total standard partial molar Gibb's free energy, $\Delta G_T^o$, is directly related to $K_P$ by the expression:

$$\Delta \bar{G}_T^o = RT\ln K_p \tag{6}$$

The total free molar energy is also given by

$$\Delta \bar{G}_T^o = \Delta \bar{G}_i^o + \Delta \bar{G}_{ex}^o = RT\ln\gamma_i P^o \tag{7}$$

where $\Delta \bar{G}_i^o$ is the ideal molar free energy (which is also the molar free energy of vaporization), $\Delta \bar{G}_{ex}^o$ is the excess molar free energy, $\gamma_i$ is the activity coefficient at infinite dilution and $P^o$ is the saturation vapor pressure of the pure solute. Equation (7) can therefore be written in the form:

$$\Delta \bar{G}_i^o = RT\ln P^o \tag{8}$$

$$\Delta \bar{G}_{ex}^o = RT\ln\gamma_i \tag{9}$$

In a similar way, the total molar ethalpy and entropy $\Delta \bar{H}_T^o$ and $\Delta \bar{S}_T^o$ respectively, can be separated into their components:

$$\Delta \bar{H}_T^o = \Delta \bar{H}_i^o + \Delta \bar{H}_{ex}^o \tag{10}$$

$$\Delta \bar{S}_T^o = \Delta \bar{S}_i^o + \Delta \bar{S}_{ex}^o \tag{11}$$

Smidsrod and Guillet (1969) have shown that the activity coefficient can be related to the specific retention volume and other parameters by the equation:

$$\gamma_i = \frac{273.2\ R}{V_g^o\ M_p\ P^o} \tag{12}$$

where $M_P$ is the molecular weight of the polymer.

Paterson *et al.* (1971) pointed out the difficulty in using Eq. (12) in the case of high molecular weight polymers and suggested the use of activity coefficients based on weight fractions rather than mole fractions. Nevertheless, we will use Eq. (12) to calculate "effective molecular weights".

## EXPERIMENTAL

### Materials

The copolymer investigated in the present study is manufactured by Monsanto Co. and appears under the trade name LOPAC$^R$. It is a 70% ACN and 30% styrene (W/W) copolymer having an average molecular weight of $10^5$ (according to the manufacturer). The ACN was a chromatographic grade (AO 350) supplied by Analab Inc.

### Methods

A F & M gas chromatograph (Model 810) equipped with a dual flame ionization detector was used in the present study. The parameters of the column are described in Table 1. The chromatographic parameters were as follows:
Flow rates (cc/min): Nitrogen (carrier gas), 60; Hydrogen, 15; Air, 300.
Temperatures ($^0$C): injection port, 120; column,30 to 126;detector, 225.

Table 1. Column parameters

| | |
|---|---|
| Dimensions: | 0.25 in. O. D. X 3 ft. 2 in., stainless steel |
| Solid Support: | Anakrom R ABS 70/80 mesh. |
| Percent Loading: | 5.5% W/W |
| Amount of Packing Material: | 7.00 g |
| Amount of Polymer in the Column: | 0.386 g |
| Surface Area[1] | 7.00m$^2$ |

[1] According to specifications of Anakrom ABS manufacturer.

The stationary phase in the column was prepared in the following manner: a preweighed amount of polymer was dissolved in 500 ml of pure acetone to give a 0.5% W/V solution. When the polymer dissolution was completed, a preweighed amount of the solid support (Anakrom$^R$

ABS 70/80 mesh, Analab Inc. New Haven, CT) was added. The mixture was refluxed for half an hour with occasional swirling to assure a complete and homogeneous coating of the support by the polymer. The acetone was then removed from the mixture using a rotating vacuum evaporator in a water bath at 40°C. The drying process of the semi-dry coated powder, that was obtained from the rotary evaporator, was completed in a vacuum oven kept at 60°C under vacuum of 28 in. mercury for 24 h. This coated powder was then packed into a column (see Table 1).

## RESULTS AND DISCUSSION

The specific retention volumes obtained for different amounts of ACN injected are presented in Fig. 1. It can be seen that $V_g^o$ strongly depends on the sample size and this dependency decreases with the temperature increase. The relation between the two parameters is nonlinear and, as can be seen from Fig. 2, can be related by a semilogarithmic equation of the form:

$$V_g^o = A + B \log m_p \tag{13}$$

where A and B are constants, and $m_p$ is sample size.

The $r^2$ values for Eq. (13) were found to be in the range of 0.97 to 0.98. Fig. 3 is a representative plot of $\ln V_g^o$ as a function of 1/T. From the slopes of the lines the total molar heats of adsorption ($\Delta\bar{H}_{ads}^o = \Delta\bar{H}_T^o$) given in Table 2 were calculated. It can be seen that the negative value of the heat of adsorption increases with the decrease in the amount of ACN injected, indicating an increase in the interaction between the polymer and monomer as the latter's concentration decreases. Table 3 contains the values of $\Delta\bar{G}_T^o$ as calculated from Eq. (5) and (6) as well as the components of $\Delta\bar{G}_T^o$, $\Delta\bar{G}_i^o$ and $\Delta\bar{G}_{ex}^o$. The values of $\Delta\bar{G}_i^o$ were calculated from Eq. (8) and from the Antoine type equation for acrylonitrile:

$$\log P^o = 7.57 \frac{1709.9}{284.2 + t} \tag{14}$$

where t is the temperature in degrees centigrade. The constants in this equation were taken from Blout and Mark (1950). $\Delta\bar{G}_{ex}$ was calculated from the difference $\Delta\bar{G}_T^o - \Delta\bar{G}_i^o$. The $V_g^o$ values used in Eq. (5) were those obtained from extrapolation of the lines in Fig. 2 to 0.21ng of ACN, representing infinite dilution. From the value of $\Delta\bar{H}_1^o = 7.33$

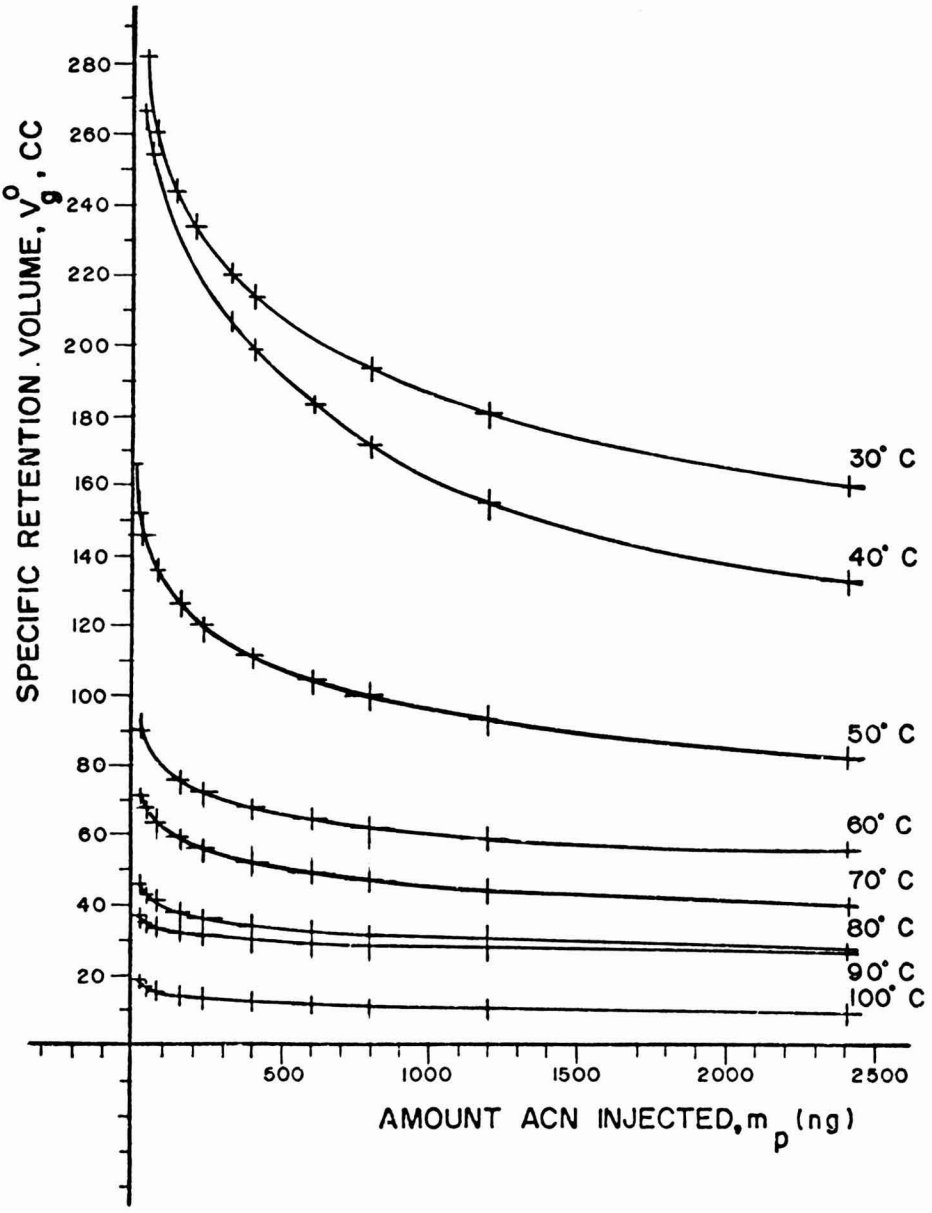


FIG. 1. THE EFFECT OF THE AMOUNT OF ACN INJECTED ON THE SPECIFIC RETENTION VOLUME

Kcal/mole and from the relation $\Delta G = \Delta H - T\Delta S$, the values of $\Delta \bar{S}^o_{ex}$ were calculated. These values are also given in Table 3. $\Delta \bar{G}^o_{ex}$ was used

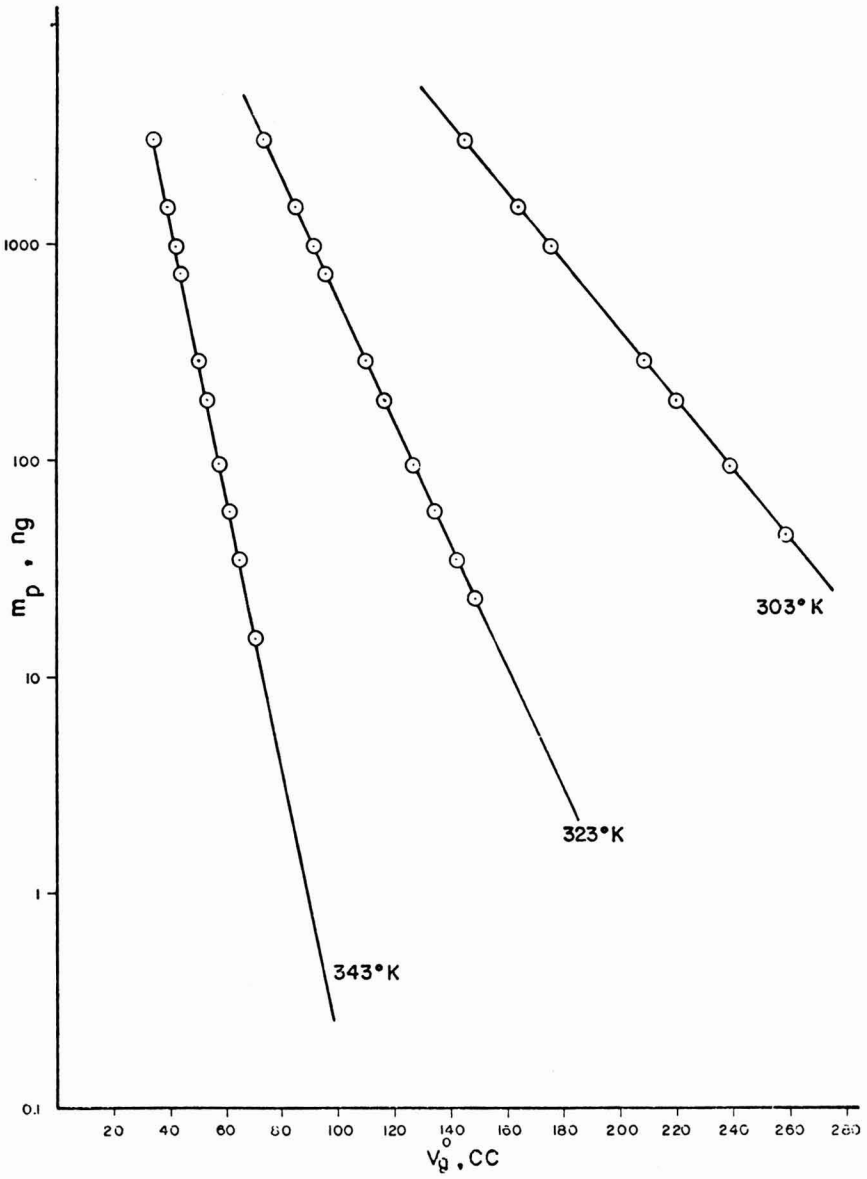


FIG. 2. THE RELATION BETWEEN THE RETENTION VOLUME AND THE AMOUNT OF MONOMER INJECTED

to calculate $\gamma_i$ by Eq. (9) and finally the effective molecular weight was calculated using Eq. (12). Also these values are given in Table 3.

From Table 3 it is evident that $\Delta\bar{G}_T^o$ becomes more negative as the

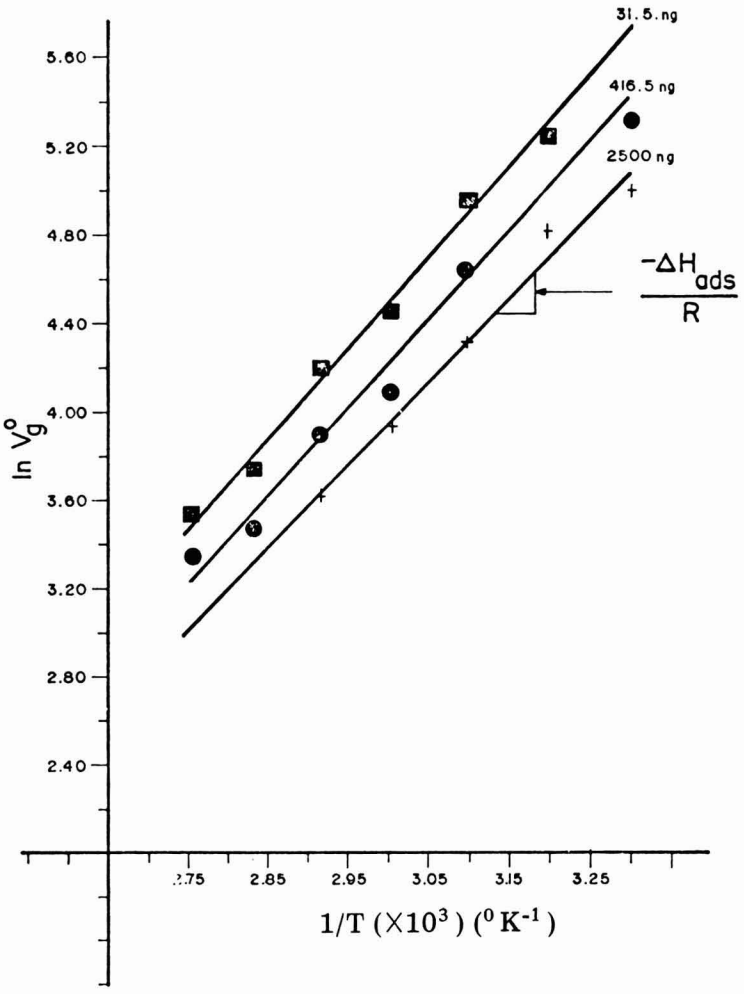


FIG. 3. HEAT OF ADSORPTION AS AFFECTED BY INITIAL AMOUNT OF ACRYLONITRILE

temperature decreases indicating a more favorable interaction between the polymer and the monomer at the lower temperatures. The value of $\Delta\bar{S}^{o}_{ex}$ is almost constant at an average value of 17.6 cal/mole indicating that there is probably no change in order. The contribution of $T\Delta S$ to $\Delta\bar{G}^{o}_{ex}$ is increasing however with the increase in temperature. The value of $\Delta\bar{H}^{o}_{ads} = -9.0$ Kcal/mole (at infinite dilution) obtained for the system investigated in the present study is considerably more negative than the value of $-4.9$ Kcal/mole obtained by Guillet and Galin (1973) for acetonitrile — polyacrylonitrile. This indicates that the interaction

Table 2. Heat of adsorption as determined from retention volume ($V_g^0$) data using Clausius-Clapeyron equation

| Amount ACN Injected (ng) | $\Delta\bar{H}^0_{ads}$ (Kcal/mole) |
|---|---|
| 21.0 | −8.61 |
| 31.5 | −8.29 |
| 52.0 | −8.29 |
| 83.5 | −8.17 |
| 166.5 | −8.19 |
| 250.0 | −8.12 |
| 416.5 | −8.00 |
| 833.5 | −7.75 |
| 1250.0 | −7.66 |
| 2500.0 | −7.51 |
| Extrapolated value | |
| 0.021 | −9.00 |

Table 3. Thermodynamic parameters evaluated from the chromatographic data extrapolated to infinite dilution. (0.021 ng ACN)

| T ($^0$K) | $\Delta\bar{G}^0_T$ (Kcal/mole) | $\Delta\bar{G}^0_i$ (Kcal/mole) | $\Delta\bar{G}^0_{ex}$ (Kcal/mole) | $\Delta\bar{S}^0_{ex}$ (cal/mole) | $\gamma_i \times 10^5$ | $M_p \times 10^{-7}$ |
|---|---|---|---|---|---|---|
| 303 | −3.87 | 2.95 | −6.83 | 17.01 | 1.20 | 2.19 |
| 323 | −3.77 | 3.63 | −7.39 | 17.71 | 0.99 | 2.36 |
| 333 | −3.52 | 3.97 | −7.49 | 17.46 | 1.22 | 2.42 |
| 343 | −3.49 | 4.31 | −7.80 | 17.86 | 1.07 | 2.50 |
| 353 | −3.27 | 4.65 | −7.91 | 17.68 | 1.26 | 2.57 |
| 363 | −3.15 | 4.99 | −8.14 | 17.80 | 1.27 | 2.63 |
| 373 | −2.89 | 5.33 | −8.22 | 17.55 | 1.53 | 2.71 |

$\Delta\bar{H}^0_{ex} = -1.67$ Kcal/mole

between the copolymer investigated in the present study and the principle monomer — ACN, is much stronger than that between polyacrilonitrile and acetonitrile. The Gibbs excess free energies obtained in the present investigation (−6.8 to −8.2 Kcal/mole) are considerably more negative than the values of −3.7 to −4.7 Kcal/mole obtained by Khalil (1976) for Barex type copolymers. The excess free entropies in the present investigation are somewhat lower but of the same order of magnitude as obtained by Khalil, while the activity coefficients are approximately two orders of magnitude lower. Although the activity coefficients given in table 3 do not change significantly with temperature their trend is to increase with increasing temperature pointing towards a decreasing interaction.

The "effective molecular weight" calculated from the activity coef-

ficients and Eq. (12) is almost constant at an average value of $2.5 \times 10^7$. As the molecular weight of the polymer (given by the manufacturer) is $10^5$, this can be interpreted as one molecule out of 250 has an active site.

If this is really the case, then the 0.386 g of polymer in the column have $(0.386 \times 6.02 \times 10^{23}) / 2.5 \times 10^7 = 9.3 \times 10^{15}$ active sites. The smallest amount of ACN injected into the GC was $21 \times 10^{-9}$ g corresponding to $(21 \times 10^{-9} \times 6.02 \times 10^{23}) / 53 = 2.4 \times 10^{14}$ molecules. The number of active sites in the polymer is therefore approximately 50 times greater than the ACN molecules injected into the GC. Thus, even if an active site is occupied by only one ACN molecule, a strong monomer/polymer interaction should be found, even when $1000_{ng}$ ACN are injected. The amount of ACN interacting with the polymer may increase considerably if more than one molecule occupies an active site. The negatively increasing free energy with decreasing temperature and the negatively increasing enthalpy with decreasing monomer concentration found in the present investigation reinforce our previously reported point of view Gilbert *et al.* (1980), that at low temperatures and low enough monomer concentrations the monomer-polymer interaction may become strong enough to prevent monomer migration from a polymeric package.

## REFERENCES

BLOUT, E. R. and MARK, H. 1950. *Monomers*, Interscience Publisher, New York.

BRAUN, J. M. and GUILLET, J. E. 1976. Inverse gas chromatography in the vicinity of $T_g$, Effect of probe molecule. Macromolecules *9*, 340.

CHUDY, J. C. and CROSBY, N. T. 1977. Some observations on the determination of monomer residues in foods. Food Cosmet. Toxic. *15*, 547.

COELHO, U., MILTZ, J. and GILBERT, S. G. 1979a. The application of inverse phase chromatography for determination of bound water in collagen. J. Food Sci. *44*, 1150.

COELHO, U. MILTZ, J. and GILBERT, S. G. 1979b. Water binding on collagen by inverse phase gas chromatography: Thermodynamic considerations. Macromolecules *12*, 284.

DANIELS, G. A. and PROCTOR, D. F. 1975. VCM extraction from PVC bottles. Modern Packaging *48* (4), 45.

DOWNES, T. W. and GILBERT, S. G. 1975. The effect of surface treatments on the migration of organotin stabilizer from polyvinyl chloride films to contacting liquid media. Deutsche lebensmittel-Rundschau, *71* (12), 422.

Federal Register, 1977. Vol. 42, No. 48, pp. 13546—13548, Fri. Mar. 11.

FIGGE, K. 1972. Migration of additives from plastic films into edible oils and fat stimulants. Food Cosmet. Toxic. *10*, 815.

GILBERT, S. G. 1976. Migration of minor constituents from food packaging materials. J. Food Sci. *41*, 955.

GILBERT, S. G. MILTZ, J. and GIACIN, J. R. 1980. Transport considerations of potential migrants from food packaging materials, J. Food Proc. Pres. *4*, 27.
GUILLET, J. E. and GALIN, M. 1973. Studies of polyacrilonitrile structure by inverse chromatography. Polymer Letters *11*, 233.
JAMES, A. T. and MARTIN, A. J. P. 1952. Biochem. J. *50*, 679.
KHALIL, H. A. 1976. A gas chromatographic study on the effect of co-and terpolymerization on the interaction of polyacrylonitrile with selected low molecular weight compounds. Ph.D. Thesis, Rutgers University, New Brunswick, N.J.
LLORENTE, M. A., MEDUINA, C. and HORTA, A. 1979. Inverse gas chromatography of Poly (cyclohexyl) methacrylate. J. Polym. Sci. Physics, *17*, 189.
MORANO, J. R. GIACIN, J. R. and GILBERT, S. G. 1977. Sorption-desorption of vinylchloride-food simulating solvent systems. J. Food Sci. *24* (1), 230.
PATERSON, D., TEWARI, Y. B., SCHREIBER, H. P. and GUILLET, J. E. 1971. Application of gas-liquid chromatography to the thermodynamics of polymer solutions. Macromolecules, *4* (3), 356.
SMIDSROD, O. and GUILLET, J. E. 1969. Study of polymer-solute interactions by gas chromatography. Macromolecules *2* (3), 272.
STEIN, A. N., GRAY, D. G. and GUILLET, J. E. 1971. Molecular probe studies of crystallinity in polyethylene. Br. Polym. J. *3*, 175.
United States Court of Appeals for the District of Columbia Circuit, 1979. No. 77—2023, 2024, 2026 and 2032, Nov. 6.

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HASSON, E. P. and LATIES, G. G. 1976. Separation and characterization of potato lipid acylhydrolases. Plant Physiol. *57*, 142–147.

ZABORSKY, O. 1973. *Immobilized Enzymes*, pp. 28–46, CRC Press, Cleveland, Ohio.

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