

STABILITY OF L-ASCORBATE AND L-ASCORBATE 2-PHOSPHATE
IN EXTRUDED WHEAT FLOUR

by

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INTRODUCTION

The requirement for vitamin C in the human diet is well recognized. Despite the widespread availability of vitamin C in fruits and vegetables, there is recent evidence of inadequate intake of vitamin C for a significant number of people. A health survey in 1971-1972 by the U.S. Department of Health, Education, and Welfare (50) showed that 10% of Americans surveyed received only one-fifth to one-seventh of the 1974 recommended dietary allowance (RDA) of vitamin C. In Canada also 10-20 per cent of the population showed an inadequate dietary intake of vitamin C (16). In Britain (26), about 10 per cent of the population had a mean daily intake of vitamin C below the British RDA (30 mg). It appears that western-style living and eating habits have a great effect on the insufficiency of vitamin C in the diet. These eating habits result in an increased consumption of animal-based foods and a lower daily consumption of fruits and vegetables. Also the increased commercial processing of potatoes probably contributes to the noted dietary deficiency of vitamin C.

Developing countries often rely on cereals directly as food, but cereals contain only traces of vitamin C. Vitamin C fortified cereal products could provide a relatively inexpensive means of alleviating inadequate intake of vitamin C where it occurs. Vitamin C fortification of infant foods could be particularly important because improperly fed mothers cannot provide a sufficient amount of vitamin C for breast-feeding their infants.

These data indicate a need to broaden the supply of vitamin C in the diet. One method of achieving this objective would be through vitamin C fortification of cereals. As already mentioned, vitamin C fortified cereals

could probably already find use in developing countries. Furthermore, unless population growth is reduced, man's increased consumption of cereals as food will dictate an ever increasing need for vitamin-enriched cereals. Why do we hear so little about dietary problems with vitamin C? It is because the health hazards of its partial deficiency have not been unequivocally established. Of course the most extreme dietary deficiencies of vitamin C, when daily intake falls below 5-10 mg, causes the easily diagnosed disease known as scurvy. But it has been established that an adult man needs 48 mg (± 15 mg) of L-ascorbic acid per day to maintain his body pool of L-ascorbic acid at approximately 1.5 g (25, 6). If 10 mg of the daily intake of vitamin C is all that is needed to eliminate scurvy, what health problems are eliminated by the other 38 mg? The relationship of vitamin C intake to health problems is well discussed in the literature (9, 19, 22, 25, 29, 33, 36, 48).

Pre-cooked cereals in the form of breakfast foods, snacks, or a base for a beverage are among the most popular cereal-based foods. When such pre-cooked cereals are fortified with vitamin C, an extra processing step is used. Vitamin C is not added prior to the cooking step, but instead is sprayed onto the surface of the processed cereal. A form of vitamin C which could be cooked with the cereal is therefore desirable.

Several reports of the loss of vitamin C during cooking of a fortified cereal have appeared (15, 38), but no detailed investigations have been published. The present study is an extensive investigation on the stability of L-ascorbic acid (vitamin C) during extrusion cooking of moistened wheat flour at various processing conditions.

Extrusion cooking was chosen because it is one of the most widely used cooking systems in processing cereal foods. With the exception of gun

puffing, extrusion cooking subjects the cereals to some of the most severe processing conditions used to produce pre-cooked cereals.

In the second part of this study, a more stable derivative of vitamin C (magnesium L-ascorbate 2-phosphate) was also cooked with wheat flour to determine its stability during extrusion.

LITERATURE REVIEW

Extrusion Cooking

The review of extrusion cooking appearing in this section is not a complete literature review. It is rather an introduction to the processing equipment used in this investigation. The reader is referred to several reviews on extrusion (12, 40, 43, 44, 46).

Food extrusion is a process in which a food material is forced to flow, under one or more of a variety of conditions of mixing, heating, and shear, through a die which is designed to form the extrudate.

There are three general classifications for cooker-extruders used in the cereal industries at the present time; cold forming, moderate temperature cooking and shaping, and short time-high temperature (S.T.-H.T.) extrusion. The functions of food extruders include cooking, mixing, shearing, sterilization, forming, and drying (43).

High temperature cooking and forming is characterized by sufficient work and heat being applied to a cereal-based product to cook and to convert the material to a "thermoplastic" mass which is shaped at the die. Equipment used for H.T.-S.T. extrusion heats the material being processed and converts it to a plastic deformable mass above 212°F and continually compresses it through a die.

The main components of a high temperature cooking-extruder are the feeder, compression screw, barrel, die, and heating system (Figure I, page 5).

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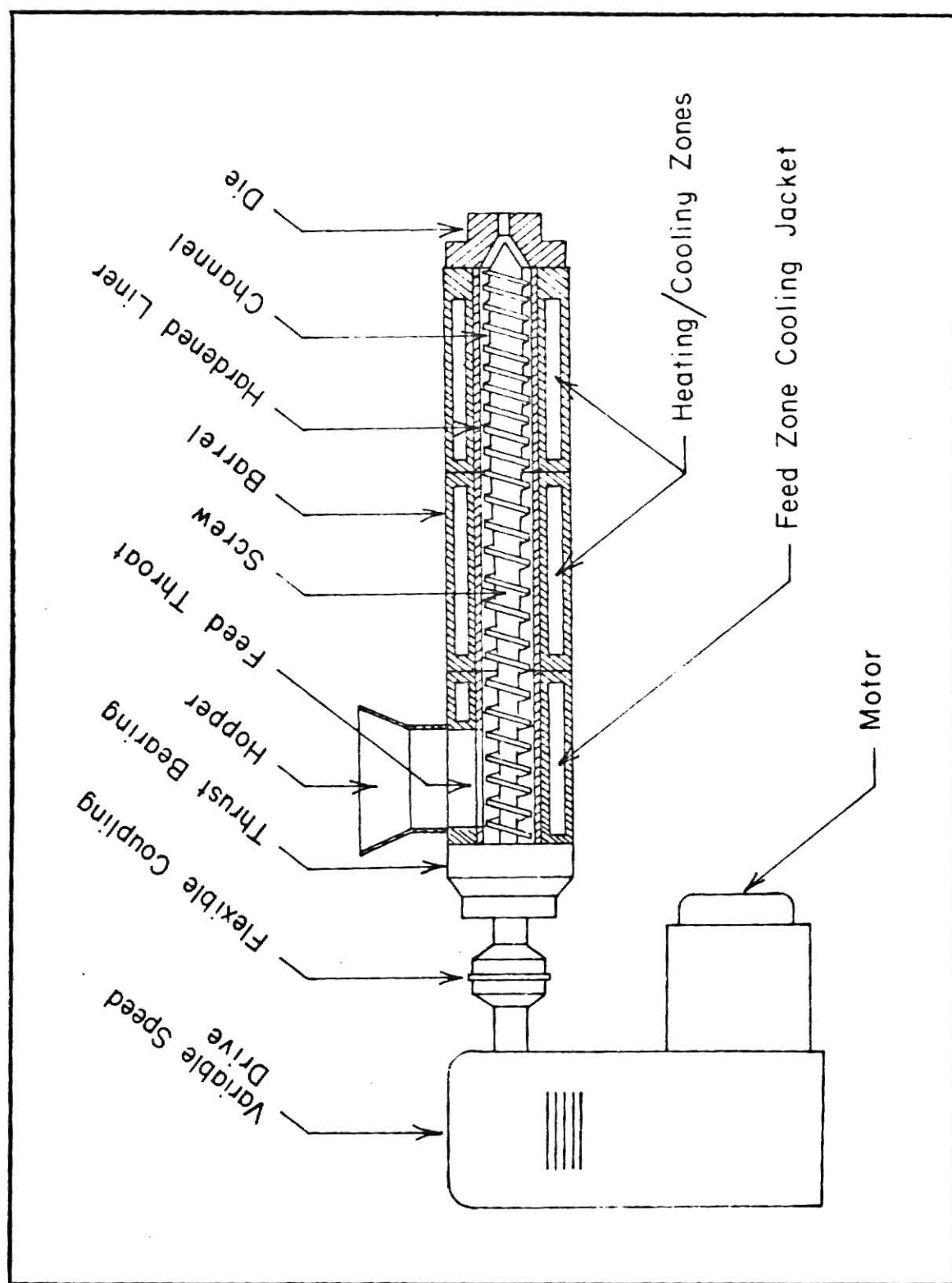


Figure 1. **ELEMENTS of a single-screw extruder**

The most important operational variables are the following:

- a. temperature, which influences the degree of cooking and the viscosity of the mass;
- b. rotational speed of the screw, which affects the residence time, the mixing and shearing of the extruded mass, and the pressure developed at the die;
- c. moisture content of the feed, which influences the viscosity of the material and thereby the die pressure and the flow rate of product;
- d. die-diameter, which affects the residence time and the die pressure.

There has been an increasingly greater use for single-screw extrusion in the food industry for the preparation of snacks and pre-cooked cereals. Smith (46) outlined the principal advantages of extrusion-cooking. They are 1) a large production capacity; 2) less labor than any other cooking system; 3) a small floor-space requirement; 4) excellent shelf-life of extrusion-cooked products; 5) a wide range of product forms, shapes, densities, and textures; 6) production of texturized vegetable protein with practically no harm to protein quality; and 8) no ecologically hazardous residue from the extrusion cooking process.

Ascorbic Acid in Health

The requirement for vitamin C (L-ascorbic acid^{*}) for human health is well known and well documented in the literature. For reviews of this subject see references (9, 19, 22, 25, 29, 33, 36, 48).

*The terms vitamin C and L-ascorbic acid are used interchangeably in this thesis. L-Ascorbic acid has 100% vitamin C potency. Other chemical forms of vitamin C are also known, such as dehydroascorbic acid and L-ascorbyl 6-palmitate.

Stability of L-Ascorbic Acid in Foods

It is well known that L-ascorbic acid is easily oxidized to dehydroascorbic acid, a compound which retains vitamin C activity but which loses biological activity by the ease of hydrolysis of its lactone ring. The oxidation of L-ascorbic acid is facilitated by such factors as oxygen, alkaline pH, and metallic ions (particularly ions of copper and iron). Of course the reaction is accelerated by increases in temperature (34). In biological systems, including foods, L-ascorbic acid may be particularly rapidly oxidized due to presence of enzyme L-ascorbic acid oxidase (3). This enzyme catalyzes the conversion of L-ascorbic acid to dehydroascorbic acid. The improving action of L-ascorbic acid in wheat-flour doughs has been explained by the presence of L-ascorbic acid oxidase in flour (3). However Carter and Page (8) indicated that in flour suspensions at pH 6.0, L-ascorbic acid is oxidized only slowly, indicating only a weak L-ascorbic acid oxidase system in the endosperm of wheat.

Vojnovich and Pfeifer (51), Lee and Labuza (32), and Cain (7), concluded that the rate of destruction of L-ascorbic acid in most foods increases as moisture content or temperature of the food increases. But in the absence of oxygen and strong mineral acids, L-ascorbic acid is a fairly stable compound (34). For example, Lopez (34) reported that boiling orange juice for 10 minutes destroyed no more than 25 per cent of the vitamin. In cold storage Bissett and Berry (5) reported that 10% and 13% of L-ascorbic acid were destroyed in orange juice after storage in glass for 4 months and one year respectively at 4.4°C. On the other hand in aerated foods, vitamin C is very unstable. Vitamin C is completely destroyed (49) when bread is baked from dough containing L-ascorbic acid at a level normally used in bread-making (200 ppm, based on flour). However, at levels of 10 mg and 212 mg of

L-ascorbic acid per 100 g of flour, 90% and 65% respectively of L-ascorbic acid is lost (38). FDA does not approve of fortification of foods when the nutrient is not stable during production of the food (20).

Only two reports have appeared in the published literature which discuss the stability of vitamin C in the extrusion-cooked cereals. DeMuelenaere and Buzzard (15) reported that the destruction of vitamin C during commercial-scale extrusion of a corn-soy mixture and during boiling in water (14.3 g L-ascorbic acid per 100 ml) for 2 minutes was 33.4% and 78.6%, respectively. Quadri et al. (38) reported a 79% destruction during extrusion of wheat flour (10 mg of L-ascorbic acid per 100 g of flour) in a laboratory scale extrusion.

L-Ascorbate 2-Phosphate

In recent years food scientists have been searching for a form of vitamin C which would be more stable in foods and vitamin premixes. Such a form could also be used in protein beverages where fortification with L-ascorbic acid gives off-flavors. Since the ene-diol structure in L-ascorbic acid is involved in the undesirable reactions in foods, chemists have made efforts to synthesize 2- or 3-substituted derivatives of L-ascorbic acid. One derivative which appears particularly promising is L-ascorbate 2-phosphate. The compound has the desired stability (35, 30) and is vitamin C active by oral or percutaneous administration (14).

To preserve the oxidation stability of L-ascorbate 2-phosphate, it is important to avoid cleavage of the phosphate group. L-Ascorbate 2-phosphate would be expected to be very stable in alkaline foods, since the doubly charged phosphate ester group on the L-ascorbate 2-phosphate is generally very stable to hydroxide attack (37).

On the acid pH side, the stability of a phosphate ester depends greatly on the structure of the alcohol portion of the ester (37). Usually strong acids (1 Molar) are required for hydrolysis, and losses in acidic foods at pH 4-6 would again be expected to be minimal. Nomura et al. (35) reported that in aqueous hydrochloric acid solution, the hydrolysis rate of L-ascorbate 2-phosphate increases linearly with the proportion of acidity (1-4 M) of the solution.

Cutolo and Larizza (14) studied the effect of pH and heat (100°C) on the stability of L-ascorbate 2-phosphate. They reported that the lower the pH, the less stable is the phosphoric ester of L-ascorbic acid. At pH 2 and after 90 minutes of heating, 70% of L-ascorbic acid was liberated. They reported that a definite improvement in hydrolytic stability is obtained at pH 4. Heating 90 minutes in neutral or slightly alkaline pH (pH 7-8) did not degrade L-ascorbate 2-phosphate.

Recently, Lee (30) compared the stability of L-ascorbate 2-phosphate to that of L-ascorbate under several conditions. In aqueous solution at 100°C with air and with nitrogen, he found that stability of L-ascorbate 2-phosphate is twenty times more stable than L-ascorbate. As expected the compound was more stable in boiling solution under nitrogen than under air. Lee (30) also found that in boiling acid at pH 13, L-ascorbate 2-phosphate had a half-life of 48 hours, but in boiling solution at pH 1, its half-life was 2.5 hours. In addition, Lee (30) showed that the compound was readily cleaved by the enzyme phosphatase from wheat germ. The preceding result indicates that foods high in phosphatase activity, such as wheat flour (39), would be expected to cause troublesome losses of the phosphate group.

It is difficult to predict the stability of L-ascorbate 2-phosphate during extrusion cooking with cereals. Thermal effects on starch do lead

to the production of acidic substances (27). The pH of a wheat flour-slurry is normally on the acid side (pH ~ 6.0).

One might speculate then that at the high temperatures used in extrusion, some acid-catalyzed hydrolysis of L-ascorbate 2-phosphate might occur. Loss of the phosphate group from L-ascorbate 2-phosphate would of course liberate L-ascorbic acid which would then be susceptible to oxygen and heat.

MATERIALS AND METHODS

Experimental Design for Response Surface Methodology Study

Response surface methodology (RSM), which has been described by Cochran and Cox (10) and promoted by Henika and Henselman et al. (23, 24) as a useful tool in food research, was used to investigate the survival of L-ascorbic acid in extruded wheat flour under a variety of cooking conditions. RSM involved use of a fractional experimental design along with a computer program to find a regression equation containing the desired response, in this case recovery of L-ascorbic acid, as a function of the independent variables (extrusion-cooking conditions). The computer program generates contour maps of the response surface at the varying levels of the independent variables.

The processing variables were temperature, moisture, screw speed, and L-ascorbic acid concentration. Each of the four variables was examined at five levels. Table 1 (page 12) gives the variables and their levels.

The four variable-five level fractional factorial design is given in Table 2 (page 13). The percentage of L-ascorbic acid recovered was used as response input data (Y) and the regression coefficients were computed by the Taylor expansion equation.

$$Y_1 = B_0X_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_4 + B_{11}X_1^2 + B_{22}X_2^2 + B_{33}X_3^2 + B_{44}X_4^2 \\ + B_{12}X_1X_2 + B_{13}X_1X_3 + B_{14}X_1X_4 + B_{23}X_2X_3 + B_{24}X_2X_4 + B_{34}X_3X_4$$

Nonsignificant B-values were eliminated by the computer program by stepwise deletion until a minimum mean square term was obtained. The

Table 1. Independent variables and their levels for the RSM study

Independent variables	Variable symbol	Code number for level				
		-2	-1	0	1	2
Temperature (°C)	X_1	150	175	200	225	250
L-Ascorbic acid concentration (mg/ounce)	X_2	5	15	25	35	45
Screw speed (rpm)	X_3	50	75	100	125	150
Moisture content (%)	X_4	17	19	21	23	25

Table 2. RSM design for four variables at five levels

Run number	Variables			
	X_1	X_2	X_3	X_4
1	-1	-1	-1	-1
2	1	-1	-1	-1
3	-1	1	-1	-1
4	1	1	-1	-1
5	-1	-1	1	-1
6	1	-1	1	-1
7	-1	1	1	-1
8	1	1	1	-1
9	-1	-1	-1	1
10	1	-1	-1	1
11	-1	1	-1	1
12	1	1	-1	1
13	-1	-1	1	1
14	1	-1	1	1
15	-1	1	1	1
16	1	1	1	1
17	-2	0	0	0
18	2	0	0	0
19	0	-2	0	0
20	0	2	0	0
21	0	0	-2	0
22	0	0	2	0
23	0	0	0	-2
24	0	0	0	2
25	0	0	0	0
26	0	0	0	0
27	0	0	0	0
28	0	0	0	0
29	0	0	0	0
30	0	0	0	0
31	0	0	0	0

X_1 = temperature

X_2 = L-ascorbic acid concentration

X_3 = rotational speed

X_4 = moisture content

computer program supplied a coefficient of determination (R^2) for the final regression equation. The final coefficients were then used to print contour maps to cover the response (Y) as a function of the extrusion variables.

Raw Materials

Flour used in this study was unenriched, unbleached, and unmalted flour milled from hard red winter wheat in the Department of Grain Science and Industry at Kansas State University. The proximate analysis of the flour was 0.51% ash, 11.5% protein, and 12.3% moisture. It should be noted that all concentrations of materials in flour were calculated on a dry basis. Moisture determination was done according to the AACC air-oven Method 44-15A (1).

Flour Deaeration

For one extrusion experiment, deaerated flour was needed. The air in flour was replaced by nitrogen as follows. The flour (300 g) was placed in a 3-necked round-bottomed flask, and the flour was sequentially subjected to vacuum and repressurized with nitrogen (prepurified grade, Matheson gas products, Joliet, Illinois). The procedure was repeated five times (30 minutes each), and flour was then stored 16 hours (tempering time) in the same flask. During extrusion of the deaerated sample of flour, the feed hopper of the extruder was filled with carbon dioxide to prevent incorporation of air with the flour particles entering the extruder.

Inactivation of Phosphatase in Wheat Flour

The inactivation of phosphatase in wheat flour was accomplished by autoclaving 200 g of wheat flour for 30 minutes at 121°C. The flour was placed in a 2000 ml erlenmeyer flask whose top was covered with aluminum foil. No visual increase in flour moisture was observed.

Tempering and Ascorbic Acid Supplementation of Wheat Flour

Powdered L-ascorbic acid (Sigma Chemical Company, St. Louis, Missouri) was used throughout this study. The desired amount of L-ascorbic acid was first premixed thoroughly for 10 minutes with 150 g of flour. The premix was then added to the rest of the flour (1850 g) which was placed in a rotating drum (18 inches in diameter, 18 inches long). The rotating drum was allowed to run for another 10 minutes before adding the water to the flour. The water was added as a mist using an atomizer while the drum was rotated. To compensate for evaporation and loss during mixing, it was found that tempering water had to be added one percentage point above the final desired level. The tempering drum was allowed to run for five minutes after the water had been added. The tempered wheat was placed in a polyethylene bag for 16 hours tempering before extrusion. The moisture content of the tempered wheat was checked before extrusion.

Extrusion

A Brabender Laboratory-Scale Extruder (Model 2503) was used in this study (Figure I, page 5). The extruder specifications are also as follows.

Barrel length	18.75 in.
Barrel diameter	0.75 in.
Length/diameter ratio	25:1
Barrel volume	47.1 in. ³
Internal surface	44.3 in. ²
Heating (3 zones)	1000 watts per zone
Cooling	air
Compression ratio	5:1
Die diameter	0.125 in.

The extruder was fitted with thermocouples at the end of each heating zone (3 zones) to measure the actual product temperature inside the barrel. The inside surface of the barrel was modified by the addition of six triangular grooves 0.125 in. depth, parallel to the longitudinal axis. The extruder was equipped with a die cap-nozzle (0.125 in. opening) which produced rod-shaped products.

The operating procedure for the Brabender extruder was followed essentially as described by Stearns (47). After the extruder was assembled, the heating zones temperatures were set for start up, so that zone one was cooled, zone two was heated to 100°C, and zone three was heated to 135°C. These temperatures were maintained until the die-cap nozzle was hot to the touch. The screw was adjusted to 100 rpm, and flour at 25% moisture was manually fed into the feeder to prevent plugging on start-up. After the start-up extrudate had been collected for 5 minutes, the flour of new moisture level was fed by hand into the hopper. The rotational speed was adjusted to the desired rpm and the temperature in zone three was also adjusted to the required temperature level. The temperatures for zones one and two remained the same as in start-up. After an equilibrium extrusion condition was indicated by continuous uniform outflow, constant voltage reading, constant amperage reading, and by the product temperature being equal to or very close to the temperature setting in zone three, three samples of product were taken at 1 minute intervals. The average production rate was recorded after weighing the one-minute samples. The samples were then placed in a polyethylene bag and stored under refrigeration until analysis of L-ascorbic acid or L-ascorbate 2-phosphate was done the next day. When an extruded sample was taken, the following readings were recorded.

- a - zone-three temperature setting
- b - product temperature in zone three
- c - rotational speed
- d - voltage--across the armature of the motor drive
- e - amperage--through the armature of the motor drive

A change to new operation parameters was accomplished by switching to the new conditions, allowing the extruder to reach equilibrium, and then sampling as described above.

L-Ascorbic Acid assay

L-Ascorbic acid in tempered flour and in extruded samples was determined according to the fluorometric procedure reported by Deutsch and Weeks (17). The method is based on the oxidation of L-ascorbic acid to dehydroascorbic acid followed by reaction of the latter with o-phenylenediamine to give a fluorescent quinoxaline.

To construct a standard curve, eighty milligrams of pure L-ascorbic acid was dissolved in 1000 ml distilled water, previously boiled. Aliquots (2.0 ml, 4.0 ml, 7.0 ml, 10 ml, and 20 ml) were taken from the stock solution and each aliquot was diluted to 100 ml with H₂O. Two milliliters were taken from each dilution and added to a small flask containing 6 ml of trichloroacetic acid (6% in water). After 10 minutes, a 7 ml aliquot of ascorbic acid/trichloroacetic acid was transferred from each flask and added to 7.0 ml of a 6% aqueous solution of metaphosphoric acid. Acid-washed charcoal (65 mg) was added to each solution, and the mixture was shaken at intervals for 20 minutes. The mixture was filtered through Whatman 2V folded filter paper, and four 3.0 ml aliquots of each filtrate were transferred to test tubes. Depending on previous aliquot sizes, the 3 ml

aliquots at this point in the assay procedure contained 0.6, 1.2, 2.1, 3.0, and 6.0 μ g of L-ascorbic acid. To two of the 3.0 ml aliquots, 1.0 ml of boric acid/sodium acetate solution (3 g boric acid dissolved in 100 ml of 50% aqueous sodium acetate) was added, and the mixture was allowed to stand for 20 minutes before adding 5.0 ml of o-phenylenediamine solution (25 mg in 100 ml water). These two tubes were designated as the sample blank. To the other two 3.0 ml aliquots, 1.0 ml sodium acetate (15%) and 5 ml o-phenylenediamine were added and mixed. These two tubes were designated as the sample. All tubes containing o-phenylenediamine were incubated for 40 minutes at room temperature, and their fluorescence was measured using a Coleman Electronic Photofluorometer Model 12-C with a primary filter (B1-S) and a secondary filter (PC-1).

Ascorbic acid in extruded flour was determined as follows:

1. Extruded wheat flour samples were ground to pass through a 40-mesh sieve using a Wiley mill. About 50g of each ground sample was collected and placed in an air-tight plastic bag and kept in refrigerated storage until analysis.
2. An amount of a ground sample containing approximately 80 mg L-ascorbic acid was placed in a 1000 ml volumetric flask, made to volume with distilled water, and stirred for 10 minutes.
3. A 2.0 ml aliquot was transferred to a 10 ml volumetric flask and made up to volume.
4. A 2.0 ml aliquot from step 3 was transferred to a small flask containing 6.0 ml of 6% aqueous trichloroacetic acid and let stand for 10 minutes.
5. Seven milliliters of the L-ascorbic acid/trichloroacetic acid

solution in step 4 was diluted and mixed with 7.0 ml of 6% aqueous metaphosphoric acid.

6. Approximately sixty-five milligrams of acid washed charcoal was added to each flask to catalyze oxidation of L-ascorbic acid to dehydroascorbic acid. The reaction flask was stoppered and shaken at intervals for 20 minutes at 25°C.
7. The solution was filtered through Whatman 2V folded filter paper.
8. Four 3.0 ml aliquots of the filtrate were transferred to test tubes.
9. To two of the 3.0 ml aliquots, 1.0 ml boric acid/sodium acetate solution (3 g boric acid dissolved in 100 ml 50% sodium acetate) was added and kept for 20 minutes.
10. At the end of the 20 minutes, 5 ml of aqueous o-phenylenediamine (25 mg in 100 ml distilled water) was added to the borate-carbohydrate complex.
11. To the two remaining 3 ml aliquots taken in step 8, add 1.0 ml of 15% aqueous sodium acetate and 5 ml of o-phenylenediamine solution, and shake. This step is done during the 20 minutes holding period of the blank sample (step 9).
12. All samples were then incubated at 25°C for 40 minutes. Their fluorescence was measured using a Coleman Electronic Photofluorometer.
13. The recovery of L-ascorbic acid was calculated from the standard curve after subtracting the blank from the sample.
14. A control sample containing a known amount of pure L-ascorbic acid was carried throughout the assay to correct for any changes occurring during a given assay run.

L-Ascorbate 2-Phosphate Supplementation of Wheat Flour

Magnesium L-ascorbate 2-phosphate was obtained through the courtesy of Mrs. Y.T. Liang, Department of Grain Science and Industry, Kansas State University. The compound was prepared as described by Lee (31). The method used in adding L-ascorbate 2-phosphate to the wheat flour was the same as that described for L-ascorbic acid. Magnesium L-ascorbate 2-phosphate (formula weight 380) was added to wheat flour in amounts equivalent on a molar basis to that of L-ascorbic acid.

L-Ascorbate 2-Phosphate Assay

The assay for L-ascorbate 2-phosphate in wheat flour and extruded wheat flour was essentially the same procedure used to analyze for L-ascorbate 2-sulphate (38). A sample containing 150 mg of magnesium L-ascorbate 2-phosphate was placed in a 1000 ml volumetric flask and made up to volume with water. The mixture was stirred for about 30 minutes, then centrifuged. Ten milliliters of the supernatant was placed on top of a microcolumn (6 x 80 mm) containing 500 mg of weakly basic, ion-exchange resin (AG3-X4A, chloride form 200-400 mesh, Bio-Rad Laboratories, Richmond, California). The column was washed with 100 ml water, and L-ascorbate 2-phosphate was then eluted with 2 M aqueous sodium sulfate (95 ml). The eluant was adjusted to pH 10 using 0.1 N sodium hydroxide, the solution was made up to volume (100 ml), and its UV absorption measured at 264 nm. The recovery of L-ascorbate 2-phosphate from the sample was calculated from the recovery determined for a control sample of pure magnesium L-ascorbate 2-phosphate dissolved in distilled water.

RESULTS AND DISCUSSION

Destruction of L-Ascorbic Acid during Extrusion of
Moistened Wheat Flour

During the first and major part of this investigation, we were interested in the loss of vitamin C during extrusion processing of wheat flour fortified with L-ascorbic acid. Since vitamin C is sensitive to oxygen and heat, its loss during extrusion processing can occur not only in the cooking stage but also during tempering of the moistened wheat flour.

There are three reactions by which L-ascorbic acid might be destroyed during the extrusion processing of wheat flour.

1. Oxidative destruction. In this pathway L-ascorbic acid is oxidized to dehydroascorbic acid, a relatively unstable form of vitamin C which converts irreversibly to an inactive molecule of diketo-L-gulonic acid (3, p. 220). Oxidative destruction of L-ascorbic acid is accelerated by higher temperatures and the presence of oxygen and traces of copper or iron cations. In the presence of a large excess of oxygen, L-ascorbic acid would be destroyed by a pseudo first-order reaction. One can calculate that 3 mg of L-ascorbic acid can be converted to dehydroascorbic acid by 1 cc of air at standard pressure and temperature (13).
2. Thermal dehydration. This destructive pathway, in which L-ascorbic acid is converted to furfural (3, 18), is promoted by high temperature and very low pH. Loss of L-ascorbic acid by this pathway would be first-order in L-ascorbic acid.
3. Free-radical mechanism. L-ascorbic acid reacts with free radicals to give 2- and 3-monodehydroascorbic acid. Two molecules of

monodehydroascorbic acid will disproportionate to produce one molecule of L-ascorbic acid and one of the unstable dehydro L-ascorbic acid (52, 4). It is known that ball-milling of starch creates free radicals (53). Thus it can be postulated that shearing of flour in an extruder produces free radicals. If the formation of free radicals in the flour is the rate-limiting step in this pathway, then L-ascorbic acid will disappear by a zero-order reaction.

Response surface methodology (RSM) is a very useful technique to investigate the loss of L-ascorbic acid during extrusion-cooking of wheat flour. That technique greatly reduces the amount of experimental work required to examine the effect of several processing variables at different levels on a given response. In this work we examined the effects of five levels of four extrusion variables, namely temperature, screw-speed, concentration of moisture, and concentration of L-ascorbic acid (see Table 1, page 12). If one was to run experiments at all possible combinations of four variables at five levels, one would have to extrude 625 times. Using RSM, the number of extrusion runs is reduced to 31. The computer does the rest. The regression equation and corresponding contour plots permit the investigator to quickly locate the optimum solution to a problem and to verify the predicted solution experimentally.

To examine the producibility of the center point of the RSM study, seven replications of the RSM zero point (temperature 200°C, moisture 21%, L-ascorbic acid concentration 25 mg/oz, and screw speed 100 rpm) were done. The seven replications gave a range of recovery values of 64.2% to 71.6% with a mean recovery of 67.0%, a standard deviation of 3.05% and a coefficient of variation of 4.5%. The producibility of the recovery values is

an accumulation of the errors in several steps in the extrusion process, including mixing, variable losses of L-ascorbate during tempering of the flour to the desired moisture level, lack of equilibrium conditions during extrusion, and accuracy of the assay for L-ascorbic acid. L-Ascorbic acid recovery was calculated from the standard curve (Figure 2, page 25) which shows the linearity of the fluorescence intensity vs concentration of L-ascorbic acid in each tube. The regression standard deviation for the standard curve ($\sigma_{y \cdot x}$) was found to be 1.152 fluorescence units/ μ g of L-ascorbic acid. It should also be noted that each recovery value is the average found for at least two experimental trials. Appendix I contains all the experimental data of the RSM experiment. The Appendix contains, besides the operating variables and recovery of L-ascorbic acid, production rate and the voltage and amperage requirements of the motor drive.

In order to understand the effects of various extruder operating conditions on the recovery of L-ascorbic acid from cooked wheat flour (the RSM experiment), it was first necessary to determine the loss of vitamin C due to tempering of the moistened flour.

For this reason, flour was tempered for 16 hours at 25°C at different moisture contents (17-25%) and different concentrations of L-ascorbic acid (5-45 mg/oz). The results are given in Table 3 (page 26). The percentage of vitamin C destroyed was not significantly affected by the moisture content or by the concentration of L-ascorbic acid. Under the tempering conditions examined, the loss of L-ascorbic acid was relatively constant at 9.4-11.7% of the initial amount of L-ascorbic acid added to the flour. This result means that variability in the losses of vitamin C which might occur during the extrusion process does not arise from losses in the

Figure 2. L-Ascorbic acid standard curve by microfluorometric method.

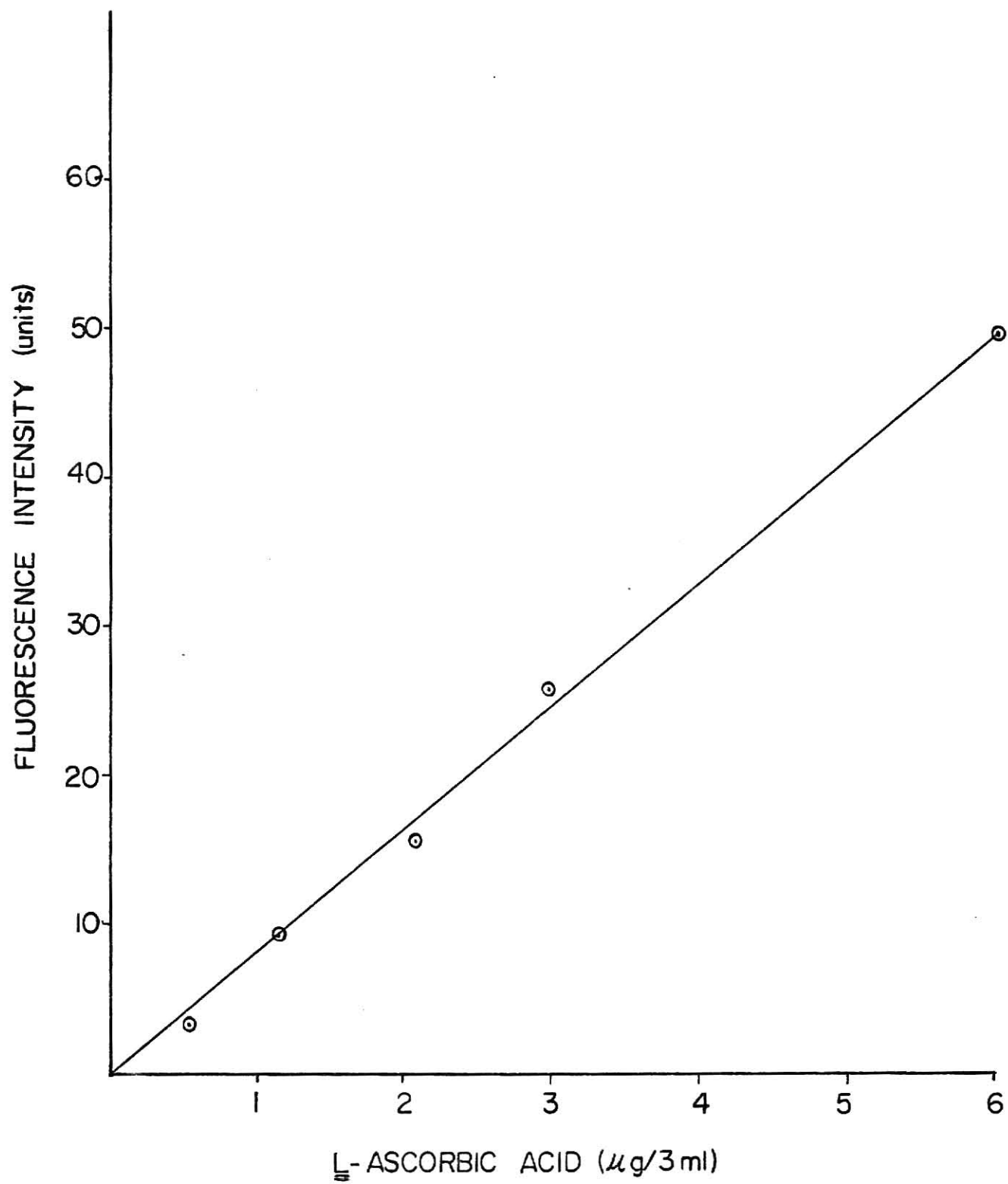


Table 3. Recovery of L-ascorbic acid from wheat flour blends after tempering 16 hours at 25°C

Moisture content %	Ascorbic acid concentration mg/ounce	Ascorbic acid recovery %
17	25	91.6
19	15	90.8
	35	89.0
21	25	90.8
23	15	90.0
	35	89.2
25	25	88.3

tempering stage, but instead is due to changes in losses during cooking at various conditions in the extruder.

Several workers have also obtained only small losses of L-ascorbic acid during storage of vitamin C fortified flour. Vojnovich and Pfeifer (51) reported a 95% retention of L-ascorbic acid in wheat flour blends (400 mg of ascorbic acid per lb of flour) at moisture levels ranging from 12.9% to 14.6% at 26°C. Klaui (28) reported a 71.2% recovery after one year storage in closed bottles.

The recovery of L-ascorbic acid* at various extrusion conditions (see Table 1, page 12 and Appendix I) was used as input data to a multiple-regression computer program. The final regression equation, which explained 90.6% of the variability in recovery of L-ascorbic acid, is as follows:

$$Y = 67.55957 - 9.516667 X_1 + 1.533332 X_2 + 2.016667 X_3 - 2.65 X_4 \\ - 2.335374 X_1^2 - 1.285373 X_3^2 - 1.285371 X_4^2 - 1.949998 X_1 X_2 + 1.587501 X_1 X_3 \\ - 1.462501 X_1 X_4 + 1.774997 X_2 X_3$$

where

Y = recovery of L-ascorbic acid recovery, %

X_1 = temperature

X_2 = L-ascorbic acid concentration

X_3 = screw speed

X_4 = moisture

* The loss of L-ascorbic acid during extrusion processing contains both the loss incurred during tempering and the loss incurred by extrusion cooking. The loss during cooking can be approximated by subtracting 10% from the processing loss.

The regression equation was used to generate all possible 150 contour plots. Several contour plots have been selected to show how the recovery of L-ascorbic acid is affected by the extrusion variables.

Figure 3 (page 31) shows the recovery of L-ascorbic acid as a function of temperature and moisture when the L-ascorbic acid concentration in flour is 25 mg/oz and when the rotational speed of the screw is 100 rpm. At any moisture level in Figure 3, L-ascorbic acid recovery decreased as temperature increased. The effect of temperature on the recovery of L-ascorbic acid was more pronounced at temperature levels higher than 200°C. A change in temperature from 150°C to 200°C is responsible for 8% destruction, while a temperature increase from 200°C to 250°C resulted in 24% destruction. At temperature levels of 185-250°C, L-ascorbic acid recovery decreased as moisture content increased, and this recovery-decrease accelerated as the moisture content increased. The role of moisture in the destruction of L-ascorbic acid could be due to one or more of the following reasons; a) the introduction of metal ions of copper and iron along with the extra water; b) the increased mobility of reactants in flour that contains more moisture, and c) the increased thermal conductivity of the moistened flour with higher moisture levels.

Two mechanisms that destroy L-ascorbic acid appear to be operating at temperatures below 175°C in Figure 3. At 150°C the recovery of L-ascorbic acid goes through a maximum at approximately 21% moisture, falling off slightly at moisture levels above or below 21%. At 150°C and 17% moisture, the viscosity of the wheat flour in the extruder would be higher than at 21% moisture, and it is hypothesized that under these conditions, the increased frictional forces would generate more free radicals leading to increased destruction by the free radical mechanisms. At 150°C and 25%

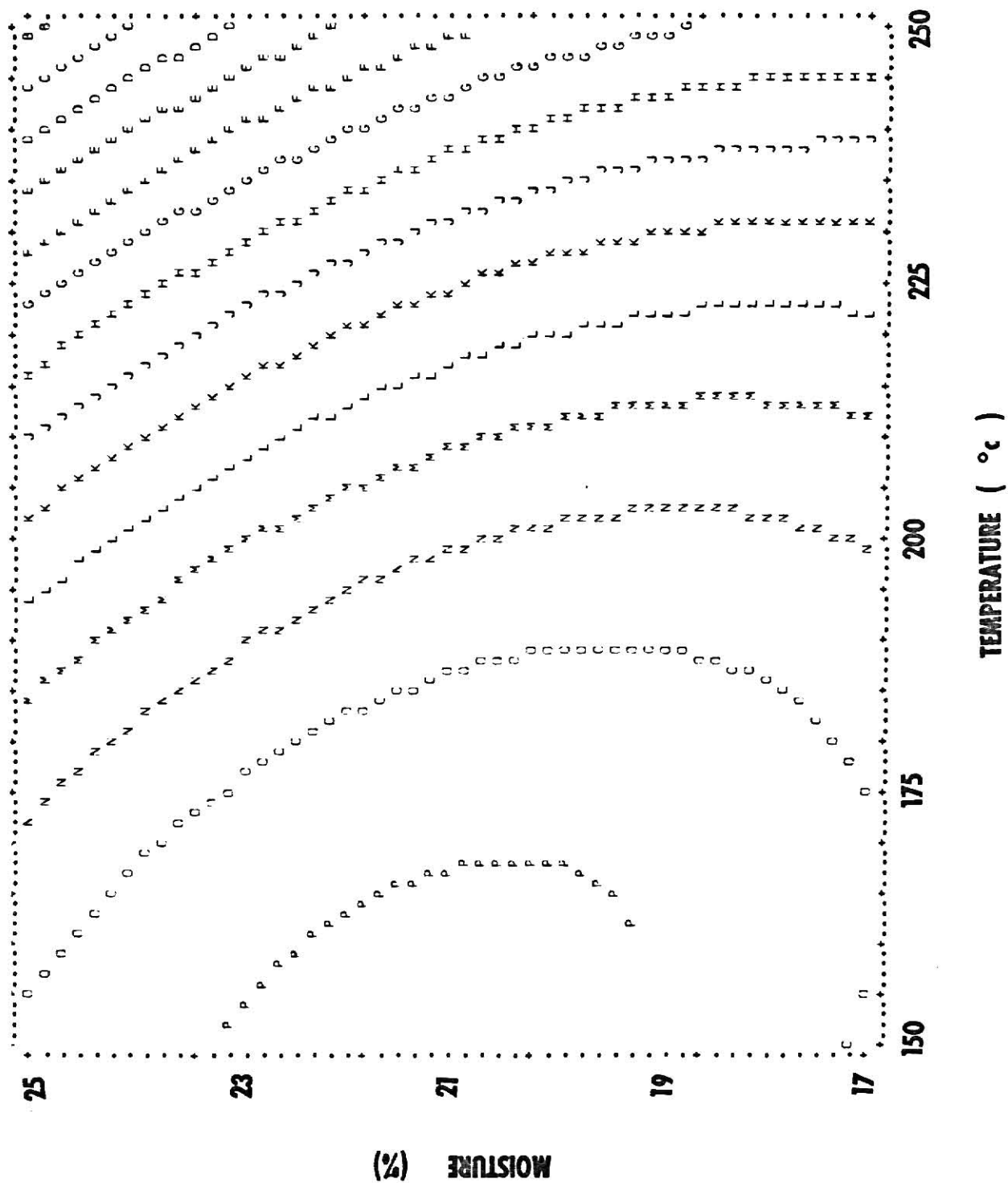
moisture, the free radical destruction of L-ascorbic acid would be less important but the oxidative destruction mechanism would increase the losses of L-ascorbic acid due to the higher moisture level of the flour. The oxidative mechanism appears to predominate at all temperatures above 185°C in Figure 3.

The effect of varying temperature and rotational speed on the recovery of L-ascorbic acid is illustrated in Figures 4, 5, and 6 (pages 34 to 39). Figure 4 shows the recovery of L-ascorbic acid as a function of temperature and screw speed when L-ascorbic acid concentration is 25 mg/oz and the moisture content is 21%. At any rotational speed, L-ascorbic acid recovery decreased as temperature increased, and the effect accelerated as temperature increased.

The effect of screw speed on recovery of L-ascorbic acid at different temperatures is best described by examining the two extremes of temperatures in Figure 4. At 250°C, the lower the screw speed, the higher the loss of L-ascorbic acid. This result is expected, since the lower screw speed means a longer residence time for the flour in the extruder. At 150°C the recovery of L-ascorbic acid with varying screw speed goes through a maximum at approximately 100 rpm. The lower than expected recovery at higher screw speeds at 150°C is again a manifestation of the probable free-radical destruction of L-ascorbic acid. Using this explanation it is assumed that the creation of free radicals at higher screw speed is a shear function of screw speed. In general, then, the magnitude of the effect of screw speed on the recovery of L-ascorbic acid is dependent on the extrusion temperature. At low temperature, rotational speed had only a slight effect on L-ascorbic acid recovery. At higher temperatures (200°C-250°C) higher screw speeds

Figure 3. Contour plot of the recovery of L-ascorbic acid at varying temperatures and moistures with rotational speed and L-ascorbic acid concentration held constant at 100 rpm and 25 mg/oz, respectively.

(B = 24, C = 28, D = 32, E = 36, F = 40, G = 44, H = 48,
J = 52, K = 56, L = 60, M = 64, N = 68, O = 72, P = 76.)



(100 rpm-150 rpm) also had little effect on L-ascorbic acid recovery, whereas lower screw speeds resulted in lower recovery.

The vitamin-recovery contour map at varying levels of temperature and screw speed changes with different levels of L-ascorbic acid concentration in the flour (moisture content constant at 21%). Figure 5 shows a contour map at the lowest level of L-ascorbic acid concentration (5 mg/oz). At the lower temperature range (150°C-200°C) in Figure 5, higher screw speeds (90 rpm-150 rpm) increased the loss of L-ascorbic acid more dramatically than in Figure 4 (page 35). This phenomenon is probably due again to the more viscous paste at a lower temperature of extrusion resulting in more free radical destruction at higher screw speeds. The contour map at the lowest level of L-ascorbic acid (5 mg/oz, Figure 5) also differs considerably from the contour map at the highest level of L-ascorbic acid (45 mg/oz, Figure 6, page 39), especially in the temperature range of 150-200°C. A possible explanation for this difference in contours in Figures 5 and 6 (pages 37 and 39) is that a greater percentage of L-ascorbic acid at the lowest concentration in flour is destroyed by the free radical mechanism. In the two flours containing 5 mg/oz or 45 mg/oz of L-ascorbic acid, the number of free radicals generated would be identical at a given screw speed, temperature, and moisture level, since the major constituents making up the flour are subjected to the same shear. If 2 mg/oz of vitamin C was destroyed by the free radical route in both flours, then only 60% of L-ascorbic acid is recovered at the lowest initial concentration of L-ascorbic acid, whereas 95.5% is recovered at the highest initial concentration. Thus, it is conjectured that the free radical destruction of vitamin C in the extruder is independent of the concentration of L-ascorbic acid in the flour (reaction rate zero-order in ascorbic acid concentration). On the other hand,

oxidative or dehydration destruction of L-ascorbic acid during extrusion is a first-order process in the L-ascorbic acid concentration. The implication of the first-order process is that extrusion of two samples of flour containing 5 mg/oz or 45 mg/oz of L-ascorbic acid under identical processing conditions would result in the same percentage recovery of L-ascorbic acid from both samples, if all destruction of vitamin C was due to the oxidative or dehydration pathways.

Figure 6 (page 39) provides additional support for our hypothesis used to explain the extrusion losses of L-ascorbic acid. It will be recalled that the contour in Figure 6 (page 39) is the recovery data for flour containing initially 45 mg/oz of L-ascorbic acid. At these higher concentrations of L-ascorbic acid, one would expect to see less effect on the contour from the small, constant, free radical losses of L-ascorbic acid. Conversely, the first order mechanisms of destruction would be expected to dominate the shape of the contour. The latter is indeed the case since for practically all temperatures in Figure 6 (page 39) the residence time of the flour in the extruder (screw speed) has a major effect on the percentage of L-ascorbic acid recovered.

L-Ascorbic acid recovery values obtained at various temperatures and initial vitamin C concentrations with screw speed and moisture content held constant are shown in Figures 7 and 8 (pages 42 and 44). In Figure 7 (page 42), where the moisture content was held at 21% and the rotational speed at 100 rpm, one can see that at any L-ascorbic acid concentration level, the recovery of L-ascorbic acid decreased as temperature increased. At temperatures ranging from 150°C to 195°C, the loss of L-ascorbic acid was dependent on the concentration of vitamin C; higher percentage recoveries of L-ascorbic acid were observed at higher concentrations of vitamin C. At temperatures

Figure 4. Contour plot of the recovery of L-ascorbic acid at varying temperatures and screw speed with L-ascorbic acid concentration and moisture content held constant at 25 mg/oz and 21%, respectively.

(B = 24, C = 28, D = 32, E = 36, F = 40, G = 44, H = 48,
J = 52, K = 56, L = 60, M = 64, N = 68, O = 72, P = 76.)

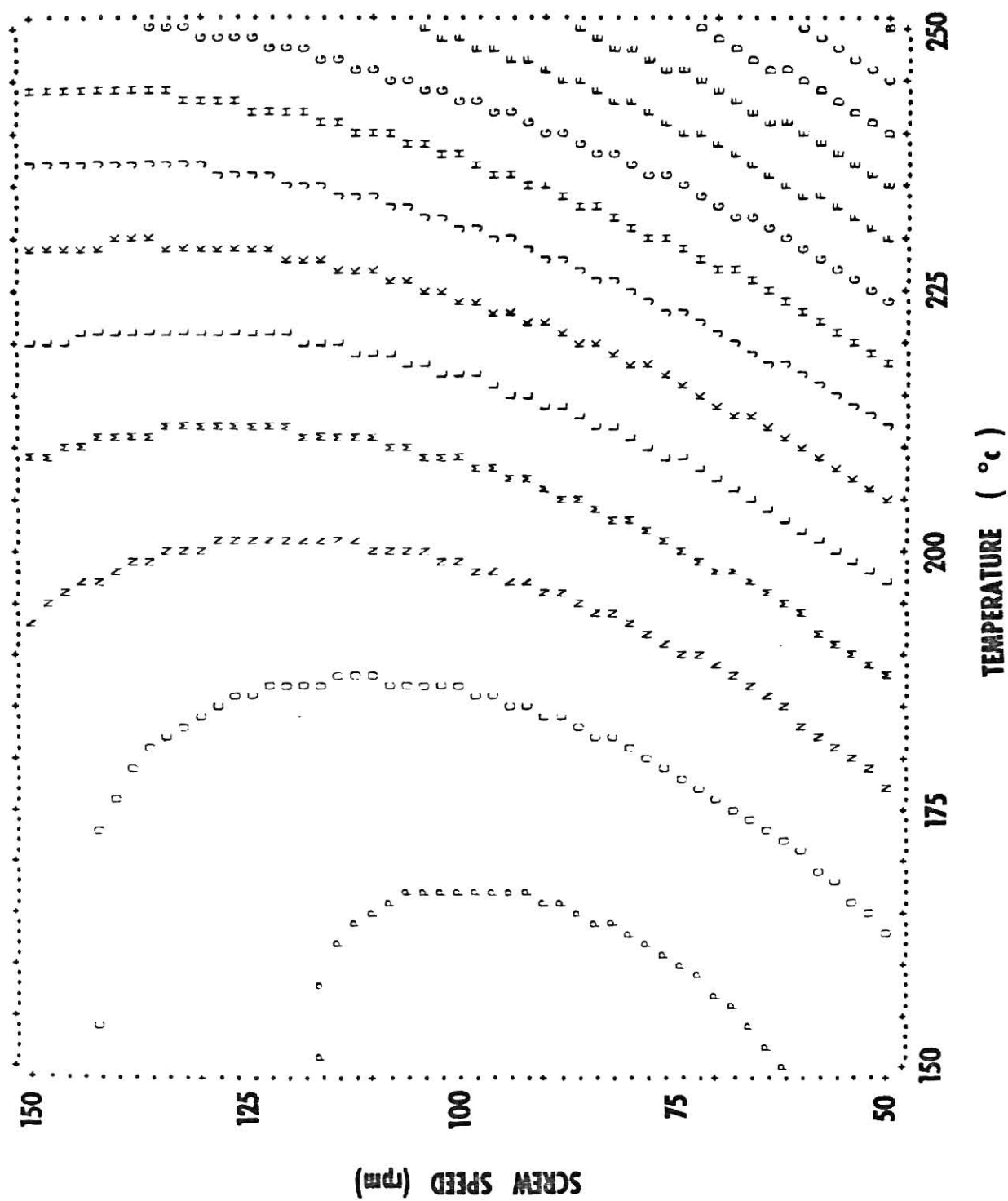
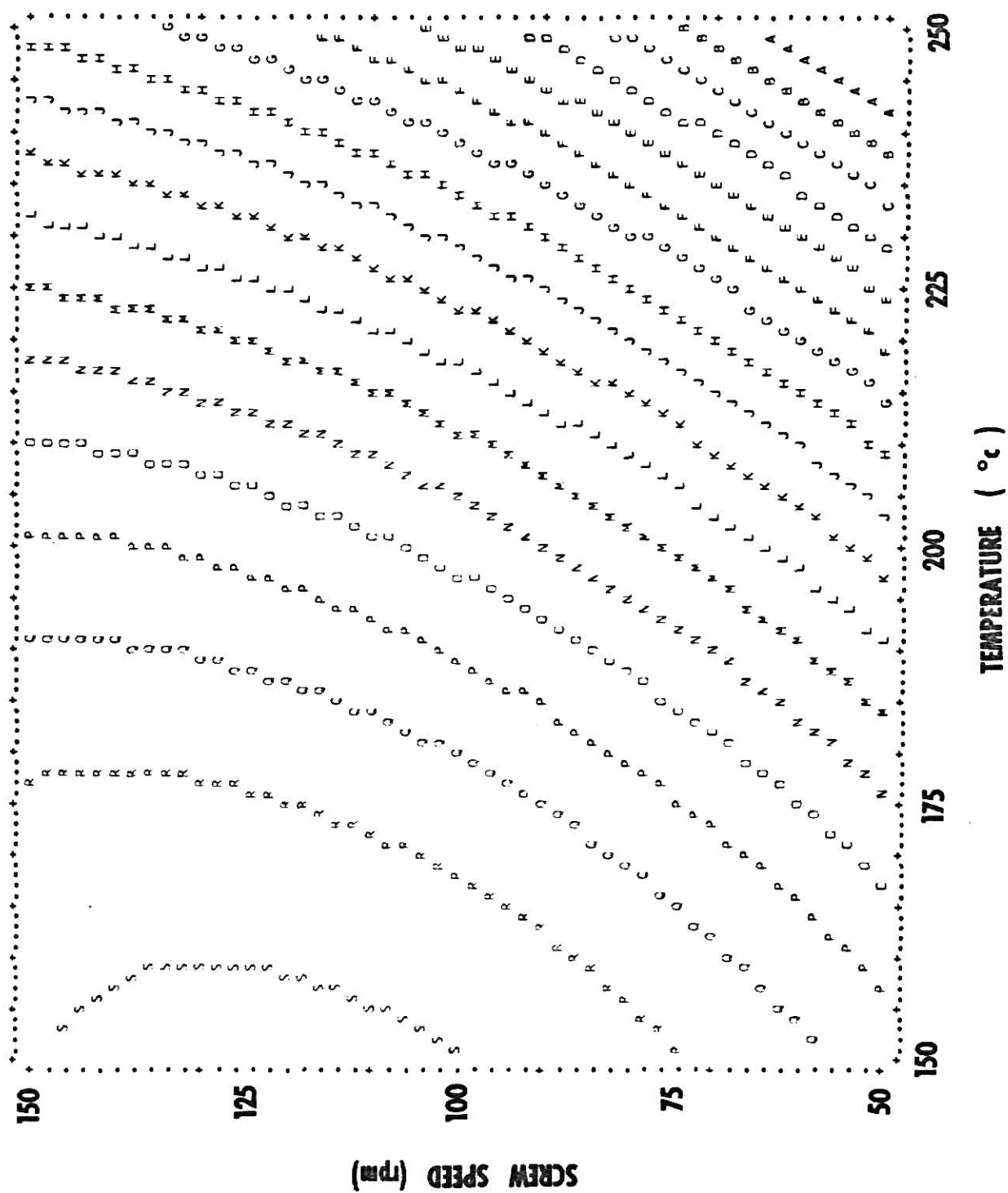


Figure 5. Contour plot of the recovery of L-ascorbic acid with varying temperatures and screw speed and with L-ascorbic acid concentration and moisture content held constant at 5 mg/oz and 21%, respectively.

(E = 36, F = 40, G = 44, H = 48, J = 52, K = 56, L = 60, M = 64, N = 68.)

Figure 6. Contour plot of the recovery of L-ascorbic acid at varying temperatures and screw speed with L-ascorbic acid concentration and moisture content held constant at 45 mg/oz and 21% respectively.

(A = 20, B = 24, C = 28, D = 32, E = 36, F = 40, G = 44,
H = 48, J = 52, K = 56, L = 60, M = 64, N = 68, O = 72,
P = 76, Q = 80, R = 84, S = 88.)



from 195-250°C, the loss of L-ascorbic acid was independent of vitamin C concentration, indicating a destruction mechanism containing a first-order loss of L-ascorbic acid. The higher temperatures reduce the viscosity of paste in the extruder, which in turn reduce the importance of free radical degradation inside the extruder.

The experimental parameters for the plot in Figure 8 (page 44) are identical to those in Figure 7 (page 42) except the screw speed was reduced from 100 rpm (Figure 7) to 50 rpm (Figure 8). The data in Figure 8 shows that at lower rpm where mechanical damage to the flour and free radical generation is lower and where the residence time in the extruder is longer, the oxidative destruction route begins to predominate at a lower temperature (175°C) than that observed at 100 rpm in Figure 7 (195°C). However, one observation in Figure 8 remains unexplained. At 250°C, the loss of L-ascorbic acid is approximately 16% higher when the initial concentration in flour is 45 mg/oz than when the initial concentration is 5 mg/oz.

The loss of L-ascorbic acid as a function of moisture and L-ascorbic acid concentration but at a constant temperature of 200°C and a constant screw speed of 100 rpm is shown in Figure 9 (page 47). The recovery data in Figure 9 shows that varying the moisture level between 17 and 21% had a little or no effect on the loss of the vitamin at any concentration of vitamin C. At higher moisture levels (21%-25%), there was a slight increase in the recovery of the vitamin as the initial concentration of vitamin C increased. At a lower extrusion temperature (150°C) (Figure 10, page 49), L-ascorbic acid recovery was dependent on the initial concentration of vitamin C in the flour. The recovery percentage increased when the initial concentration of vitamin C increased at any given moisture level (Figure 10). The higher percentage recovery at higher levels of vitamin C in the

Figure 7. Contour plot of the recovery of L-ascorbic acid at varying temperatures and L-ascorbic acid concentration with screw speed and moisture content held constant at 100 rpm and 21%, respectively.

(E = 36, F = 40, G = 44, H = 48, J = 52, K = 56, L = 60, M = 64, N = 68, O = 72, P = 76, Q = 80, R = 84, S = 88.)

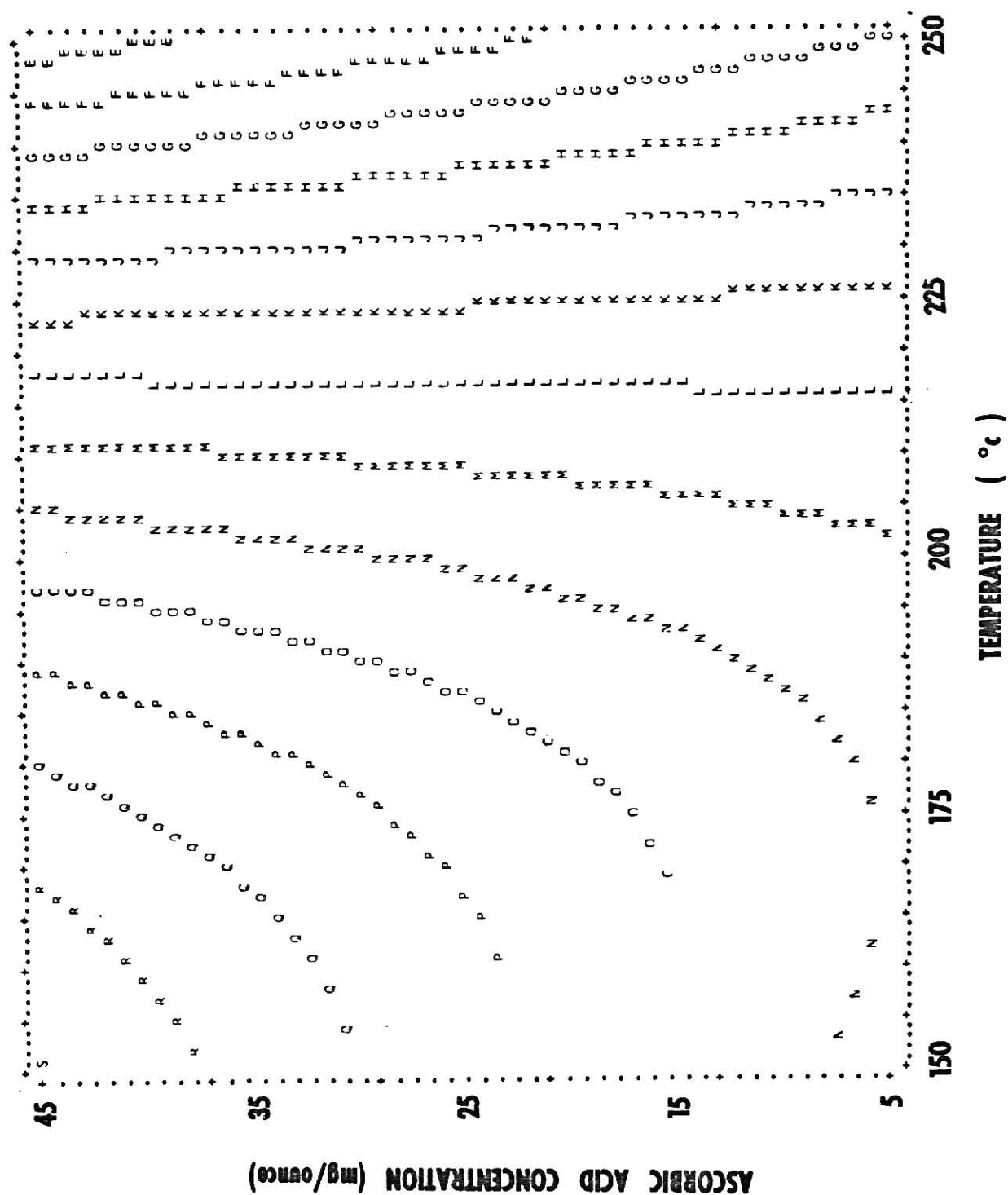
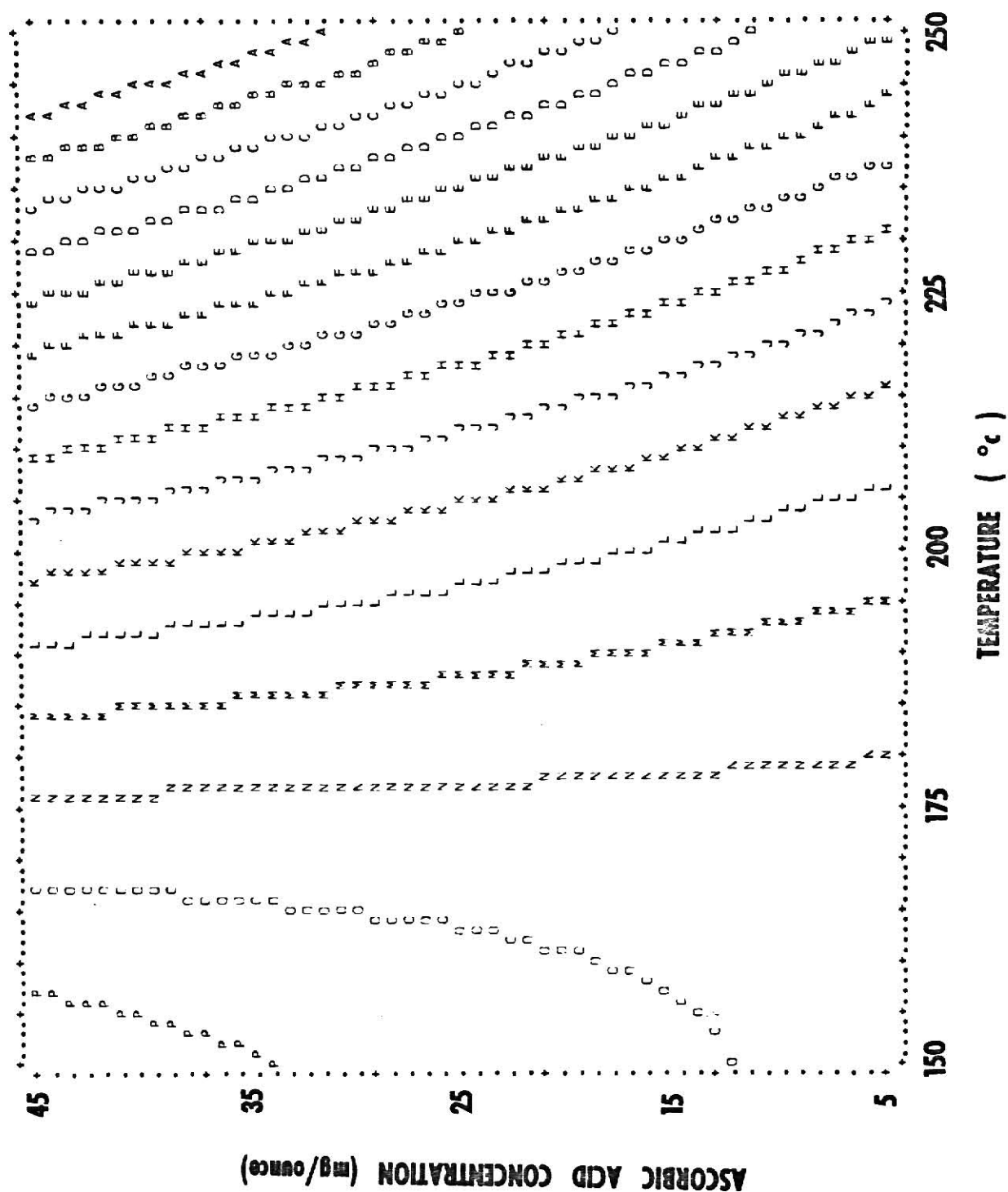


Figure 8. Contour plot of the recovery of L-ascorbic acid at varying temperatures and L-ascorbic acid concentrations with screw speed and moisture content held constant at 50 rpm and 21%, respectively.

(A = 20, B = 24, C = 28, D = 32, E = 36, F = 40, G = 44,
H = 48, J = 52, K = 56, L = 60, M = 64, N = 68, O = 72,
P = 76.)



flour extruded at 150°C and 100 rpm is likely the result of the proposed increased importance of the free-radical destruction of vitamin C at a lower temperature of extrusion. The viscosity of the flour-paste will be high at 150°C especially at a lower moisture level. If more water is added at 150°C and 100 rpm to decrease the viscosity, then the loss of L-ascorbic acid is again increased as previously discussed.

At the highest temperature of extrusion of 250°C (Figure 11, page 51) the first-order destruction mechanisms become dominant, and at this temperature and at all concentrations of L-ascorbic acid, the recovery of the vitamin decreased with increasing moisture. The reason only moisture levels in the flour could affect recovery have been given in the first part of this discussion (page 28).

Contour plots showing the effects of varying L-ascorbic acid concentrations and screw speeds on the loss of L-ascorbic acid are given in Figures 12, 13, and 14 (pages 53 through 58). Figure 12 (page 54) shows the recovery of L-ascorbic acid as a function of those two variables when the extrusion temperature is 200°C and the flour moisture is 21%. At concentration levels ranging from 5-23 mg/oz of L-ascorbic acid, there was only a slight effect on the recovery of L-ascorbic acid at any screw speed. At the highest initial concentration of L-ascorbic acid (45 mg/oz) there was a more noticeable effect of screw speed; the loss of vitamin C decreased when screw speed increased. If one examines Figure 12 with another approach, then at high screw speeds (110-150 rpm), L-ascorbic acid recovery increased as the vitamin concentration increased, whereas at low screw speeds (50-100 rpm), recovery of L-ascorbic acid was practically independent of vitamin C concentration in the flour. These results are again consistent with two mechanisms of destruction of vitamin C in the extruder. At low initial concentrations

Figure 9. Contour plot of the recovery of L-ascorbic acid at varying L-ascorbic acid concentration and moisture content with temperature and screw speed held constant at 200°C and 100 rpm, respectively.

(K = 56, L = 60, M = 64, N = 68.)

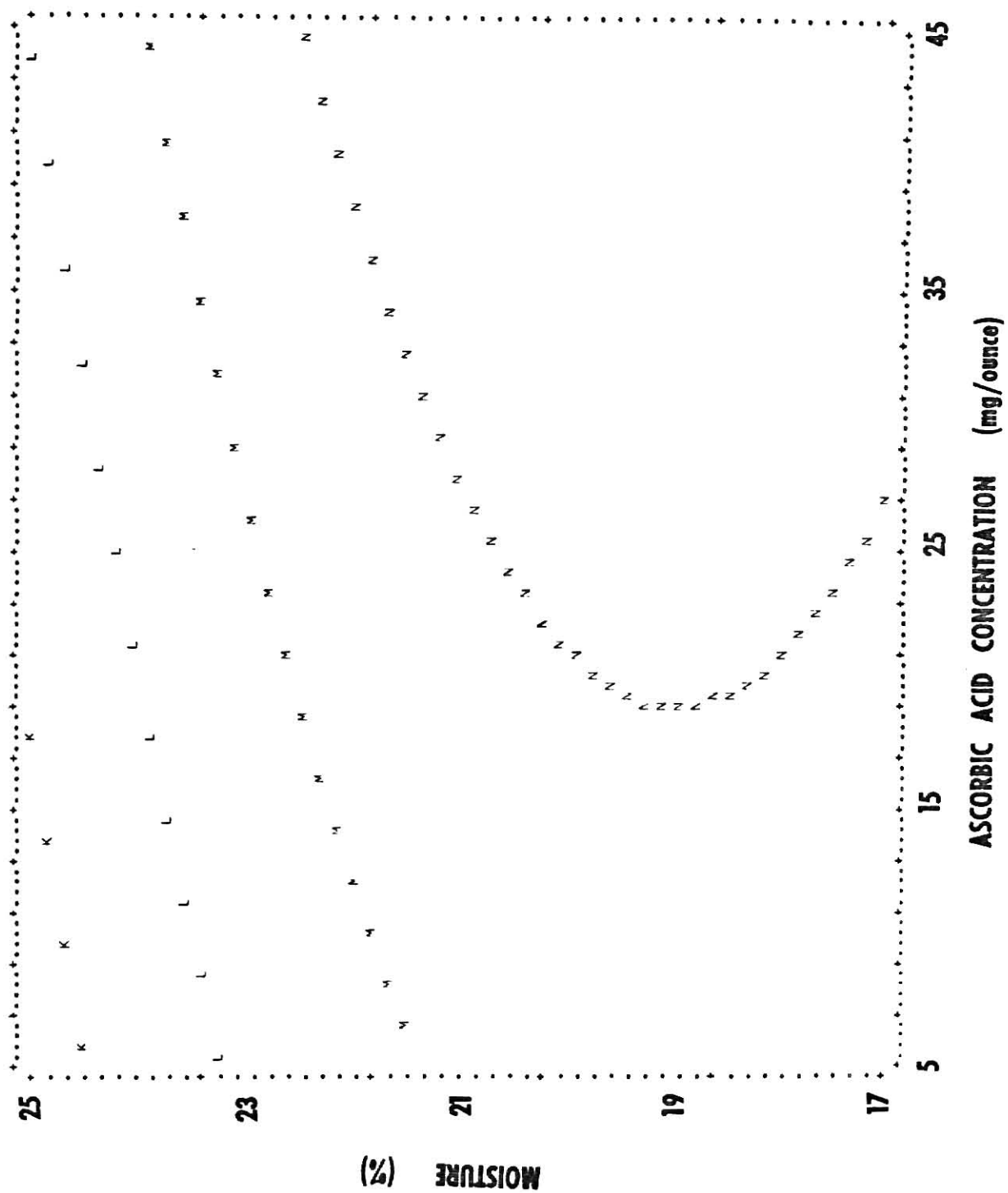


Figure 10. Contour plot of the recovery of L-ascorbic acid at varying L-ascorbic acid concentration and moisture content with temperature and screw speed held constant at 150°C and 100 rpm, respectively.

(M = 64, N = 68, O = 72, P = 76, Q = 80, R = 84, S = 88.)

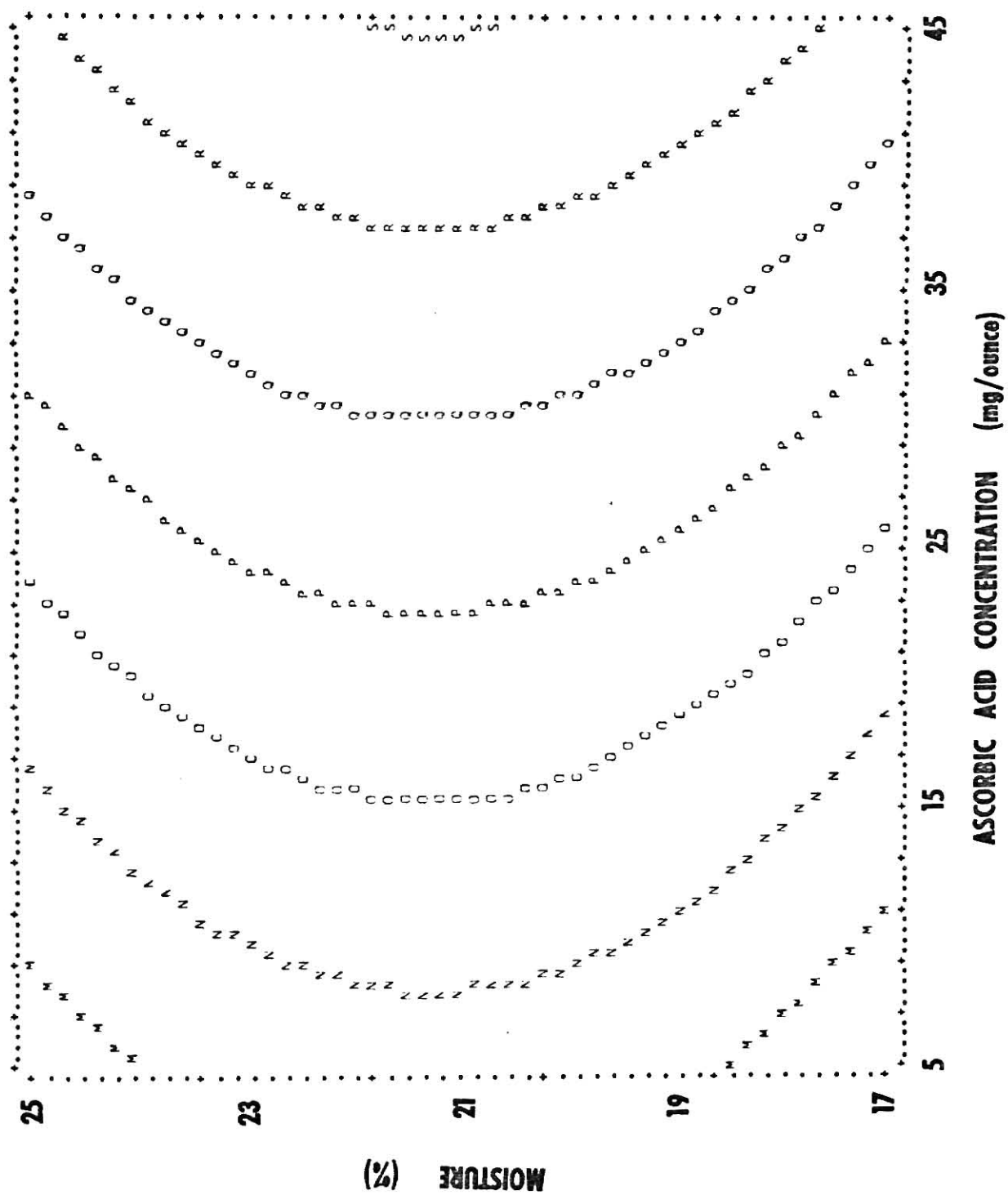
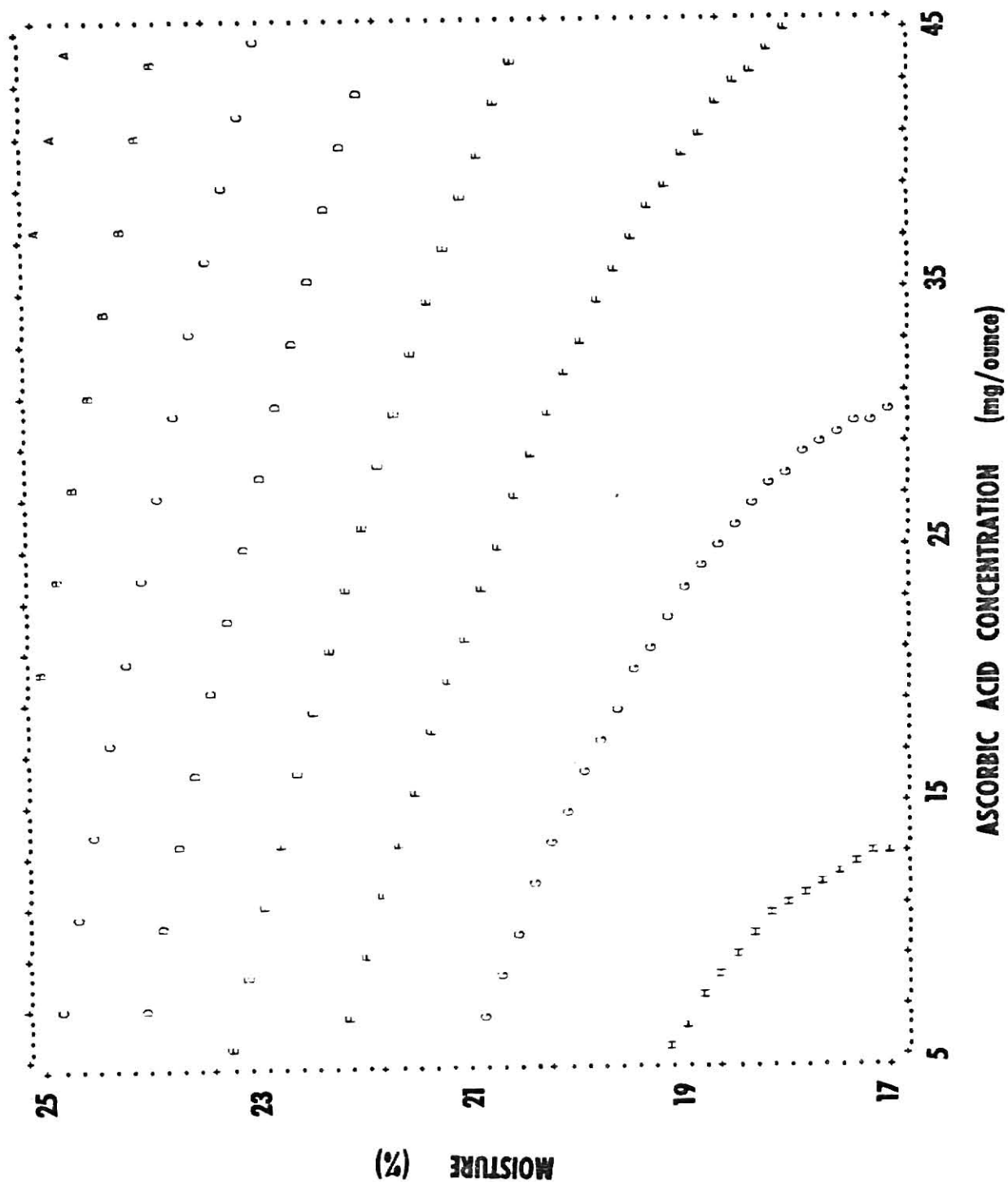


Figure 11. Contour plot of the recovery of L-ascorbic acid at varying L-ascorbic acid concentration and moisture with temperature and screw speed held constant at 250°C and 100 rpm, respectively.

(A = 20, B = 24, C = 28, D = 32, E = 36, F = 40, G = 44, H = 48.)



of L-ascorbic acid (5-23 mg/oz) and at 200°C and 21% moisture, the increase in free-radical destruction caused by a higher screw speed is off-set by a decrease in oxidative destruction due to the shorter residence time in the extruder. The net result is not much dependence of loss of vitamin C on screw speed at the 5-25 mg/oz of initial vitamin C levels. At higher levels of vitamin C (23-45 mg/oz), the oxidative loss, which is first order in vitamin C concentration, dominates and as expected a lower residence time in the extruder caused by a higher screw speed means higher recovery of vitamin C.

When the temperature of extrusion was increased to 250°C (Figure 13, page 56), the screw speed exerted a more noticeable effect on the recovery of L-ascorbic acid. L-Ascorbic acid recovery increased as rpm increased. Furthermore, the same effect was more pronounced as the initial concentration of L-ascorbic acid increased. The shape of the recovery contours in Figure 13 are again consistent with two destruction mechanisms for L-ascorbic acid.

Dropping the temperature of extrusion to its lowest level (150°C) Figure 14 (page 58), more clearly shows the effect of the proposed free-radical destruction. At low levels of L-ascorbic acid concentration, particularly 5-10 mg/oz, the recovery of L-ascorbic acid decreased with increasing screw speed. This drop-off of the recovery in this area of the contour map is much more noticeable in extrusion at 150°C (Figure 14, page 58) than at 250°C (Figure 13, page 56), or even 200°C (Figure 12, page 54). At the highest level of L-ascorbic acid concentration (45 mg/oz) in Figure 14, we note that the recovery of L-ascorbic acid has become almost independent of screw speed. The free-radical destruction of vitamin C is a smaller percentage of the higher levels of L-ascorbic acid in the flour. At the

Figure 12. Contour plot of the recovery of L-ascorbic acid at varying L-ascorbic acid concentrations and screw speeds with temperature and moisture content held constant at 200°C and 21%, respectively.

(K = 56, L = 60, M = 64, N = 68, O = 72, P = 76.)

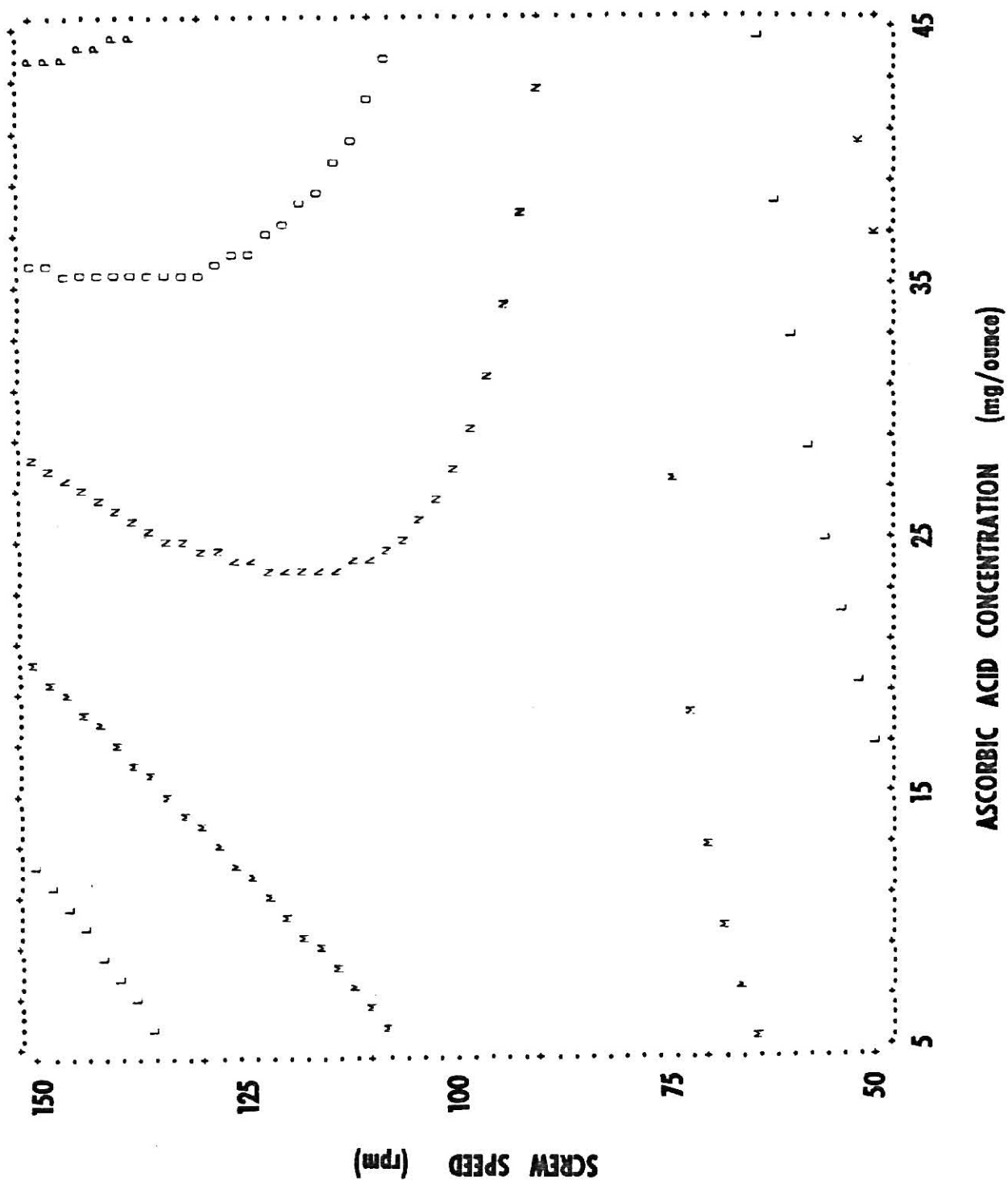


Figure 13. Contour plot of L-ascorbic acid recovery at varying L-ascorbic acid concentrations and screw speeds with temperature and moisture held constant at 250°C and 21%, respectively.
(A = 20, B = 24, C = 28, D = 32, E = 36, F = 40, G = 44.)

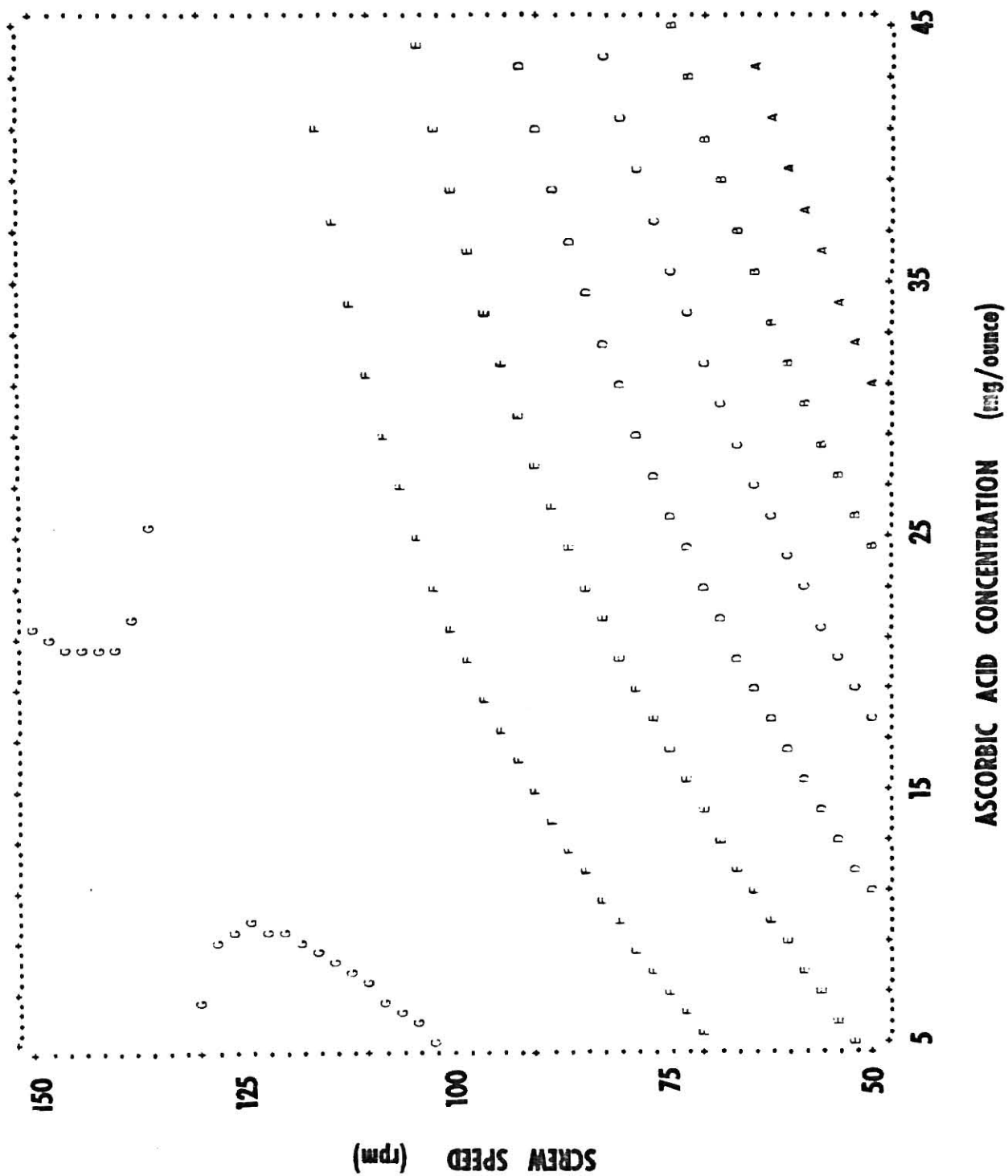
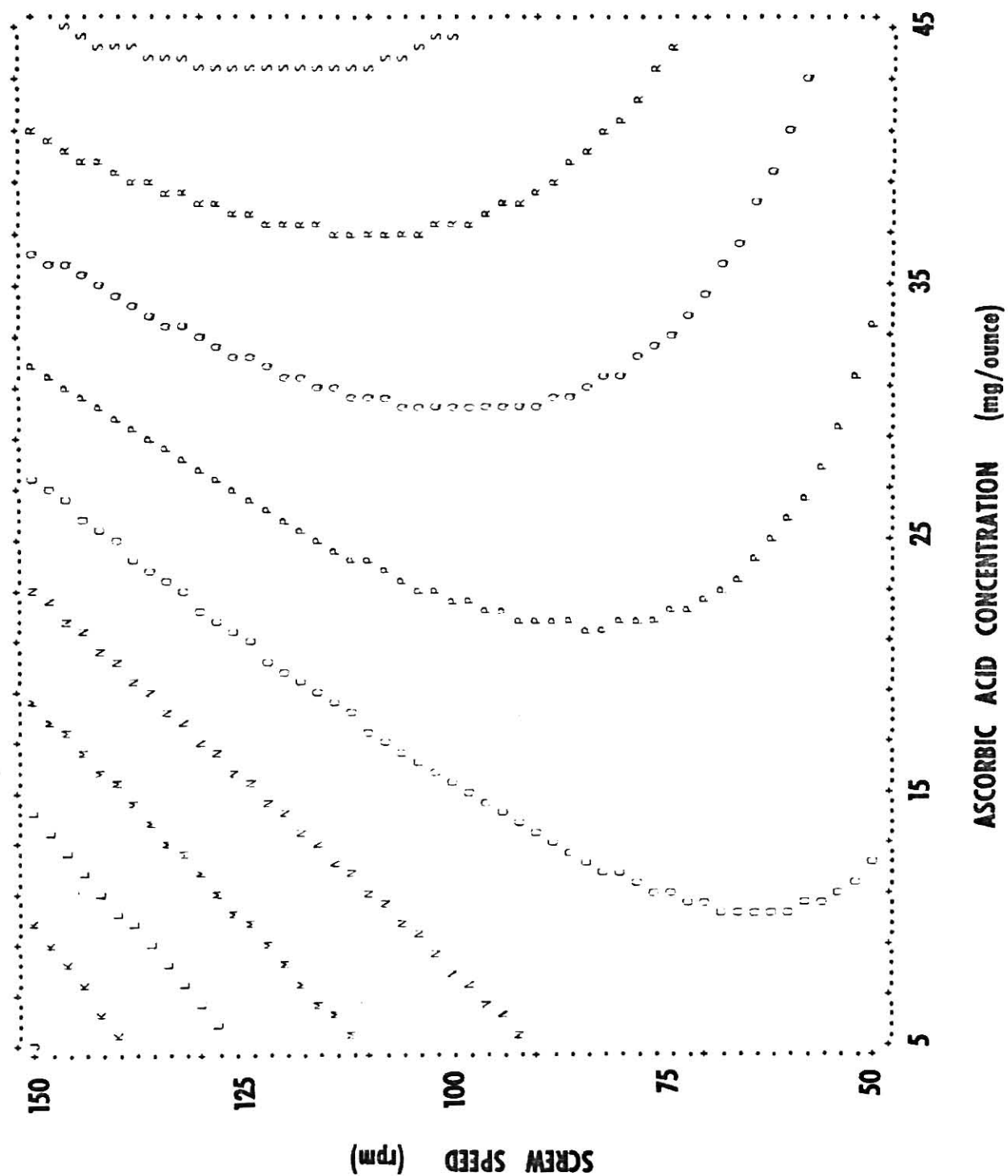


Figure 14. Contour plot for the recovery of L-ascorbic acid at varying L-ascorbic acid concentrations and screw speeds with temperature and moisture held constant at 150°C and 21%, respectively.

(K = 56, L = 60, M = 64, N = 68, O = 72, P = 76, Q = 80, R = 84, S = 88.)



same time, the lower residence time at higher screw speeds means reduced oxidative destruction of vitamin C. Since the screw speed affects the oxidative destruction inversely to its effect on free-radical destruction, the two effects tend to cancel each other.

The contour in Figure 15 (page 61) shows the effect of varying the screw speed and the moisture content on the recovery of L-ascorbic acid when the concentration of L-ascorbic acid is held constant at 25 mg/oz and the temperature is 200°C. It is apparent from Figure 15 that moisture and screw speed had only a minor to moderate effect on the loss of the vitamin at all values of screw speed and moisture levels when the extrusion temperature was 200°C and the L-ascorbic acid concentration was 25 mg/oz. Holding either variable constant, there was no more than 8% difference in the recovery of L-ascorbic acid at any level of the other variable. Upon decreasing the temperature of extrusion to 150°C (Figure 16, page 63), we find the same results. But upon increasing the extrusion temperature to 250°C (Figure 17, page 65), the moisture and screw speed play a more significant role in the loss of L-ascorbic acid. At any screw speed in Figure 17, L-ascorbic acid recovery decreased as moisture increased. At 25% moisture, increasing the screw speed from 50 to 150 rpm increased the recovery of L-ascorbic acid by approximately 8%. But at 17% moisture the same increase in screw speed increased the recovery of L-ascorbic by approximately 16%.

In summary, the RSM study concerning the stability of L-ascorbic acid in extruded wheat flour showed the following:

1. The temperature of extrusion was the most important variable, with higher temperatures causing more loss of vitamin C.

Figure 15. Contour plot of the recovery of L-ascorbic acid at varying screw speeds and moisture until temperature and L-ascorbic acid concentration held constant at 200°C and 25 mg/oz, respectively.

(J = 52, K = 56, L = 60, M = 64, N = 68.)

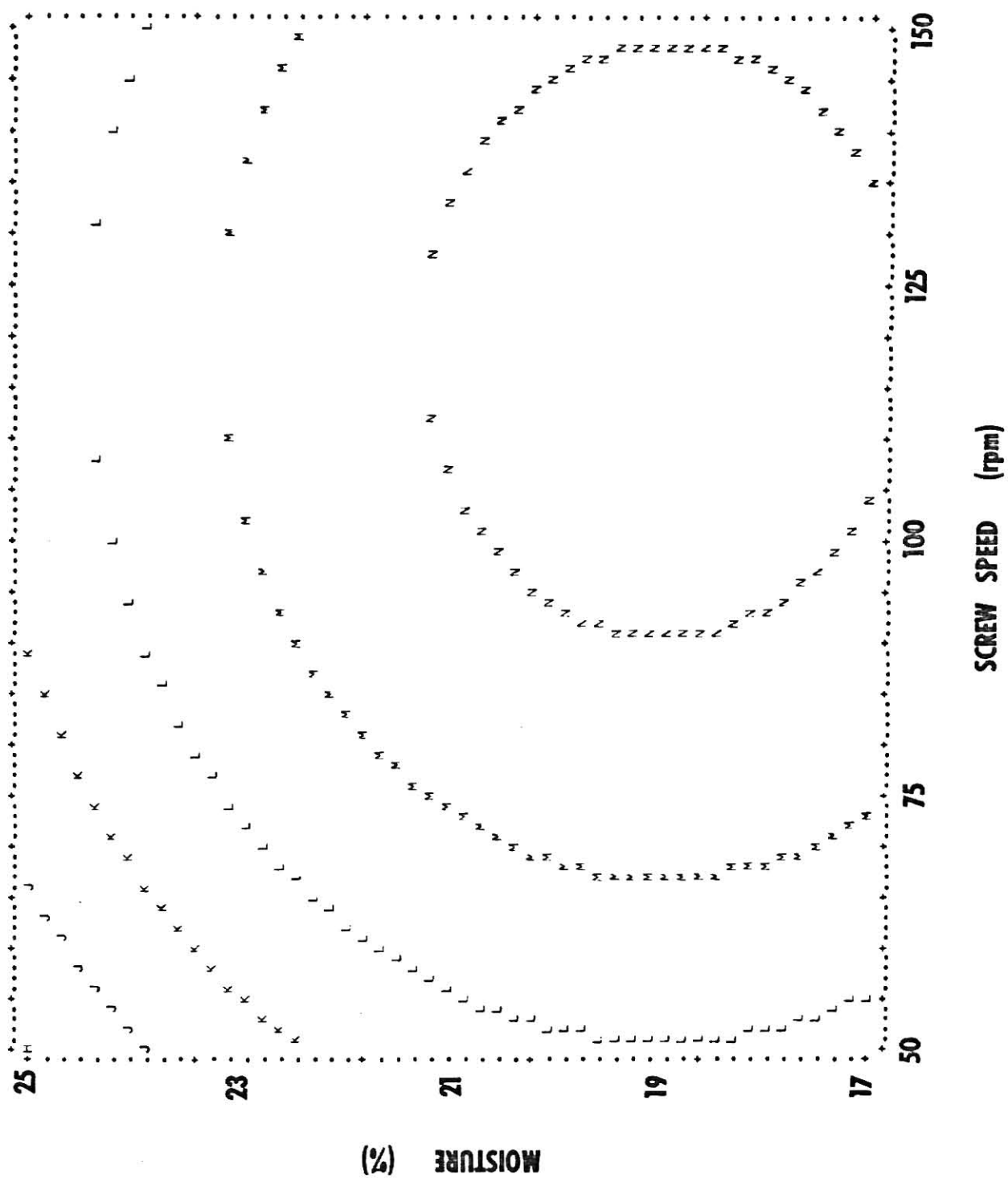


Figure 16. Contour plot of the recovery of L-ascorbic acid at varying screw speeds and moisture with temperature and L-ascorbic acid concentration held constant at 150°C and 25 mg/oz, respectively.

(N = 68, O = 72, P = 76.)

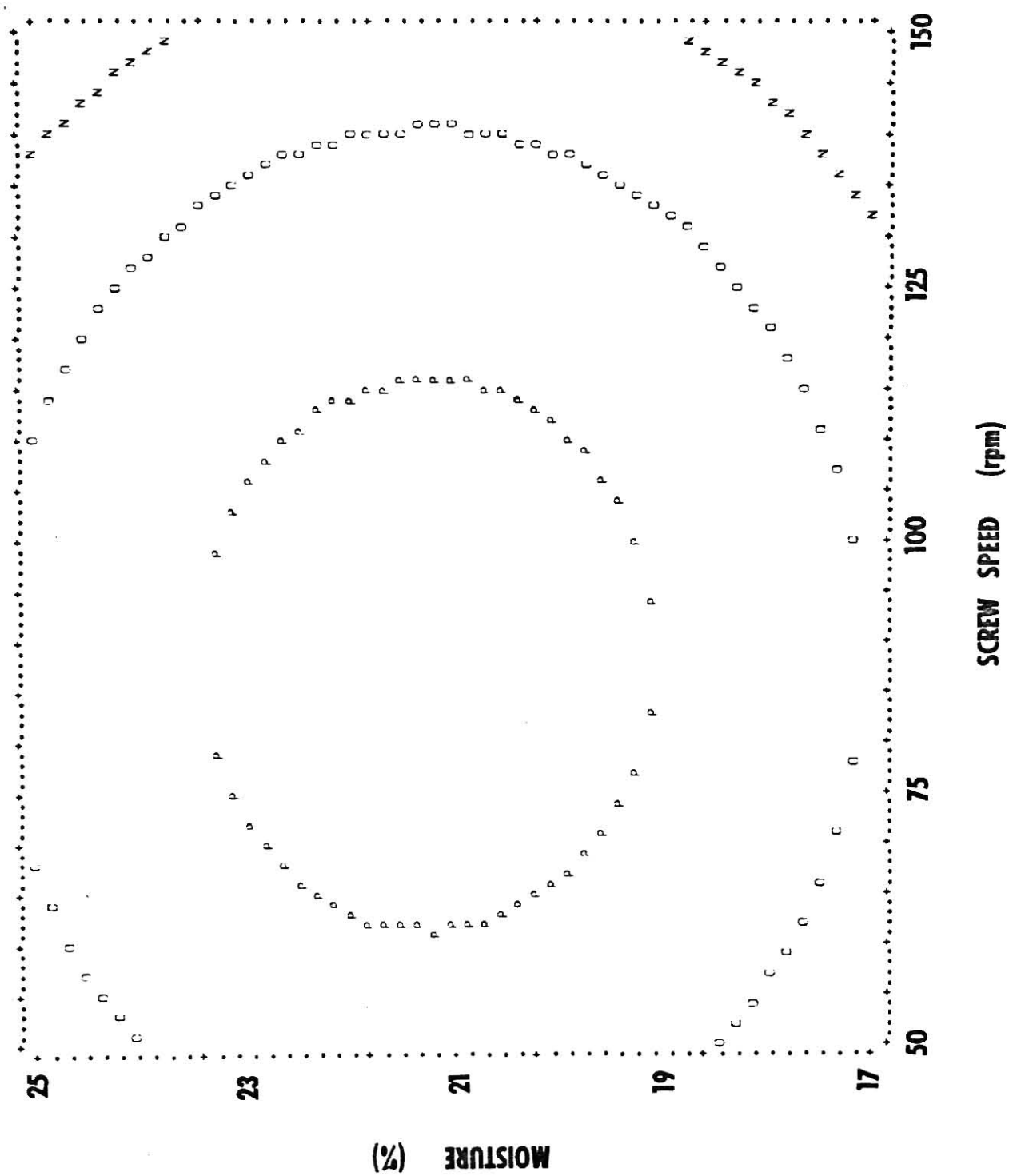
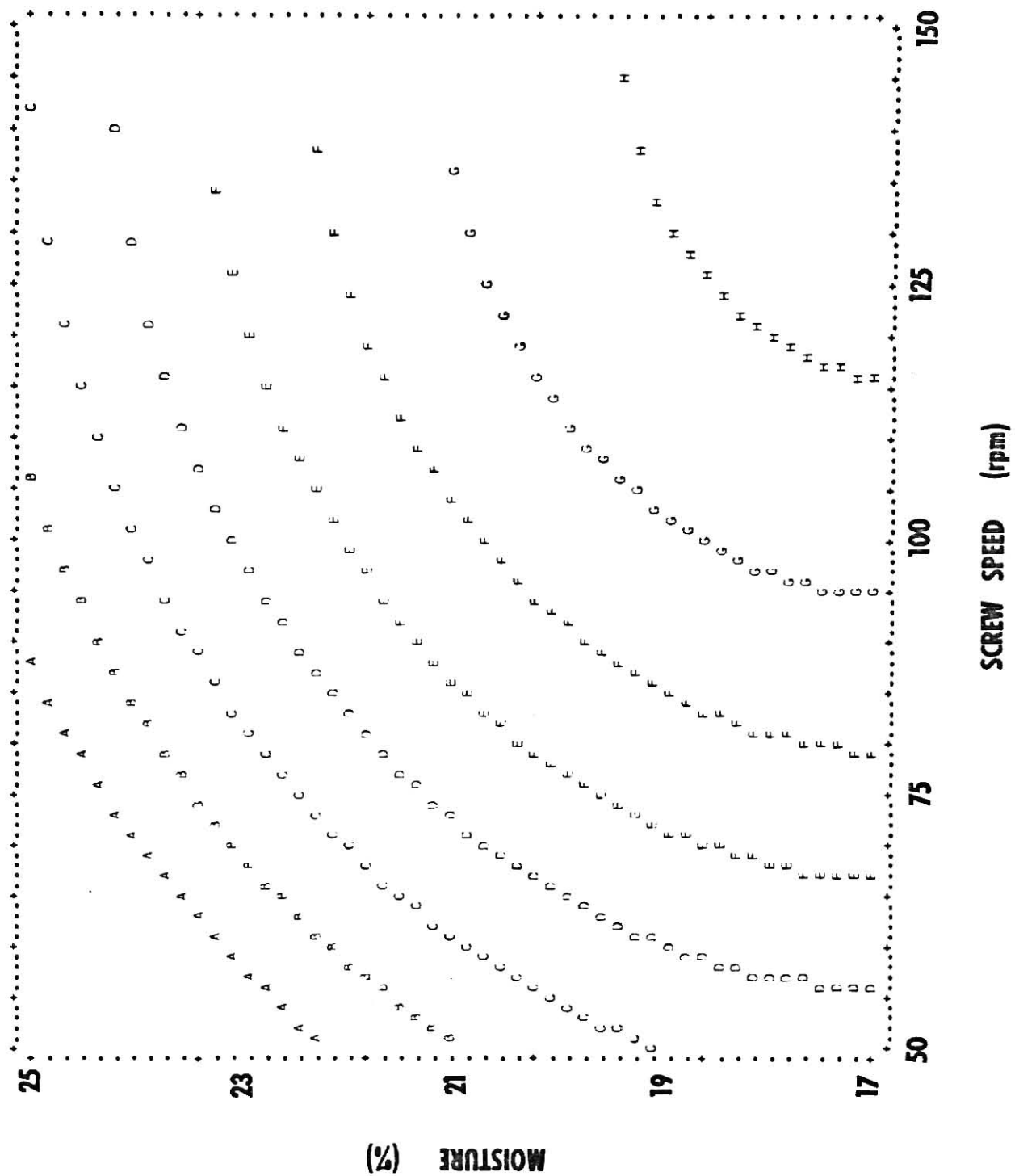


Figure 17. Contour plot of the recovery of L-ascorbic acid at varying screw speeds and moisture with temperature and L-ascorbic acid concentration held constant at 250°C and 25 mg/oz, respectively.

(A = 20, B = 24, C = 28, D = 32, E = 36, F = 40, G = 44, H = 48.)



2. Higher moisture levels in the wheat flour generally led to greater destruction of vitamin C.
3. Increasing the screw speed either increased or decreased the loss of vitamin C. Under conditions where the hot paste in the extruder would be very viscous, such as low temperature and low moisture, increasing screw speed increased the destruction of vitamin C. However, under conditions which favored the oxidative and/or dehydration destruction of vitamin C, such as high temperature and high moisture content, increasing screw speed decreased the destruction of vitamin C.
4. Generally, the loss of L-ascorbic acid in extrusion cooked wheat flour was first-order in L-ascorbic acid when the initial concentration of L-ascorbic acid in the flour was approximately 21-45 mg/oz.
5. It appears that at least two pathways are active in the destruction of vitamin C during extrusion.

Other Variables Affecting the Destruction of L-Ascorbic Acid During Extrusion of Wheat Flour

Processed cereals are often fortified with added vitamin and mineral nutrients. It is well known that iron, which is one of the common nutrients added to cereals, stimulates the oxidative destruction of vitamin C (34). But vitamin C should somehow still be added to fortified cereal products, since this vitamin almost triples the absorption of iron from the human gut (22, 9).

Since no data has been published concerning the effect of mineral elements on the destruction of vitamin C during extrusion, the effect of ferrous ions and several other metallic nutrients on the stability of

vitamin C in extruded wheat flour was therefore investigated. The extrusion conditions were as follows; 200°C, 21% moisture, a screw-speed of 100 rpm, and an initial L-ascorbic acid concentration of 25 mg/oz. The recoveries of L-ascorbic acid observed in this experiment are given in Table 4 (page 68). A statistical analysis* of the data in Table 4 showed that the recoveries of vitamin C from the test samples did not differ significantly from the recovery value of the control sample of the extruded wheat flour (no added salt). The least significant difference between the recovery values was calculated to be 8.0% at the 95% confidence level. These data show that addition of ferrous iron or the metallic ions of zinc, magnesium, calcium, or sodium, do not accelerate the destruction of vitamin C. Furthermore, it indicates that the main mechanism of destruction of vitamin C during extrusion of wheat flour is not limited by the amount of iron salts in the flour, but is limited instead by the concentration of L-ascorbic acid in the flour.

Although the results in Table 4 indicated that salts had little effect on the recovery of L-ascorbic acid, we thought it prudent to check whether the source of the tempering moisture changed the recovery. The data in Table 5 (page 69) shows that the recovery from extruded flour premoistened with tap water was the same as the recovery of vitamin C from a control sample premoistened with distilled water.

Another variable which might affect recovery of L-ascorbic acid is the pH of the tempering water. Dehydration of L-ascorbic acid is increased at

* The analysis of variance of these seven different extrusion treatments of flour fortified with L-ascorbic acid and minerals was done along with three other extrusion treatments given in Table 5 (page 69). The analysis of variance data is given in Appendix II.

Table 4. Effect of selected metal ions on the stability of L-ascorbic acid in extruded wheat flour^a

Salt added	Concentration of salt mg/ounce	Recovery of ascorbic acid, %
Ferrous chloride	10	68 *
	40	64 *
Zinc chloride	10	61.6*
Magnesium chloride	200	64.0*
Sodium chloride	900	65.5*
Calcium chloride	900	65.5*
Control sample	---	67.0*

^aAll samples were extruded at 21% moisture, 200°C, 100 rpm, and initial L-ascorbic acid concentration of 25 mg/oz.

* No statistical difference between values at the 95% confidence level.

Table 5. L-Ascorbic acid recovery from extruded wheat flour^a

Treatment	<u>L</u> -Ascorbic acid recovery, %
Control (pH 6) ^b	67.0
Tap water	67.5*
5% acetic acid solution (pH 5.1) ^b	63.3*
Vacuum deaeration ^c	83.3

* Not statistically different ($\alpha = 0.05$).

^a All samples extruded at 200°C, 21% moisture, 100 rpm screw speed, and 25 mg/oz L-ascorbic acid.

^b pH measured before extrusion by a pH meter.

^c Air in flour sample replaced by nitrogen and the sample then extruded using a CO₂ atmosphere in the extruder feed hopper.

low pH (18). Furthermore, Stearn (47) found that the pH of extruded wheat starch depended on the severity of the cooking condition. The higher the temperature of cook, the lower the pH of the extrudate. It was decided to examine how a lower initial pH in the tempered wheat flour would affect the recovery of L-ascorbic acid from an extruded sample. Accordingly, 5 per cent acetic acid solution was used as tempering medium instead of water. The added acid changed the pH of the initial flour slurry from pH 6.0 to 5.1. The pH of the extruded wheat flour dropped from pH 5.5 to 4.4. The additional acidity in the wheat flour resulted in a 3.7% lowering of recovery (Table 5, page 69). However, a statistical analysis (Appendix II) indicated this difference was not significant.

The major pathway for destruction of L-ascorbic acid in the extruder appears to be oxidative. The results of the RSM investigation and the pH experiment just discussed support this conclusion. Additional evidence was obtained by showing that air plays a major role in the loss of vitamin C during extrusion. A sample of wheat flour was fortified with vitamin C and tempered in the normal manner. The sample was then deaerated and extruded (200°C, 21% moisture, 100 rpm, and 25 mg/oz L-ascorbic acid) using an atmosphere of carbon dioxide in the hopper at the extruded inlet. With all or most of the oxygen eliminated from the inlet flour, the recovery of L-ascorbic acid increased by 16.3% (83.3% for test sample vs. 67% for control sample, Table 5, page 69). The observed difference of 16.3% recovery between the control and the deaerated sample was significant at the 95% confidence level.

It was shown previously (Table 3, page 26) that a 9.2% loss of L-ascorbic acid occurred during mixing and tempering of flour containing 21% moisture and 25 mg/oz of L-ascorbic acid. Since extrusion of the

deaerated flour sample at 200°C, 21% moisture, 100 rpm, and 25 mg/oz L-ascorbic acid resulted in a 16.7% destruction of vitamin C, 7.5% of the loss (approximately 2 mg of L-ascorbic acid) was unaccounted for. This loss of 2 mg could represent the amount of L-ascorbic acid lost by the free-radical destruction mechanism, although the possibility of some oxidative destruction cannot be discounted. Another possible explanation is a loss of vitamin C due to lack of complete extraction of the vitamin from the sample.

Stability of L-Ascorbate 2-Phosphate During Extrusion of Wheat Flour

Because of losses of vitamin C during extrusion of wheat flour, it was of interest to examine the stability of a more stable form of vitamin C, namely L-ascorbate 2-phosphate. Tempering and fortification of flour with magnesium L-ascorbate 2-phosphate were done identically to the methods used for L-ascorbic acid. The stability of L-ascorbate 2-phosphate during extrusion cooking was examined at the most severe cooking conditions used on L-ascorbic acid. These conditions which resulted in 66.7% destruction of vitamin C, were 250°C, 21% moisture, 100 rpm, and 53.9 mg/oz of L-ascorbate 2-phosphate.

The data in Table 6 (page 73) shows a low recovery of L-ascorbate 2-phosphate when the latter is added to and tempered with wheat flour (sample 1). One can suggest two reasons for the low recovery of L-ascorbate 2-phosphate; 1) phosphatase activity in wheat flour (39); and/or 2) incomplete extraction of L-ascorbate 2-phosphate from wheat flour during assay.

To help determine which reason is correct, we investigated the stability of L-ascorbate 2-phosphate in autoclaved wheat flour. The data in Table 6 (sample 4) shows 100% recovery of L-ascorbate 2-phosphate from

autoclaved wheat flour (inactivated phosphatase). This result is evidence for enzymatic loss of L-ascorbate 2-phosphate.

Phosphatase action on L-ascorbate 2-phosphate should release stoichiometric amounts of L-ascorbic acid. Assay of sample 1 (Table 6) showed that 66.6% of L-ascorbate 2-phosphate added to the flour was converted to L-ascorbic acid. Total recovery of the amount of L-ascorbate 2-phosphate added to flour in sample 1 (Table 6) amounted to 93.6%.

The destruction of L-ascorbate 2-phosphate and the liberation of L-ascorbic acid from sample 1 (Table 6) can result from losses occurring during the tempering process or the assay method.* Elimination of the tempering period (sample No. 2, Table 6) did not affect the amount of L-ascorbate 2-phosphate recovered from the wheat flour at 21% moisture. Furthermore, a decrease in tempering moisture from 21% to 12% (sample No. 3, Table 6) did not change the recovery of L-ascorbate 2-phosphate. These results lead us to conclude that the loss of L-ascorbate 2-phosphate occurs during the assay procedure.

One of the first steps in the assay method for L-ascorbate 2-phosphate involves an aqueous extraction of a sample for 20 minutes. The question arises as to how much enzymic cleavage of L-ascorbate 2-phosphate occurs during this extraction. In a separate experiment flour was extracted with water so that the final extract contained the same concentration of phosphatase enzyme that occurs in the extraction step of the assay method. The rate of cleavage of L-ascorbate 2-phosphate in the aqueous extract at room temperature is shown in Figure 18 (page 75). After 20 minutes of

* It is possible that extraction of L-ascorbate 2-phosphate from wheat flour could be incomplete.

Table 6. Recovery of magnesium L-ascorbate 2-phosphate and L-ascorbic acid from wheat flour and extruded wheat flour

Sample number	Sample ^a treatment	Recovery of <u>L</u> -ascorbate-2-phosphate %	Recovery of <u>L</u> -ascorbic acid %
1	Tempered ^b (21% moisture)	27.0	66.6 ^c
2	No tempering period (21% moisture)	28.5	62.5
3	Tempered ^b (12% moisture)	27.5	--
4	Autoclaved flour, ^d tempered (21% moisture)	100.0	--
5	Tempered ^e (21% moisture) and extruded	29.0	--
6	Autoclaved flour, tempered (21% moisture) and extruded ^f	48.0	28.3

^aAll samples contained initially 53.97 mg/oz of magnesium L-ascorbate 2-phosphate (equivalent to 25 mg/oz of L-ascorbic acid).

^bMagnesium L-ascorbate 2-phosphate added to wheat flour and the moisture of the flour brought to 21% or 12%. Sample then stored for 16 hr at approximately 25°C.

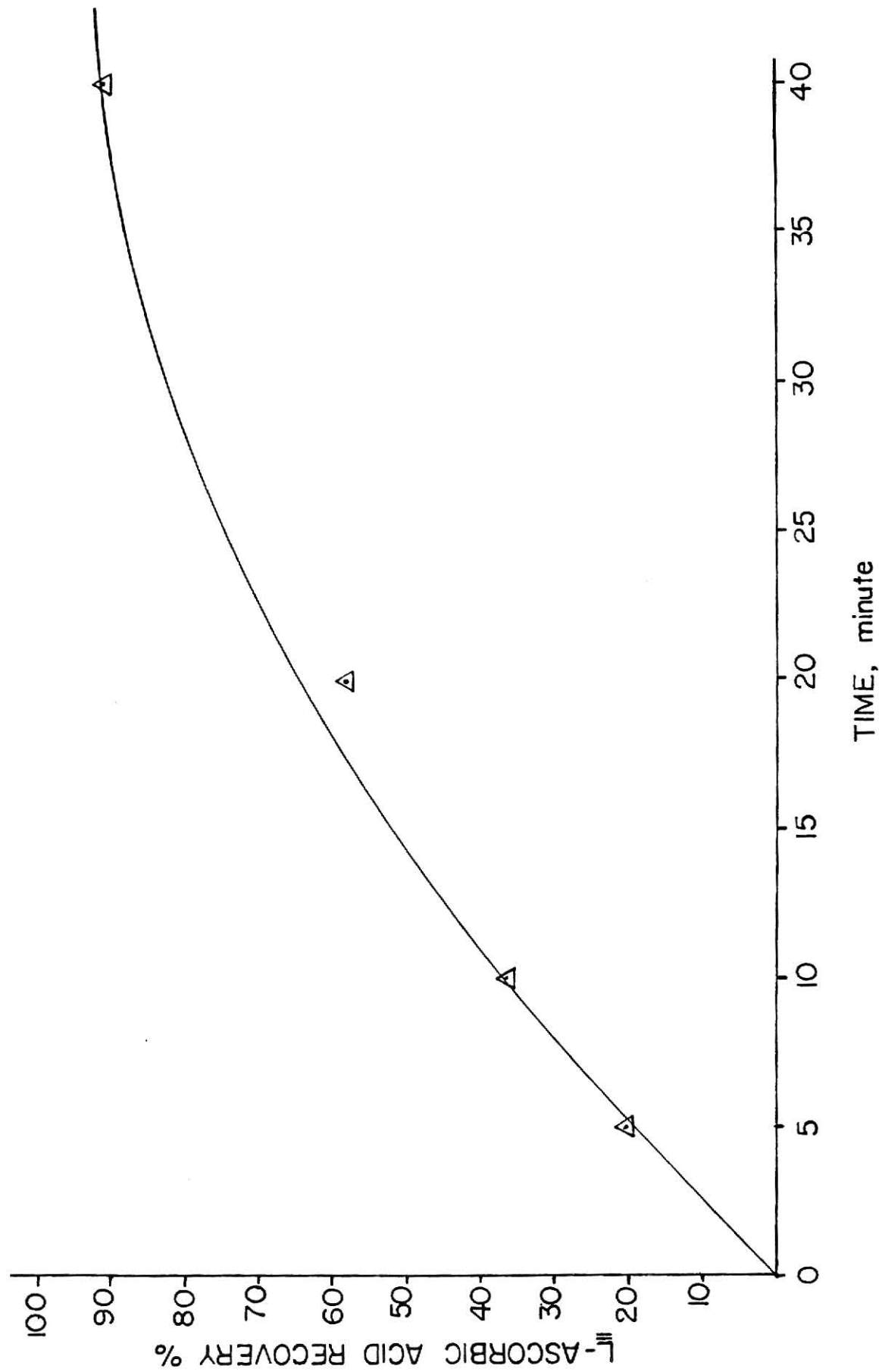
^cA control sample, containing 13 µg of magnesium L-ascorbate 2-phosphate per 3 ml of solution, showed no interference with the assay method for L-ascorbic acid. Percentage recovery of L-ascorbic acid based on the moles of added L-ascorbate 2-phosphate.

^dFlour autoclaved in a closed container at 121°C for 30 min. Sample then fortified with magnesium L-ascorbate 2-phosphate, moisture level adjusted, and stored 16 hr at 25°C.

^eExtruded at 250°C and 100 rpm after being treated identically as sample number 1.

^fSame sample of flour as sample number 4, then extruded.

Figure 18. Recovery of L-ascorbic acid from treatment of magnesium
L-ascorbate 2-phosphate with aqueous flour extract.



reaction time, approximately 60% of L-ascorbate 2-phosphate was converted to L-ascorbate. Thus most of the loss of L-ascorbate 2-phosphate which was found in samples No. 1 and 2 of Table 6 (page 73) occurred during assay. Since 6.4-9.0% of L-ascorbate 2-phosphate added to flour in sample 1 and 2 was unaccounted for, it is difficult to determine the exact loss of the phosphate ester during tempering.

Since the phosphatase activity in wheat flour presents a problem in the assay method for L-ascorbate 2-phosphate, an autoclaved sample of wheat flour was used to examine the stability of L-ascorbate 2-phosphate during extrusion. Sample number 6 in Table 6 (page 73) shows a 52% loss of L-ascorbate 2-phosphate during extrusion cooking (250°C and 100 rpm). Even in the absence of phosphatase activity, L-ascorbate 2-phosphate is cleaved to L-ascorbate and phosphate due to the high extrusion temperature and slightly acidic pH of the flour (pH of extruded flour was 5.5). In fact, 28.3% of the initial amount of L-ascorbate 2-phosphate in sample number 6, Table 6 (page 73) was converted to L-ascorbate during extrusion. Thus total vitamin C recovery in sample number 6 was 76.3%. This recovery is more than twice as great as the recovery of L-ascorbate (33.3%) from an extruded flour (sample 18, Appendix I) fortified prior to extrusion with an initially equivalent level of L-ascorbic acid.

SUMMARY OF CONCLUSIONS

Losses of L-ascorbic acid during blending and tempering with moistened wheat flour were not significantly affected by the concentration of L-ascorbic acid or moisture in the flour. The blending and tempering loss was approximately 10% of the initial amount of L-ascorbic acid in the flour when the flour contained 5-45 mg/oz of L-ascorbic acid and 17-25% moisture.

The RSM study concerning the stability of L-ascorbic acid during extrusion cooking of wheat flour led to the following conclusions.

1. Extrusion temperature was the most important variable affecting the recovery of L-ascorbic acid. L-Ascorbic acid recovery decreased as temperature increased.
2. Generally, increasing moisture resulted in decreasing L-ascorbic acid recovery.
3. Increasing the screw speed either increased or decreased the loss of vitamin C, depending on the moisture content and extrusion temperature. Under high viscosity conditions, such as low temperature and low moisture, increasing screw speed increased the loss of L-ascorbic acid. However, under conditions where viscosity is low, such as high temperature and high moisture content, increasing the screw speed decreased the loss of vitamin C.
4. Generally, the loss of L-ascorbic acid in extrusion-cooked wheat flour appears to be a first-order process in L-ascorbic acid concentration when the initial concentration of L-ascorbic acid in the flour was approximately 21-45 mg/oz.

5. At least two pathways appear to be active in the destruction of vitamin C during extrusion.

The fortification of extruded wheat flour with metallic ions of iron, zinc, magnesium, calcium, or sodium did not affect the loss of L-ascorbic acid. Neither the source of the moistening water (distilled or tap) nor the pH of the tempering medium (pH 5.1) affected the recovery of L-ascorbic acid during extrusion. On the contrary, the loss of vitamin C during extrusion was shown to be strongly influenced by oxygen.

Recovery of L-ascorbate 2-phosphate from wheat flour containing deactivated phosphatase (autoclaved flour) was 100%, whereas L-ascorbate 2-phosphate recovery from untreated wheat flour was quite low (27%). The total vitamin C activity in extruded, autoclaved-wheat flour fortified with L-ascorbate 2-phosphate was twice as great (76.3%) as the vitamin recovered from extruded wheat flour (33.3%) originally fortified with L-ascorbic acid.

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APPENDIX I

L-Ascorbic acid recovery, production rate, amperage, voltage and temperature of product in extruded wheat flour

Sample number	Temperature °C	L-ascorbic acid concentration mg/oz	Screw speed rpm	Moisture content %	Temperature of product °C	Voltage	Amperage	Production rate g/min	L-ascorbic acid recovery %
1	175	15	75	19	170	65	3.5	55	71.0
2	225	15	75	19	220	65	4.0	54	59.1
3	175	35	75	19	170	65	3.5	58	73.3
4	225	35	75	19	220	65	4.0	56	49.2
5	175	15	125	19	170	105	3.5	90	70.0
6	225	15	125	19	220	105	4.0	90	57.5
7	175	35	125	19	170	105	3.5	89	73.3
8	225	35	125	19	220	105	4.0	88	66.8
9	175	15	75	23	170	65	3.5	59	67.5
10	225	15	75	23	215	65	4.0	59	53.3
11	175	35	75	23	170	65	3.5	58	74.2
12	225	35	75	23	215	65	4.0	56	45.0
13	175	15	125	23	170	105	3.5	98	67.5
14	225	15	125	23	215	105	4.0	90	55.0
15	175	35	125	23	170	105	3.5	95	75.8
16	225	35	125	23	215	105	4.0	91	53.3
17	150	25	100	21	140	85	5.0	82	80.8
18	250	25	100	21	235	85	3.0	66	33.3
19	200	5	100	21	195	85	4.0	68	61.6
20	200	45	100	21	195	85	4.0	70	75.0
21	200	25	50	21	195	45	4.0	52	55.8
22	200	25	150	21	195	140	4.0	123	66.7
23	200	25	100	17	200	85	5.0	65	70.0
24	200	25	100	25	190	85	3.0	77	52.5
25	200	25	100	21	195	85	4.0	70	65.0
26	200	25	100	21	195	85	4.0	69	71.6
27	200	25	100	21	195	85	4.0	70	65.0
28	200	25	100	21	195	85	4.0	68	67.5
29	200	25	100	21	195	85	4.0	72	65.0
30	200	25	100	21	195	85	4.0	66	64.2
31	200	25	100	21	195	85	4.0	70	70.8

APPENDIX II

Analysis of variance for recovery values of vitamin C obtained from the 10 different extrusion treatments described in Tables 4 and 5 (pages 68 and 69)

Source of variation	Degree of freedom	S.S.	M.S.	F	Alpha
Treatments	9	341.95	37.99	4.094	.05
Error	6	55.69	9.28		

LSD at $\alpha = .05$ for comparing recovery at each treatment to the recovery of the control = $\pm 7.97\%$

STABILITY OF L-ASCORBATE AND L-ASCORBATE 2-PHOSPHATE
IN EXTRUDED WHEAT FLOUR

by

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requirements for the degree

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1976

Moistened wheat flour was fortified with L-ascorbic acid and cooked in a laboratory-sized extruder. The loss of vitamin C during cooking was examined for four extrusion variables using response surface methodology (RSM). The RSM variables were the concentration of L-ascorbic acid in the flour, the moisture content of the flour, the screw speed, and the cooking temperature. Temperature was the most important factor affecting the loss of vitamin C during extrusion. The higher the extrusion temperature, the greater the loss of vitamin C. It appears that the loss of L-ascorbic acid during extrusion cooking with wheat flour results mostly from oxidative destruction of the vitamin. Wheat flour fortified with a more stable form of vitamin C (magnesium L-ascorbate 2-phosphate) gave twice the recovery of vitamin C in the extruded product as compared to flour fortified with L-ascorbic acid.