PUNGENCY CHANGES IN FROZEN ONIONS

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
WILLIAM WALLACE BALLANTYNE
1967

ABSTRACT

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by William Wallace Ballantyne

This study was undertaken to determine the changes in pungency potential of fresh and frozen onion rings, to compare fresh and frozen onion rings with dehydrated flakes, and to determine whether the pungency of the dehydrated product could be improved by the addition of either enzyme or substrate to the product before use.

Pungency was measured by the amount of pyruvate formed by the action of the enzyme alliinese on the substrates S-methyl- and S-propyl cysteine sulfoxides. Onion rings were held at various storage temperatures for varying lengths of time and then tested for pungency.

Results indicate that onion rings can be held successfully at 35°F (without excessive pungency losses) for at least one month but that after 2 to 3 weeks their appearance becomes poor. Pungency retention in onion rings stored at -30°F was about 52 per cent after 28 days of storage and the rings were useable. Rings frozen and stored at 0°F lost 80 per cent of their pungency and there was complete breakdown of tissue structure upon thawing.

Compared on the basis of pungency, both fresh and frozen rings are better than dehydrated flakes. Flakes are low in pungency because of their lack of substrate.

PUNGENCY CHANGES IN FROZEN ONIONS

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science

1967

ACKNOWLEDGEMENT

The author would sincerely like to thank Dr. Clifford L. Bedford for his advice and guidance throughout the duration of the study. He appreciates very much the help given by Dr. Walter M. Urbain, Dr. Charles M. Stine, and Dr. Donald J. Ricks, in the preparation of this manuscript. He would also like to thank Mr. Moshe Tishel for his suggestions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The total value of onions in Michigan is now greater than that of any other single vegetable. Michigan is located in a very favorable position with respect to markets, and technological developments seem to indicate that Michigan's prospects for the future are ideal. However, for Michigan to develop its full potential, it will be essential that onion varieties that are suitable for processing as dehydrated and frozen products become available. Onions suitable for these processed products should have high solids and high pungency.

Many investigations of vegetable flavor chemistry have been carried out with members of the genus Allium. There is little doubt that pungency in Allium cepa, onion, is intimately related to flavor or aroma. Onions are a classic example of enzymatic development of flavor in which the initial products of reaction are highly unstable and undergo further changes. These initial compounds cause the pungent or sharp, stinging, and painful sensation associated with onions. The flavor substrates for enzymatic action are sulfoxide amino acids. The most important products contain sulfur, and include hydrogen sulfide, thiols, disulfides, trisulfides, thiosulfinates, and the lachrymatory factor, propenyl sulfenic acid.

This study was undertaken to determine the changes in pungency potential of fresh and frozen onion rings, to compare the pungency potential of fresh and frozen onion rings with dehydrates onion flakes, and to determine whether the pungency of the dehydrated product could be improved by the addition of either enzyme or substrate to the product before use.

LITERATURE REVIEW

Substrate

The earliest quoted reference on the analysis of onion odors is Semmler (25). He studied the Allium species, garlic in particular, and reported allyl n-propyl disulfide as the principle odor constituent. He assumed the same to be true for onions. Kohman (9) believed he had found this compound in onions, and suggested thioaldehyde as the lachrymatory factor.

There were numerous reports of odor analysis and of pyruvic acid accumulation in the Allium species, but the basic principle of pungency formation was not proposed until the work of Stoll and Seebeck (28)(7). They were able to isolate and characterize both the precursor and the enzyme of the reaction that is probably the major source of garlic odor. The substrate, which they called alliin, is acted upon by an enzyme, alliinase, to form allicin, pyruvic acid, and ammonia. (Figure 1.) This reaction occurs with the masceration of the cells. Allicin is not very pungent, but is unstable and decomposes to other more characteristic volatile sulfides.

Renis and Henze (16) were among the first to apply the above theory to onions. Using chromatographic methods, they found S-allyl cysteine and S-propyl cysteine sulfoxide as onion pungency precursors, but could not find alliin. The S-propyl cysteine sulfoxide was proposed as the precursor of n-propyl mercaptan, the compound they considered most important for odor.

Virtanen (31), while studying the antimicrobial effect of crushed onion, isolated S-methyl cysteine sulfoxide and S-propyl cysteine sulfoxide. (Figure 2.) He showed that these two compounds

Figure 1. Pungency formation in garlic.

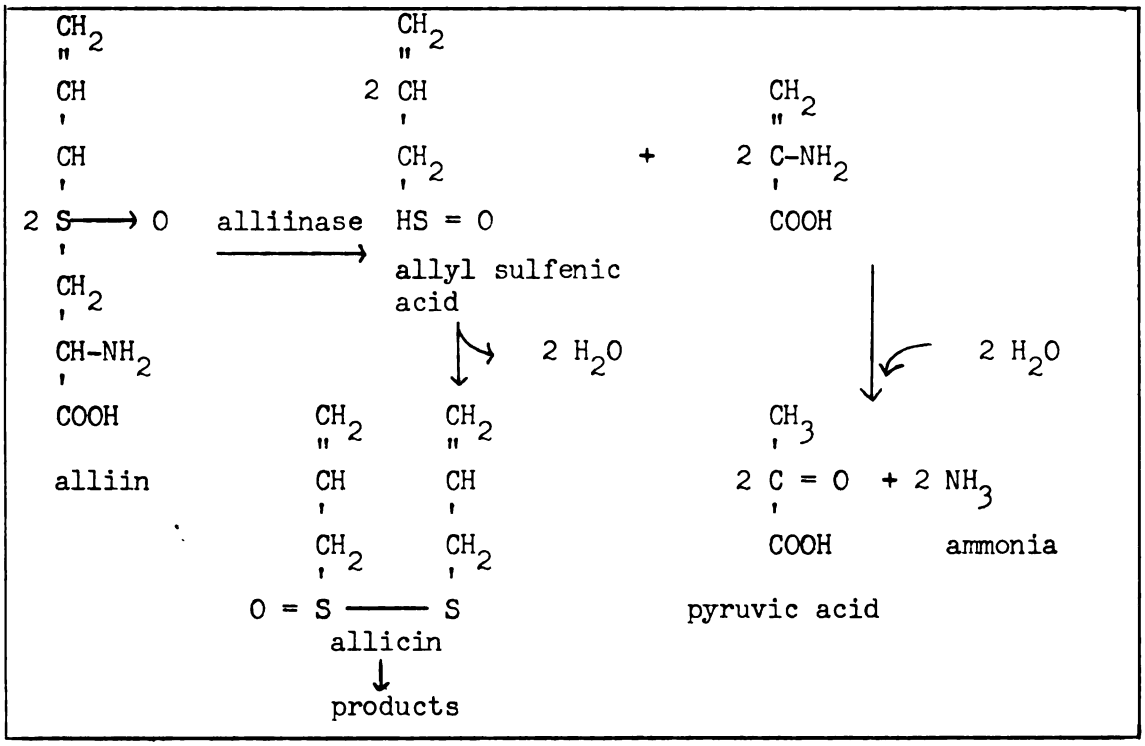
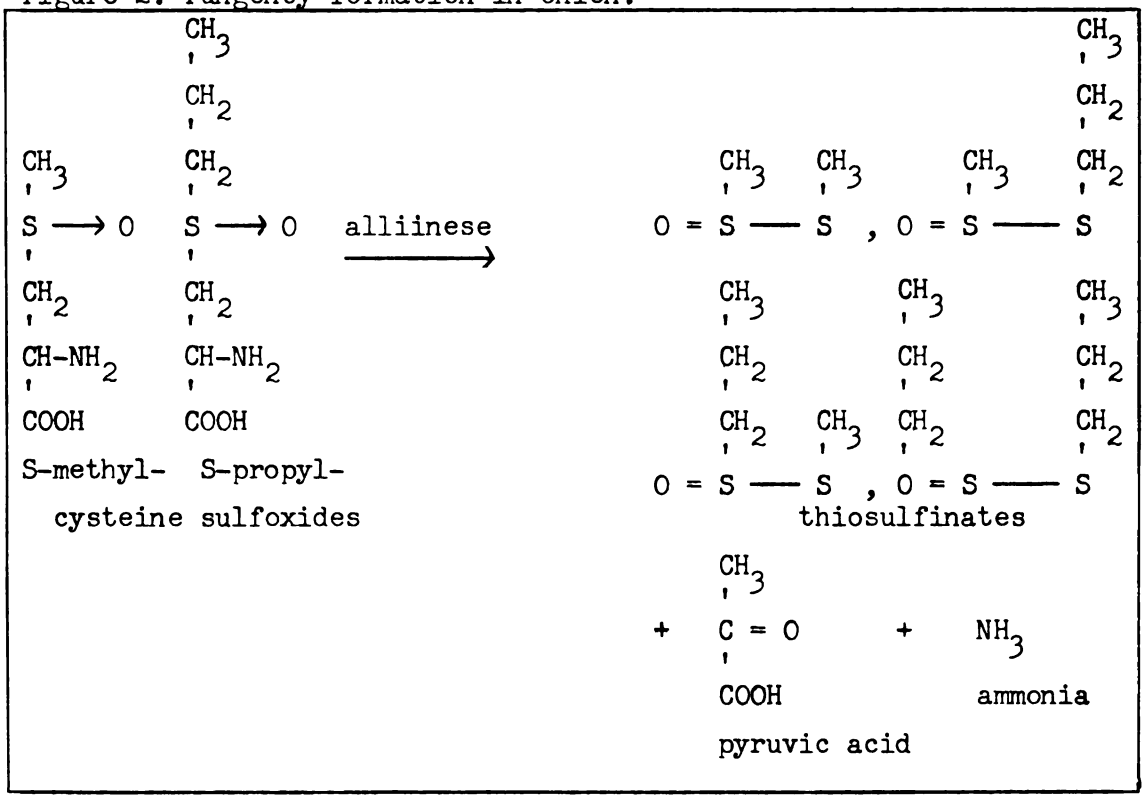


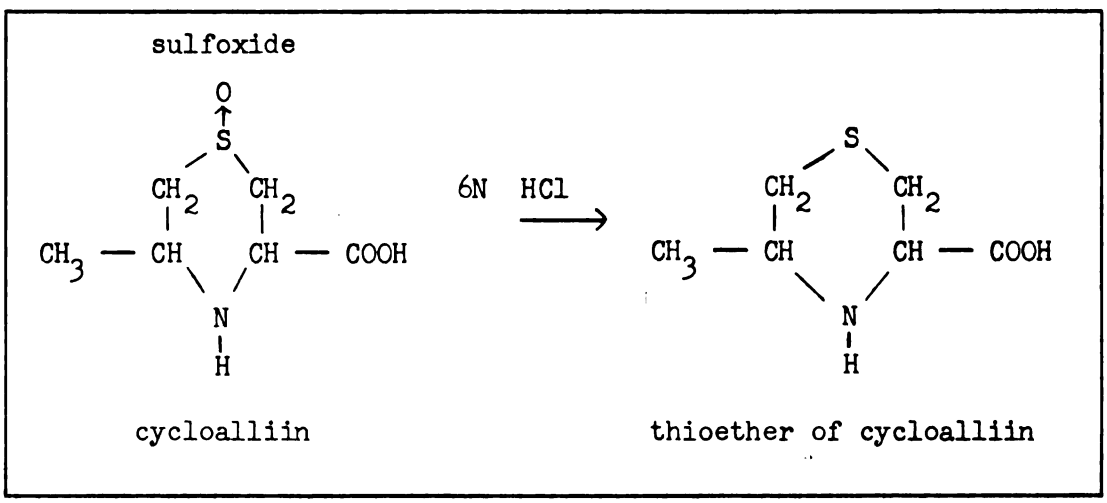
Figure 2. Pungency formation in onion.



were pungency precursors, and that the thiosulfinates that cause odor could be enzymatically formed from them. (Figure 2.) Carson and Wong (4) confirmed this, and showed the existence of these precursors as the dextrorotatory sulfoxides of L-cysteine.

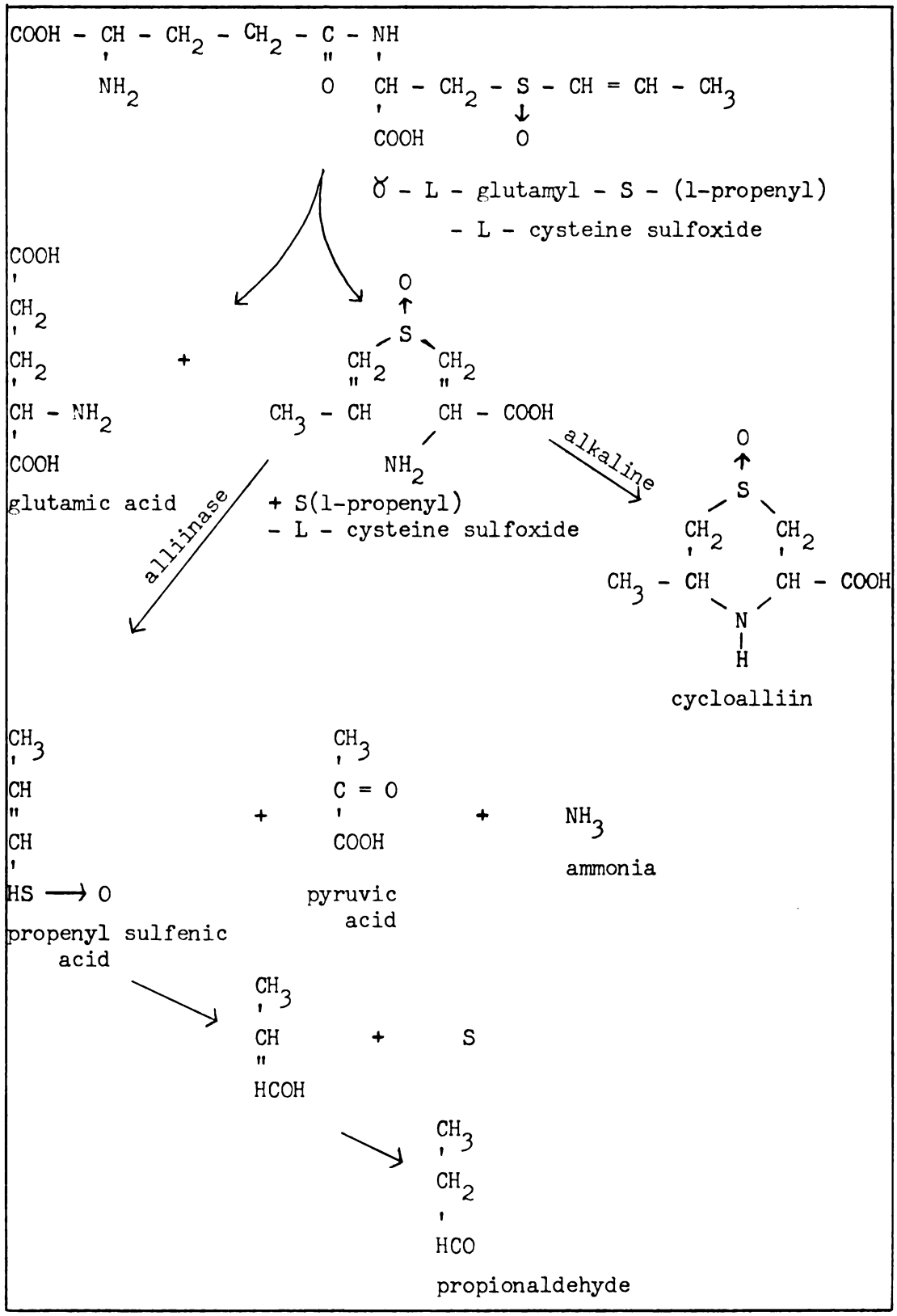
Virtanen (30) also reported the presence of a sulfur-containing amino acid in the hydrolyzed onion homogenate. On oxidation it yielded a compound he called cycloalliin because of its similarity to, and possible formation from, alliin. (Figure 3.) Cycloalliin has been suggested as a possible alternate source of pungency as measured by the pyruvate test. This is important because there seems to be more pyruvate produced than methyl- and propyl cysteine sulfoxide precursor present during the test.

Figure 3. An alternate pungency precursor in onion.



Spare (27) (32) studied the lachrymatory factor. He proposed S-(1-propenyl) cysteine sulfoxide, a derivative of a glutamyl peptide, as the immediate precursor. The precursor is cleaved by an enzyme of the alliinase type to pyruvic acid, ammonia, and the lachrymatory factor, propenyl sulfenic acid. In alkaline solutions, the lachrymatory precursor is converted to cycloalliin. (Figure 4.)

Figure 4. Formation of the lachrymatory factor.



Kuon (10), using different techniques, isolated the pungency precursors S-methyl- and S-propyl cysteine sulfoxide, and the gamma glutamyl peptide from which the lachrymatory precursor is derived. He did not find S-allyl cysteine.

Neigish (13) utilized the results of Stoll and Seebeck to study the volatile constituents of onions. Using mass spectrometry and infrared spectroscopy, he identified methyl alcohol and n-propyl mercaptan, and suggested the presence of propionaldehyde, acetaldehyde, and carbon dioxide, and possibly propyl alcohol, sulfur dioxide, hydrogen sulfide, and n-propyl disulfide. Allyl propyl disulfide, which had been considered the major component of onion odor, was not present.

Carson (5) (18), with gas-liquid chromatography and precipitation methods, isolated sixteen compounds from onions. Of these, the most important for odor contained sulfur. However, because of the extreme lability of the materials responsible for pungency and the methods used, the compounds isolated more nearly approached those from cooked onions than those from fresh. Significant observations include the lack of allyl-n-propyl disulfide, the predominance of methyl and n-propyl derivatives of cysteine sulfoxides, and the presence of trisulfides. This confirmed the theory that S-methyl- and S-propyl cysteine sulfoxides are reduced to mixed or asymmetric thiosulfinates, pyruvic acid, and ammonia. The thiosulfinates decompose to thiosulfonates, di- and trisulfides, and other products. The products depend upon the conditions of the enzymatic reaction.

Saghir (17) developed a method for chromatographing odors

obtained immediately after masceration of the onions, thereby avoiding the "cooked flavors only" label associated with Carson's study. N-propyl disulfide was the principal pungency component of onion odor which Saghir was able to closely duplicate with synthetic n-propyl disulfide. He also expressed belief that other factors greatly influence onion odor awareness, for example: sugars, total solids, crispness, and lachrymatory factor concentration.

Table 1. Compounds of onion odor

methyl disulfide	acetaldehyde
methyl-n-propyl disulfide	propionaldehyde
n-propyl disulfide	n-butyraldehyde
methyl trisulfide	methyl alcohol
methyl-n-propyl trisulfide	n-propyl alcohol
n-propyl trisulfide	ethyl alcohol
hydrogen sulfide	isopropyl alcohol
sulfur dioxide	acetone
n-propyl mercaptan	methyl ethyl ketone

Enzyme

Studies of the enzyme causing the reaction demonstrated its great substrate specificity. Kupiecki (11) found that only sulfoxides derived from cysteine are cleaved, and that the alliin of garlic is the most reactive, followed by ethyl-, methyl-, and propyl cysteine sulfoxides. Maximum activity occurred in a phosphate buffer at a pH of 7.4.

Schwimmer (19) followed the production of pyruvic acid from synthetic S-propyl-L-cysteine sulfoxide, and observed a rapid initial reaction which occurred because the (+) form was hydrolyzed much

faster than the (-) form.

Schwimmer and Mazelis (23) qualified enzyme specificity. Only L-cysteine derivatives in which S is oxidized to S $\longrightarrow$ O can act as substrate. Dextrorotatory diastomers of the derivative act as better substrate than the levo diastomers.

Storage of Rings

Pungency changes have been studied in dehydrated flakes (24) and onion juice (33), but no data are available for fresh or frozen rings or diced product. Shimazu (26), in a study of rehydration of onion cubes, remarked that those prepared by lyophilization (frozen at -29°C in a moving air stream) came closest to original volume, a fact he attributed to the presence of irregular capillary spaces in the tissue caused by expanding ice crystals.

Early freezing methods resulted in the growth of large ice crystals which ruptured cell walls (29). Present rapid methods of freezing should, however, result in more internal ice crystal formation and less cell wall damage and denaturation of cell constituents. Onions, although high in moisture, have large cells that should be less damaged by ice crystals than other plant materials.

The major problem is that damage to onion cells, whether from freezing, chipping for dehydration, or bruising, results in substrate-enzyme interaction. Within five minutes at room temperature, ninety per cent of the potential pungency of a damaged cell will have been produced (18). That is, as the effect of the lachrymatory factor is observed, the chain of reactions from the important substrates,

S-methyl- and S-propyl cysteine sulfoxides, rapidly occurs. If the volatiles produced are not "caught" at this time, they are lost, leaving only some of the slower acting and less pungent end products.

Associated with this problem is the effect that loss of turgidity will have on pungency production in the fresh storage of onion rings.

Flakes

Because dehydrated onion flakes are at present the major form of processed onion (14), they are used as a comparison to rings and whole onions with respect to pungency potential. Schwimmer (19) studied the relationship between pyruvate content and odor strength of reconstituted onion powder, and found a close correlation. He also proposed that the substrate was the limiting factor in pungency formation. This he demonstrated by adding S-propyl cysteine sulfoxide, or alternately enzyme preparation, to flakes before rehydration. Increases in pyruvate concentration were observed with substrate, but not enzyme addition.

Powder

The addition of enzyme powder to processed foods to restore fresh food flavors has been studied (22). Onion enzymes added back to autoclaved fresh onion will cause distinct onion-like odors, however, the lachrymatory effect is not produced.

Attempts have been made to make a pungency concentrate from volatile distillations and to make juices (33), but none of these have produced acceptable onion pungency. The distillation destroys the enzyme that acts on the S-methyl- and S-propyl cysteine

sulfoxides, and the production of the juice causes the interaction between enzyme and substrate which releases the pungency.

If the enzyme and substrate could be separated and preserved in an active form, they might be capable of beginning the pungency reaction when recombined.

Testing Method

Methods of testing for onion pungency have varied greatly. For centuries, both garlic and onion have been used for medicinal purposes, and antimicrobial effect has been associated with pungency (31).

Until about twenty years ago, most chemical determinations involved measurement for sulfur. Platenus (15), studying onion pungency by measuring volatile sulfur content, found that pungency could be affected by varietal characteristics, growing temperatures, soil type, water supply, and storage. Kohman (8) developed a procedure for separation of volatile components in which sulfur compounds were oxidized by bromine to sulfates. Other methods with sulfur include a reaction with N-ethyl maleimide, with thiamine, and determination by gas chromatography (20) (12). Ammonia production has also been measured.

Bennet (2) showed that pyruvic acid accumulates in onions, but did not relate it to pungency formation. Morgan (12) showed, by mascerating one-half of an onion in water and the other half in trichloroacetic acid (TCA), that no pyruvate accumulates in intact onions.

Schwimmer (20) used a method developed for garlic which

incorporated the theory of enzymatic pungency formation whereby a combination of products, ammonia, and pyruvic acid were formed. Carbonyl compounds contained in onion homogenate will react with 2,4-dinitrophenylhydrazine. Not only pyruvate, but all keto groups will react, thus necessitating a control group. The control group, however, remained very constant (21). Highly significant correlations of enzymatically produced pyruvate and olefactory measurements of onion pungency were observed (21).

METHODS AND MATERIALS

Materials

The onions used in this study were obtained from the Food Stores and the Horticulture Department of Michigan State University. Those from Food Stores were common commercial varieties grown in Michigan, for example Yellow Globe. Those from the Horticulture Department - Trapp Downing, 2399 X 611, Spartan Banner, and Spartan Gem - were test varieties from the 1965 and 1966 crop years. Only unsprouted onions were used. Onions were stored at 32°F to 36°F before use.

The dehydrated flakes used were "off the shelf" commercial varieties.

Methods

Preparation of Sliced Rings

The onions were washed and peeled, then either tested immediately to determine pungency of whole onions, sliced and then tested, or sliced and put in storage until needed for testing. A Qualheim cutter was used to make the slices which were pressed into rings as quickly as possible. It was hoped that slicing with the Qualheim would be a shearing rather than a tearing action, thus minimizing cell damage. Slices were cut $\frac{1}{4}$ inch thick. Rings of about twice this thickness did not appear to have less pungency loss.

Initially, slicing was done at 35°F in an attempt to reduce enzymatic action, but the time of slicing was short and there was no more activity at 70°F, so the latter temperature was used for the majority of the slicing. The slices were pressed into rings at 35°F. The rings to be frozen at 0°F were spread on trays, placed

in a 0°F walk-in freezer for 6 hours, then bagged and stored. Those to be stored at -30°F were spread on trays and placed in a -30°F freezer for 2 hours, then bagged and stored. Those to be stored at 35°F were sealed immediately into plastic bags. Moisture vapor-proof plastic bags were used.

Low Temperature Storage

Rings were stored at 35°F and -30°F to determine the approximate pungency loss with short time storage. Tests were then done at 35°F, 0°F, and -30°F to compare losses in pungency between refrigerated and frozen product.

Pungency determinations were made on the whole onion and on the slices immediately following slicing. The slices held at 35°F were tested 1, 2, 4, 7, 14 and 28 days after slicing. Those held at 0°F were tested 1, 4, 7, 14, and 28 days after slicing. Those held at -30°F were tested 1, 4, 7, and 28 days after slicing.

In an attempt to simulate plant conditions that might occur, rings were held at room temperature before storage. Data was obtained for whole onions and for onions immediately following slicing. The rings were held for 2 hours at 70°F, tested, and stored at 35°F, 0°F, and -30°F. Pungency was determined after 2 hours, 24 hours, 7 days, and 28 days of storage.

The physical appearance of the rings was noted during storage, and in the case of the frozen product, after thawing.

High Temperature Storage

Rings were stored at 68°F and 95°F to observe the effects of higher temperature on pungency potential. Because the moisture losses from the rings were likely to become excessive, test lots were stored in individual plastic bags. The rings were stored until the net pyruvate concentration became zero in the 95°F storage lots, and for one month for the 68°F lots. The lots stored at 68°F were tested after 1, 4, 7, 14, and 28 days. The lots stored at 95°F were tested after 1, 4, 7, and 14 days.

Flakes

The flakes were rehydrated during the test according to the recommended proportion, 1 ounce of flakes equal to ½ pound of fresh peeled onion.

The pungency potential of the dehydrated flakes was determined after blending the flakes in water at 50°F for 5 minutes in a Waring Blender. Flakes mascerated in boiling water and tested were used as a control.

A simple test was devised to determine whether enzyme or substrate was lacking in the flakes. Both boiled and unboiled onions were mascerated in equal weights of water, and the juice extracted. Pyruvate levels were determined in both. Pyruvate was also determined in the reconstituted flakes. Then 15 grams of flakes were blended with 50 milliliters of water, 50 milliliters of either the boiled or unboiled onion juices, and the pyruvate measured. The pyruvate content after addition compared to the cumulative contents of the juice and the flakes would

indicate any interaction between the two.

Preparation of Powder

An attempt was made to separate enzyme and substrate separately from the onion as powders, using an acetone precipitation method.

Twenty-five grams of onion were ground with 250 milliliters of acetone (at -40°F) for 25 seconds in a Waring Blender. The mixture was filtered rapidly in a Buchner funnel, and the residue reblended for 10 seconds with an additional 100 milliliters of -40°F acetone. After refiltering, the residue, which should contain the enzyme, was air dried. The combined filtrates were evaporated with water in a flash evaporater to approximately 25 milliliters of product.

The activity of the concentrates was determined by recombining the two, adding a proportionate amount of water, and comparing the results of the pyruvate test to a test on a blank made by adding the concentrates to trichloroacetic acid.

Pyruvate Measurement of Pungency

Pungency was measured by a determination of carbonyl compounds present in the onion after masceration. A sample size of 300 grams of rings and whole onions was used; a proportionate amount of flakes or powder plus water was also used. The sample was mixed with an equal weight of water and ground for five minutes at room temperature in a Waring Blender. After a reaction time of 20 minutes, the homogenate was filtered and duplicate 5 milliliter samples were added to 5 milliliters of trichloroacetic acid. The mixture was held for 1 hour, then filtered through a Buchner funnel and washed with water

to a total volume of 200 milliliters.

The control sample was prepared by boiling 300 grams of the same form of onions in an equal weight of water for 10 minutes, then blending and continuing with the above test. Whole onions were halved longitudinally, with one half used to determine pungency and the other half used as control.

One milliliter of the sample was placed in a test tube with ½ milliliter of water and 1 milliliter of a solution of 0.0125 per cent 2,4-dinitrophenylhydrazine in 2N HCl. This was heated for 15 minutes at 37°C, then 7.5 milliliters of 0.4N NaOH were added. The color developed was measured in an Evelyn colorimeter set at 420 millimicrons against a blank similarly prepared with water substituted for the sample. The color was stable for a period of one hour.

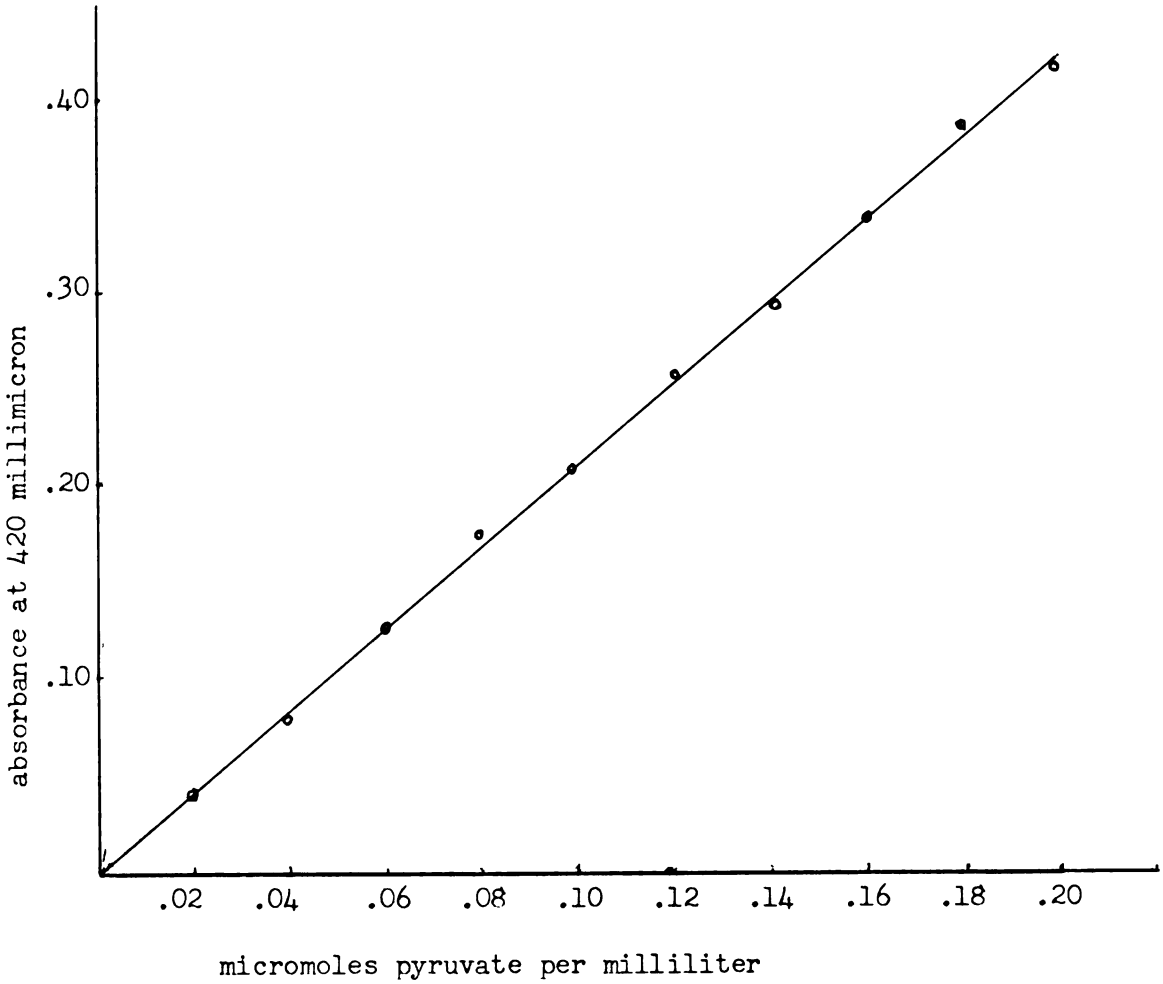
Pyruvate was calculated by comparison to a standard curve prepared with known concentrations of sodium pyruvate. Absorbance is linear with sodium pyruvate concentrations of 0 to 0.20 micromoles per milliliter (20).(Figure 5.)

The amount of pyruvate in the onion samples, determined as sodium pyruvate, was calculated from the formula

$$\frac{A}{K} \times \frac{\text{dilution}}{\text{weight of sample}}$$

where A is the measured absorbance at 420 mμ and K is the comparison of absorbance to concentration of pyruvate determined for the standard. The ratio of dilution to weight of sample was 80 with the test procedures followed here. Two standards were run with each test.

Figure 5. Pyruvate Concentration vs. Absorbance (concentrations from 0.02 to 0.20 micromoles of pyruvate per milliliter)



$$K = \frac{A}{C} = 2.09$$

RESULTS AND DISCUSSION

Pungency Losses During Storage of Rings

There was a loss of pungency during slicing which varied from 11.9 to 20.7 per cent. (Table 2.)

Table 2. Pungency losses with slicing.

Test	Per cent loss
4-day test at 35°F	14.8
7-day test at -30°F	11.9
28-day test at 35°, 0°, -30°F	15.6
28-day test -2 hours hold	20.7

This loss was immediate because of the substrate-enzyme interaction when the cells were broken during slicing. The variation might be due to differences in cell size, and perhaps in onion size. Slight variations in the time lapse before testing could also be a contributing factor. Slicing at 35°F rather than at room temperature (68 - 70°F) did not decrease the loss of pungency. Since only about 15 minutes was required to wash, peel, and slice the onions, this might be expected.

Initial results indicated that onion rings retained from 85 to 88 per cent of their pungency immediately after slicing. (Table 3.) Those stored at 35°F showed no further loss after 4 days, while those frozen at -30°F retained only 60 to 62 per cent of their pungency after 7 days storage.

Table 3. Pungency of onion rings after storage at 35° and -30°F.

Condition	Per cent retention	
	35°F	-30°F
Before slicing	100 %	100 %
After Slicing	85	85
24 hours after slicing	89	60
2 days after slicing	91	
4 days after slicing	85	
7 days after slicing		62

Longer storage periods (28 days) resulted in no significant loss of pungency in the rings stored at 35°F. (Table 4.)

Table 4. Pungency of onion rings after storage at 35°, 0°, and -30°F.

Condition	Per cent retention		
	35°F	0°F	-30°F
Before slicing	100 %	100 %	100 %
After slicing	84	84	84
24 hours after slicing	88	57	65
2 days after slicing	85		
4 days after slicing	88	56	52
7 days after slicing	84	41	49
14 days after slicing	86	43	
28 days after slicing	80	21	51

At 0°F there was a continual loss of pungency throughout the storage period. At 28 days only 21 per cent of the original pungency

was retained. The pungency of the slices stored at -30°F decreased to 52 per cent of the original pungency after 4 days, and then remained constant.

Pungency retention is intimately related to cell disturbance and destruction. Any change that results in enzyme activity will reduce the available supply of substrate.

With 35°F storage, there was little loss of pungency potential, which indicates that only minor structural changes had occurred.

Rings stored at 0°F had a rapid drop in potential within 24 hours after freezing. Slow freezing at 0°F will result in large ice crystal formation, and therefore cell breakdown. The effects of slow freezing are apparent in Table 5. After 2 hours of storage at 0°F there was little pungency potential loss, but the loss after 24 hours was extensive. Continued losses at this temperature may be due to changes in crystal size caused by slight temperature fluctuations. Also, this temperature may slow enzyme activity, but is not likely to stop it.

Storage at -30°F resulted in a slower immediate potential drop, and a levelling of potential to a constant value after 4 days. This immediate loss can again be explained by ice crystal formation. Freezing at -30°F causes smaller crystals and less damage than freezing at 0°F. The results in Table 5 show that freezing had occurred within 2 hours and ice crystal formation had ceased. Enzyme activity was also probably minimal.

In normal plant processing, the onions might be held at

room temperature before storage. Table 5 gives the results of holding the onions at 70°F for 2 hours prior to storage.

Table 5. Pungency of onion rings after storage at 35°, 0°, and -30°F with a 2-hour hold at 70°F.

Condition	Per cent retention		
	35°F	0°F	-30°F
Before slicing	100 %	100 %	100 %
Immediately after slicing	79	79	79
After 2 hours at 70°F	80	80	80
After 2 hours of storage	76	79	63
After 24 hours of storage	77	46	60
After 7 days storage	82	43	77
After 28 days storage	67	32	61

This appears to cause increased loss of pungency potential at all three storage temperatures when compared to the results in Table 4. The greatest variation is at storage temperatures of 35°F. However, other factors may influence these differences, as the tests were conducted later in the season on different lots of onions.

A study of pungency loss in onion rings becomes more significant when physical characteristics are observed. Generally, the rings stored at 35°F stored very well for two weeks. Until this time, the rings were crisp and white, with minimal browning and no apparent microbial spoilage. By 28 days, although very little free liquid had accumulated, turgidity was reduced, browning was

noticeable and the rings were visually unacceptable.

The rings held at 0°F retained good color and appearance during storage; however, they were somewhat brittle and had to be carefully handled to avoid breakage. Damage done to them while they were frozen could result in additional pungency losses during thawing. After thawing, these rings suffered a complete loss of shape and structure. Attempts to use some of them for deep fat frying failed because of their weak structure.

Rings stored at -30°F had a better, less crystalline appearance but were more brittle than those stored at 0°F. Their color and appearance remained constant during storage, and the rings, when thawed, were limp but useable.

Possibly, freezing the rings very quickly to -10°F and storing at this temperature would result in smaller crystals and less reduction of pungency potential.

The rings stored at -30°F retained about half of their pungency potential. When thawed, they did not, however, retain pungency for extended periods of time. (Table 6.)

Table 6. Pungency after thawing of rings held at -30°F.

Condition	Per cent retention
After 7 days storage	55 %
After 7 days storage plus 24 hours at 70°F	2
After 14 days storage	27
After 14 days storage plus 2 hours at 70°F	25
After 14 days storage plus 24 hours at 70°F	13

The study of pungency retention in frozen and refrigerated rings indicated that enzyme activity was effective in the loss. Therefore, tests were conducted at 68°F and at 95°F in order to observe the effect of enzyme activity at these temperatures.

Table 7. Pungency of onion rings after storage at 68° and 95°F.

Condition	Per cent retention	
	68°F	95°F
Before slicing	100 %	100 %
Immediately after slicing	100	100
24 hours after slicing	92	86
4 days after slicing	89	63
7 days after slicing	92	26
10 days after slicing		0
14 days after slicing	79	
28 days after slicing	73	

Rings stored at 68°F retained 73 per cent of their pungency after 28 days. Those stored at 95°F lost all of their pungency after 10 days.

At a temperature of 68°F, the rings were considerably wilted in 14 days, and the color was very poor. In addition, mold growth had begun. The large amount of water in the bags indicated that great structural breakdown had occurred. However, as observed from Table 7, there was no corresponding drop in measured pyruvate. The pyruvate test may be invalid at this temperature, perhaps because of the presence of microbial flora. Also, the total pyruvate

here did not decrease with time as in the previous tests. This may indicate that the pyruvate was bound at cooler temperatures, but remains free at 68°F.

The pungency potential of onions held at 95°F decreased to 0 in 10 days, as would be expected from the results obtained with storage at lower temperatures. That is, as the cell structure breaks down, the pungency as measured by pyruvate production will decrease. In 4 days, the onions were very brown and their cell structure had broken. Large amounts of free water had collected.

Flakes

A brief study of flakes was undertaken to compare their pungency levels with those of fresh onions and frozen rings. An attempt was also made to show what causes the lack of pungency in flakes.

Table 8. Pungency comparison of whole onions, fresh and frozen rings, and dehydrated flakes.

Sample	Micrograms of pyruvate per gram of onion
High pungency California onions	20
Medium pungency California onions	12
High pungency Michigan onions	12
Medium pungency Michigan onions	6
Fresh rings, Michigan onions	6-9
Frozen rings, Michigan onions	3-6
Dehydrated flakes	1.8

As shown in Table 8, an average of results for various commercial grades of flakes rehydrated according to package directions gave a net pyruvate value which, compared to the values for whole onions, was relatively small. Both fresh and frozen rings had very acceptable pungency when compared to flakes. The high pyruvate levels (20 micrograms) of the California onions compared to the Michigan onions (12 micrograms) combined with their color advantage make California onions more desirable for the production of dehydrated flakes. However, on the basis of the pungency potentials of the flakes compared to the potential of the frozen onion rings, Michigan processed onions are acceptable.

Because the rehydrated flakes exhibited very low pungency, a test was devised to determine whether enzyme or substrate was lacking. Flakes were added to juice from boiled and unboiled onions. The juice from boiled onions should have substrate, but no enzyme. Juice from unboiled onions should have enzyme, but should have been depleted of its substrate. Pyruvate levels of the flakes and of the juices of boiled and unboiled onions were taken. Combinations of these should give cumulative results if there is no further substrate-enzyme activity.

Table 9. Pungency of flakes added to boiled and unboiled onion juice measured as micrograms of pyruvate per gram of onion.

Flakes added to	Theoretical cumulative value	Actual value	Net
Juice of boiled onion	5.2	10.2	+5.0
Juice of unboiled onion	11.4	12.3	+0.9

The results of Table 9 show a doubling of pungency levels above an additive effect when flakes were added to the juice of boiled onions. Addition to the juice of unboiled onions gives only minor increases. That is, the enzyme in flakes still has considerable activity, but substrate is lacking.

A development from this might be the production of onion juice. If boiled onion is a good source of natural substrate, then the juice of this, when added to an enzyme powder preparation, should have pungency potential proportional to the substrate remaining in the juice. However, the substrate may be destroyed or made inactive by heat, thus introducing a limiting factor.

Powder

The onion industry wants to produce a concentrated form of onion flavor that is high in pungency, easily handled, consistent in strength, and inexpensive.

Since pungency develops when enzyme and substrate interact, attempts were made to separate these two components without pungency release and waste, to dry them to powder form, and then to rehydrate them to obtain odor.

Results of the acetone preparation are given in Table 10. Net pyruvate was similar in concentration to dehydrated flakes when measured at pH 7.4, the optimum pH for this enzyme. As would be expected at pH 5.8, the normal pH of onions, the results were lower.

Table 10. Pungency of rehydrated powder in micrograms of pyruvate per gram of onion.

	Control	pH 5.8	pH 7.4	Net
Test 1.	2.9	4.0		1.1
	2.9		4.6	1.7
Test 2.	1.9	2.2		0.3
	1.9		2.7	0.8

Although this procedure was not too successful, it is likely that a technique which could separate the enzyme and the substrate in active forms could supply the answer to the onion pungency problem.

CONCLUSIONS

1. Onion rings can be held successfully at 35°F without excessive pungency losses. The appearance of the rings becomes poor after 2 to 3 weeks.

2. Pungency retention with frozen storage is acceptable at -30°F, but is poor and continually decreases at 0°F.

3. Rings frozen and stored at -30°F could be useable when thawed, but those stored at 0°F will not be.

4. For short periods of storage, that is, less than 2 weeks, fresh storage would be better than -30°F storage, but for longer periods, -30°F storage would be better.

5. A possible solution to the problem of freezing might be to quick freeze the rings to -10°F and hold them at this temperature.

6. Compared on the basis of pungency, both fresh and frozen rings are better than dehydrated flakes.

7. Substrate is lacking in flakes, causing low pungency.

8. A method of separating enzyme and substrate in an active form, without interaction, may be a possible solution of the pungency problem.

APPENDIX

APPENDIX TABLE 1

Net pungency measured as
micrograms of pyruvate per gram of onion

Rings held at 35°F.

Condition	Total	Control	Net
Before slicing	13.4	1.2	12.2
Immediately after slicing	11.8	1.4	10.4
24 hours after slicing	12.1	1.2	10.9
48 hours after slicing	12.2	1.1	11.1
96 hours after slicing	11.4	1.0	10.4

APPENDIX TABLE 2

Net pungency measured as
micromoles of pyruvate per gram of onion

Rings held at -30°F.

Condition	Total	Control	Net
Before slicing	13.1	1.3	11.8
Immediately after slicing	11.8	1.4	10.4
24 hours after slicing	9.9	2.8	7.1
7 days after slicing	10.1	2.8	7.3

APPENDIX TABLE 3

Net pungency of stored rings
as micrograms of pyruvate per gram of onion

Rings stored at 35°, 0°, and -30°F

	35°F			0°F			-30°F		
Condition	Total	Control	Net	Total	Control	Net	Total	Control	Net
Before slicing	13.4	1.2	12.2	13.4	1.2	12.2	13.4	1.2	12.2
After slicing	11.7	1.4	10.3	11.7	1.4	10.3	11.7	1.4	10.3
24 hours after slicing	12.1	1.3	10.8	8.7	1.7	7.0	10.3	2.4	7.9
48 hours after slicing	11.5	1.1	10.4	8.7					
4 days after slicing	11.5	0.8	10.7	8.7	1.9	6.8	8.8	2.5	6.3
7 days after slicing	11.2	0.9	10.3	6.8	1.9	4.9	8.5	2.5	6.0
14 days after slicing	11.7	0.7	10.5	7.0	1.7	5.3			
28 days after slicing		1.9	9.8	4.5	1.9	2.6	8.8	2.6	6.2

APPENDIX TABLE 4

Net pungency of stored rings
as micrograms of pyruvate per gram of onion

Rings stored at 35°, 0°, and -30°F and held at 68°F for 2 hours after slicing before storage.

	35°F			0°F			-30°F		
Condition	Total	Control	Net	Total	Control	Net	Total	Control	Net
Before slicing	9.4	1.2	8.2	9.4	1.2	8.2	9.4	1.2	8.2
After slicing	8.2	1.7	6.5	8.2	1.7	6.5	8.2	1.7	6.5
After 2 hours hold at 68°F	8.3	1.7	6.6	8.3	1.7	6.6	8.3	1.7	6.6
After 2 hours storage	7.9	1.7	6.2	8.2	1.7	6.5	7.1	1.9	5.2
After 24 hours storage	7.7	1.4	6.3	6.6	2.3	3.8	6.6	1.7	4.9
After 7 days storage	7.9	1.2	6.7	6.1	2.6	3.5	7.4	1.1	6.3
After 28 days storage	6.9	1.4	5.5	6.1	3.5	2.6	7.3	2.3	5.0

APPENDIX TABLE 5

Net pungency of rings stored at -30°F
as micrograms of pyruvate per gram of onion

Rings tested 1 and 2 weeks after slicing, after 2 and 24 hours thawing.

Condition	Total	Control	Net
Before slicing	10.5	2.3	8.2
Immediately after slicing	11.4	3.1	8.3
24 hours after slicing	8.6	4.8	3.8
7 days storage	8.9	4.4	4.5
7 days storage 24 hours thaw	3.8	3.6	0.2
14 days storage	7.6	5.4	2.2
14 days storage 2 hour thaw	6.5	4.4	2.1
14 days storage 24 hour thaw	4.7	3.6	1.1

APPENDIX TABLE 6

Net pungency of rings stored at 68°F
as micrograms of pyruvate per gram of onion

Condition	Total	Control	Net
Before slicing	7.6	2.2	5.6
Immediately after slicing	7.6	2.2	5.6
24 hours after slicing	7.0	1.8	5.2
4 days after slicing	6.8	1.8	5.0
7 days after slicing	7.2	2.0	5.2
14 days after slicing	6.8	2.4	4.4
28 days after slicing	6.4	2.3	4.1

APPENDIX TABLE 7

Net pungency of rings held at 95°F
as micrograms of pyruvate per gram of onion

Condition	Total	Control	Net
Before slicing	9.1	2.3	6.8
Immediately after slicing	9.5	2.7	6.8
24 hours after slicing	7.5	1.8	5.7
4 days after slicing	5.3	1.9	3.4
7 days after slicing	2.1	1.7	0.4
10 days after slicing	0	0	0

APPENDIX TABLE 8

Pungency potential
of
rehydrated, mascerated onion flakes

	Micrograms of pyruvate per gram of onion
Flakes plus boiling water	1.5
Flakes plus cold water	3.3
Net pyruvates per gram	1.8

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