EFFECT OF COOKING ON ASCORBIC ACID RETENTION AND PALATABILITY OF FROZEN OKRA

by

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INTRODUCTION

Vegetables are important sources of vitamins which are required for efficient functioning and growth of the body. In some parts of Nigeria, vegetables are the largest source of ascorbic acid because of the seasonal production of fruits. Some of the vegetables which provide ascorbic acid are bitter leaf (Vernonia amygdalina), water leaf (Talinum triangulare), Indian Spinach (Basella alaba), okra (Hibiscus esculentus) and red peppers.

In a recent survey at Igbo-Ora in Western Nigeria, Kotnis and Houssain (1) showed that the intake of ascorbic acid varied from about 280% of the recommended allowance for 3 to 5 year old children to about 400% for pregnant women. Recommended daily intake for children and pregnant women are 20 mg. and 50 mg. respectively (2). On the other hand Nicol (3) found that lack of fresh fruit and leaves in Northern Nigeria resulted in varying degrees of dietary deficiency of ascorbic acid. In general the diets of the Nigerians in the Southern part of the country are not deficient in ascorbic acid because of the large consumption of red peppers.

Many methods of vegetable cookery are known; these include boiling, panning, waterless cooking, baking, steaming, cooking in pressure saucepan and, more recently, microwave cooking. The general method of vegetable cookery in Nigeria is boiling. In order to derive the maximum ascorbic acid value from vegetables such as okra, it is necessary to find a cooking method which will give an acceptable product as well as minimize the ascorbic acid loss.

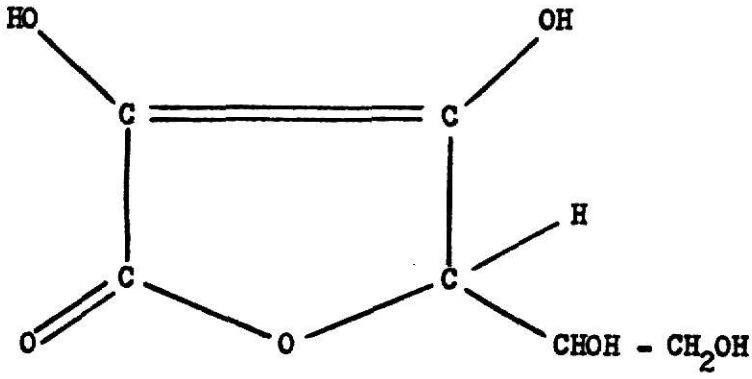
This study therefore investigated the effect of variation in amount of cooking water and length of cooking time on ascorbic acid retention and palatability of frozen okra pods.

REVIEW OF LITERATURE

Ascorbic acid

Natural occurrence. Ascorbic acid is found almost exclusively in foods of plant origin. Aside from liver, no other animal food is considered a significant source. In fruits and vegetables the greatest concentrations are found in those parts directly concerned with growth. The ascorbic acid content of plants is extremely variable since it is affected by a number of factors; seasonal, climatic and agronomic. Soil conditions and the use of soil nutrients affect its content as does the degree of maturity. In fruits ascorbic acid accumulates throughout the ripening process, therefore the longer the fruit remains on the tree the higher its ascorbic acid content (4). Skin of fruits has the highest ascorbic acid content. Immature seeds such as peas and beans, contain some ascorbic acid but lose most of it at maturity. Andree (5) reported that ascorbic acid increases during growth of asparagus from 35 to 58 mg/100 g of the vegetable.

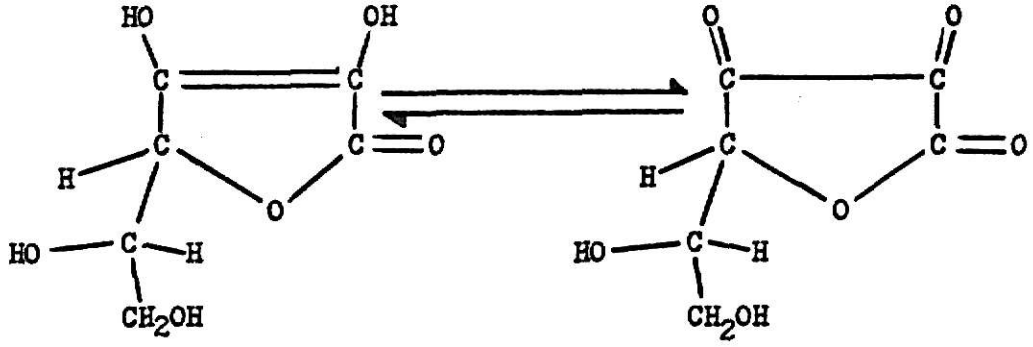
Chemistry and Physical Properties. L-ascorbic acid ($C_6H_8O_6$) is a fairly strong monobasic acid closely related to the hexoses. Extensive structural and degradation studies of the molecule have shown it to be an unsaturated hydroxylated γ-lactone of the following structure (4).



L-ascorbic acid

The outstanding feature of this structure is the presence of an α-keto-ene-diol system, the chromophore responsible for the high acidity and well known reducing properties of l-ascorbic acid. L-ascorbic acid is a powerful reducing agent in acid and neutral solutions. In addition to reducing Fehling's solution and ammoniacal silver nitrate in the cold, it reacts with such regents as phenylhydrazine to yield a phenylhydrazone. It reacts quantitatively with one molar equivalent of iodine or other halogens and with selenium dioxide. Most methods for the determination of ascorbic acid are based upon its reducing properties.

L-dehydroascorbic acid is the first oxidation product of ascorbic acid.



L-ascorbic acid L-dehydroascorbic acid

This reaction is reversible since l-dehydroascorbic acid can be converted to l-ascorbic acid by reduction with hydrogen sulfide or hydrogen iodide. Further oxidation of l-dehydroascorbic acid leads to extensive degradation with the formation of oxalic acid. L-dehydroascorbic acid is as active biologically as l-ascorbic acid, and it has been shown that some of the naturally occurring vitamin exist in the oxidised form.

Jackel et al. (6) showed that ^{14}C-d-glucose, labelled uniformly in all positions, and given to rats by subcutaneous injection, caused excretion of l-ascorbic acid uniformly labelled. Harowitz and King (7) reported that, in the rat, d-glucose labelled with ^{14}C at position one, produced l-ascorbic acid labelled mainly at position six. The similarity of radioactive l-ascorbic acid in the urine gave further support to the view that the sixth carbon of glucose entered into ascorbic acid via the same molecular pathway that was followed by carbon one. Thus evidence was furnished for direct conversion of d-glucose to l-ascorbic acid in rats.

Ray (8) reported on the synthesis of ascorbic acid during germination of pea seeds. He found that in plants there was an overall conversion of sugars (d-glucose, d-fructose and d-mannose) to l-ascorbic acid in cotyledonless pea seedlings. In similar study Isherwood (9) postulated that a compound such as glucose was developed as an intermediate in germinating cress seedlings.

Estimation of Activity. Although ascorbic acid was among the first of the vitamins to be characterized, its determination in some foods still

presents several problems. No ideal method of stabilizing the vitamin in all foods is available (4). Foods that have been cooked or processed are often low in ascorbic acid and contain reducing substances which interfere with the usual methods of analysis. Methods of differentiating the biologically active ascorbic and dehydroascorbic acids from the inactive 2,3-dioxogulonic acid are cumbersome, lengthy and lack precision.

Chemical methods for the determination of reduced ascorbic acid are based upon its reducing properties. The indophenol method of Bessey (10) is an example. This method is not specific for ascorbic acid since such reducing substances as sulfhydryls, sulfides, thiosulfates, pyridine, reduced forms of nicotinic acid derivatives, riboflavin, and inorganic and organic ferrous and ferric compounds interfere with the determination.

Numerous modifications to increase the specificity of the indophenol method have been proposed by Robinson and Stotz (11) and Lowry et al. (12). Two of the most common are: a) the addition of about 20% acetone, which effectively removes sulfites, and b) titration in presence of formaldehyde, which is not always successful in suppressing interference from reducing agents.

The most popular of the methods for determining ascorbic acid plus dehydroascorbic acids is probably that developed by Roe and Kuether (13). In their procedure, ascorbic acid is oxidized with active carbon to dehydroascorbic acid which is then condensed with 2,4-dinitrophenylhydrazine at 37°. The resultant osazone is dissolved in sulphuric acid forming a red color which is measured spectrophotometrically. Any 2,3-dioxogulonic acid which may be present also reacts and increases

the apparent ascorbic acid. This method is claimed to be unaffected by metal ions and is free from interference by plant pigments but it does not eliminate the effect of reductones.

Schaffert and Kingsley (14) proposed a method for determining reduced, dehydro and total ascorbic acid in materials of animal and plant origin. In this method, l-ascorbic acid is first oxidised to dehydroascorbic acid by the addition of Norit to the filtrate, the dehydroascorbic acid then mutarotates to 2,3-diketogulonic acid. When the dehydroascorbic acid is incubated with 2,4-dinitrophenylhydrazine at 100° for ten minutes in the presence of thiourea, an orange-red precipitate which is soluble in 85% sulfuric acid, is formed. With this method there is no significant interference of 2,3-diketogulonic acid upon the coupling reaction of dehydroascorbic acid with 2,4-dinitrophenylhydrazine.

Functions and Requirements. Ascorbic acid is essential for the growth and development of most animal species. As an activator of tissue growth, it is required for normal development of such organs as the endocrine glands, liver and kidney (15). Ascorbic acid is present in highest concentration in areas of great metabolic activity and is found in the retina, pituitary gland, corpus luteum, brain, heart, muscles, erythrocytes and plasma (16).

One of the most important roles of ascorbic acid involves the formation of collagen in teeth, bone, cartilage, connective tissue and skin. It promotes normal development of the teeth, including tooth pulp and dentine; but apparently does not influence the occurrence of dental caries in man. Its function extends from the formation of

cartilage, callus and osteoid tissue of the bones to the union of fractures and the healing of wounds and burns (16). Hines et al. (17) reported that ascorbic acid is essential for regeneration of damaged nerve tissue.

Reactions involving ascorbic acid as a carrier for hydrogen transfer have been demonstrated in plant tissue, and the existence of those reactions in animal tissues has been postulated. The oxidation of glutathione in crude kidney homogenates has been described in terms of reaction in which both diphosphopyridine nucleotide and ascorbic acid were concerned (18). Ascorbic acid plays an important role in the metabolism of protein, particularly in the final oxidation of the aromatic acids; tyrosine and phenylalanine (16). The absorption of iron from the intestine is reported to be enhanced by ascorbic acid (16).

Ascorbic acid is required for the conversion of folic acid to folinic acid in vivo and vitro. Although the exact role of ascorbic acid in the folic-folinic acid interconversion is still unknown, the interplay of these vitamins is illustrated by the observation that megaloblastic anemia in the vitamin C deficient monkey can be cured either by ascorbic acid or folinic acid (4).

Human requirements for ascorbic acid have been determined by establishing the minimum amount needed to prevent scurvy. In the adult a daily intake of 10 mg is the minimum amount required for protection against deficiency disease, but several times this amount is needed to maintain a margin of safety (19, 20).

Table 1 shows the daily dietary allowances of ascorbic acid recommended by the Food and Nutrition Board of the National Research Council in 1968 (21). The infant's requirements for ascorbic acid can

TABLE 1

Recommended Daily Dietary Allowances for Ascorbic Acid (21)

	AGE	ASCORBIC ACID (mg.)
Infants	1 - 3 Months	35
	4 - 9 "	35
	10 - 12 "	35
Children	1 - 3 Years	40
	4 - 9 "	40
	10 - 12 "	40
Males	10 - 12 "	40
	12 - 14 "	45
	14 - 18 "	55
	18 - 22 "	60
	22 - 35 "	60
	35 - 55 "	60
	55 - 75 +	60
Females	10 - 12 Years	40
	12 - 14 "	45
	14 - 16 "	50
	16 - 18 "	50
	18 - 22 "	55
	22 - 35 "	55
	35 - 55 "	55
	55 - 75 +	55
Pregnancy		60
Lactation		60

be filled by the amount present in the milk of a well-nourished mother whose increased need for the vitamin is provided for by diet and adequate supplementation. It is generally acknowledged that any drug or condition which accelerates the metabolic rate also increases the need for ascorbic acid. Pauling (22) suggested the use of large doses of ascorbic acid for the treatment of the common cold. His prescription is 250 to 10,000 mg/day as a preventive measure and 1 to 15 g/day for therapeutic treatment.

Technical Applications. Ascorbic acid plays an important role in the control of enzymatic browning of fruits. The enzyme responsible for oxidative browning of fruits is in most cases polyphenoloxidase (23).

Ascorbic acid itself is not an inhibitor of polyphenoloxidase, it must be oxidized indirectly by the enzyme before it can inhibit the activity. While the enzyme will oxidise its natural substrate, the oxidation product will be continuously reduced by ascorbic acid, and as this process proceeds it is accompanied by a decrease of the enzyme activity until complete inactivation has been reached. This mechanism explains why ascorbic acid has to be present in a sufficiently large amount to prevent browning. Etheridge and Hard (24) recommended the addition of 150 to 200 mg of ascorbic acid to each liter of fruit juice.

The use of ascorbic acid has proved beneficial as a stabilizer in the dairy industry. The amounts of ascorbic acid or sodium ascorbate used varies between 20 and 50 mg per liter of milk, 30 to 40 mg usually being sufficient for fresh tank milk (25). Tamsma et al. (26) found a stabilizing effect of ascorbic acid in foam-dried whole milk.

Effect of Cooking and Processing. The water soluble and heat labile nature of ascorbic acid predispose it to destruction by cooking and processing. It is oxidised in air under alkaline conditions and the oxidation is powerfully catalysed by traces of copper. Great losses of ascorbic acid are due to leaching in the processing water (16).

Kylen et al. (27) reported that a sample of cabbage cooked in 10 volumes of water lost 10% ascorbic acid by destruction and 80% to the cooking water; and retained 10% in the food. Noble (28) reported that cooking green beans, broccoli, brussel sprouts, cabbage and cauliflower in boiling water "until tender" and five, ten and fifty minutes beyond this time decreased the ascorbic acid. Mean ascorbic acid retention decreased significantly as cooking period increased, but mean percentage ascorbic acid dissolved in the cooking water did not change with longer cooking times. About 38% of the vitamin was recovered from the cooking water and another 15% was presumed changed to biologically inactive substances during cooking.

Oke (29) reported that some vegetables such as bitter leaf (Vernonia amygalina), Telfare occidentale and Solanum spp lose as much as 70% of their ascorbic acid on boiling while others such as Indian Spinach (Basella alaba) and water leaf (Talinum triangulare) lose only 20 to 50% of their ascorbic acid on boiling as done in Nigeria. He also found that okra leaves lose 60% of the ascorbic acid on boiling. Sweeney et al. (30) reported that cooking 20 oz broccoli for fifteen minutes in 236 or 472 g of boiling water produced an overdone vegetable. They also showed that the ascorbic acid content of uncooked frozen broccoli was somewhat lower than that of fresh broccoli and there was a significant loss on cooking.

Ascorbic acid keeps remarkably well in frozen foods, apart from the losses incurred in the preliminary blanching process. Noble and Gordon (31) showed that broccoli and beans cooked fresh, retained 50% of their ascorbic acid while the blanched product, stored for 6 months at 0°F, retained 30% of this vitamin after cooking. In another experiment Eheart (32) reported that broccoli lost 35% of its ascorbic acid during blanching, 7% during freezing and storage for 52 weeks and a further 10% on cooking.

Ascorbic acid content of fruits and vegetables is slowly lost during storage and is a useful index of freshness, particularly if the food has been bruised during handling and at elevated temperatures (25). Sun drying is a method of vegetable preservation in Nigeria and Oke (29) reported that 87% of ascorbic acid was lost on sun drying for three hours. Bruising and wilting allow ascorbic acid oxidase to come into contact with its substrate and destroy the vitamin. Losses are reduced by factors that reduce wilting such as high humidity and cool storage conditions, but freezing may burst the cells and bring the enzyme into contact with the vitamin. Leafy vegetables cannot be stored at temperatures below the freezing point without causing damage to the tissue. In an examination of hospital diets, Platt et al. (33) showed that when peeled potatoes were soaked in water overnight, they lost 45 to 60% of their ascorbic acid. They pointed out that this loss was due to the damage caused by mechanical peeling as leaching does not occur if the tissues are undamaged. Hand peeling followed by fourteen hours soaking in water caused an average loss of 9% ascorbic acid, and machine peeling for one minute followed by soaking caused an 18% loss.

With the introduction of microwave cooking and its potential use on a large scale, its effect on nutritional losses is a matter of considerable interest. Eheart and Gott (34) reported that there were no significant differences in ascorbic acid retention when frozen broccoli, peas, spinach and fresh potatoes were cooked with and without water in the microwave oven. Frozen spinach retained significantly more ascorbic acid when cooked by microwave methods than by the conventional method. However for peas, broccoli and potatoes, the differences were not significant between microwave and conventional methods when the amount of cooking water was constant.

Large scale preparation of food often results in larger ascorbic acid losses than when food is prepared at home. Peppler and Cremer (35) reported that cabbage and spinach lost 62% and 66% ascorbic acid respectively during household preparation compared to 84% and 87% loss, respectively, during canteen preparation.

EXPERIMENTAL

Selection of Treatments. No previous studies on okra had been done in this laboratory, hence preliminary work was necessary to select the treatment to be used and standardize the methods. Six treatments used in cooking 10 oz of okra in each test were selected which consisted of three amounts of cooking water and two cooking times. The three amounts of water were designated low (669 g), medium (1,003 g), and high (1,338 g). The medium amount of water is approximately that which is used to cook 10 ounces of okra in Nigeria; higher and lower amounts were chosen for comparison. Cooking times selected were ten and fifteen minutes, as was done in the study of Sweeney et al. (30) on frozen broccoli.

Experimental Design and Analyses. A balanced incomplete block design type III as shown below was followed (36). One ten ounce package of frozen okra furnished all the data for one treatment in one replication as shown in table 2.

TABLE 2

EXPERIMENTAL DESIGN

EVALUATION PERIOD	TREATMENTS		
1.	2A	2B	2C
2.	2B	1A	1C
3.	1A	1B	2C
4.	1A	1C	2C
5.	2A	1A	2B
6.	1B	2A	1A
7.	1C	2A	2C
8.	1B	2B	2C
9.	1C	1B	2B
10.	1B	1C	2A

1,2 designate 10 and 15 minutes cooking time, respectively

A, B, C designate low (669 g), medium (1003 g), and high (1,338 g) amounts of cooking water, respectively

Analysis of variance was run on data for each measurement according to the following:

Source of variation		df
Treatments		5
Amount of water	2	
Cooking time	1	
Water X cooking time	2	
Blocks		9
Error		15
Total		29

Least significant differences (LSD) at 0.05 were determined when F-values were significant.

Cooking Procedure. Cut frozen okra pods[1] used in this study were purchased in 10 ounce packages from a retail grocery firm and stored in a freezer at -10°F until needed. Three packages of frozen okra were cooked during each evaluation period and before cooking four g of the frozen okra pods were removed and analyzed for ascorbic acid. Each 10 ounce package of frozen okra was cooked in the designated amount of water and for either ten or fifteen minutes according to the design of the experiment.

At the end of the cooking period, the okra pods were drained and weighed. Four g of the cooked okra pods were analyzed for ascorbic acid. The weight of the cooking water also was determined. The pH of the cooking water was tested and four ml were analyzed for ascorbic acid.

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[1] TV quick frozen brand from Arensberg IGA Store

Taste Panel Evaluation. Samples of the cooked okra were judged for color, flavor, texture and over-all acceptability by a panel of six judges. Panelists judged the same samples for color under the Macbeth Skylight. The scoring range used was 1 to 5 with 5 being the highest score. A sample of the score card used is shown in Fig. 1 (appendix).

Determination of Ascorbic Acid. A curve representing standardization of l-ascorbic acid (5 to 60 µg) in 4.0 ml of 4% trichloroacetic acid solution was prepared using the method of Schaffert and Kingsley (14). (See appendix for details of method). This curve was used to calculate the micrograms of l-ascorbic acid in the okra samples and the cooking water.

A modification of the method developed by Schaffert and Kingsley (14) was used to obtain values for total ascorbic acid content of the okra pods and cooking water. The changes made for the present study were: (1) an increase in the size of the okra sample from 2 g to 4 g, (2) a decrease in the volume of 0.5% oxalic acid from 98 ml to 96 ml, (3) an increase in volume of cooking water from 1 ml to 4 ml, (4) a decrease in volume of 4% trichloroacetic acid from 19 ml to 16 ml, (5) the substitution of a Klett-Summerson photoelectric colorimeter[1] with a filter with 500-570 nm wavelength for the Coleman spectrophotometer model 6 with a filter with 515 nm wavelength.

Four g of the frozen or cooked okra pods were blended in a Waring blender for 5 minutes with 96 ml of 0.5% oxalic acid. Four ml of the cooking water were also mixed with 16 ml of 4% trichloroacetic acid. To 20 ml of the solution in an Erlenmeyer flask, ½ teaspoon of

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[1] Klett-Summerson photoelectric colorimeter, Klett Manufacturing Company, New York, USA.

Norit was added and shaken vigorously for 1 minute. The solution was filtered through Whatman no. 42 filter paper.

Four milliliters of the filtrate were pipetted into cuvettes, one drop of 10% thiourea and 1 ml of 2,4-dinitrophenylhydrazine solution were added. The tubes were placed in a boiling water bath for exactly 10 minutes. At the end of that time, the tubes were placed in crushed ice. Five ml of 85% sulfuric acid were added slowly drop by drop and mixed by twirling. The tubes were allowed to stand for ten minutes. They were read in the colorimeter against a blank (prepared in exactly the same manner, except for the omission of the 2,4-dinitrophenylhydrazine until after the addition of 85% sulfuric acid). The blanks were set at 100% light transmission with 500-570 nm wavelength. Duplicate samples were prepared for each treatment.

Corresponding percent transmittance values for the colorimeter readings were obtained from the Klett-Summerson handbook. Concentration in micrograms of l-ascorbic acid contained in the okra pods or cooking water were determined from the standard curve. Milligrams of total ascorbic acid per 100 ml cooking water or 100 g okra pods were calculated using the formula shown below:

$$\text{mg/100 g sample} = \frac{\text{g ascorbic acid X total dilution X 100}}{\text{1000 X ml aliquot X ml sample}}$$

The concentration of total ascorbic acid in the frozen okra, cooked okra and cooking water were calculated using their respective weights. Total percent retention was calculated using the following formula:

$$\frac{\text{Ascorbic acid in cooked okra + Ascorbic acid in cooking water X 100}}{\text{Ascorbic acid in frozen okra}}$$

Color Difference Determination. Color difference of the cooked okra samples were determined using a Gardner Automatic Color Difference meter (model AC-2A Series 200).[1] The instrument was standardized using a ceramic tile with calculated values of Rd (darkness) 9.6, $\bar{a}$ (greeness) 2.2, b+ (yellowness) 20.8. A 15-g portion of the cooked okra pod was blended with 20 ml of distilled deionised water and packed into a sample cup. The cup was placed over the aperture of the instrument so that the center of the cup was directly over and covering the opening. An initial set of readings were taken, the sample cup was then rotated 90° clockwise direction and another set taken. The average of these readings were considered as the color value.

RESULTS AND DISCUSSION

Ascorbic Acid Content of Frozen Okra. A lot of variation in the ascorbic acid content of the frozen okra pods was found. Values ranged from 9.7 to 22.2 mg/100 g for individual packages of okra. The mean values for the five packages used for each treatment are given in table 3 and data for all replications of each treatment are given in the appendix (table 7).

Total Ascorbic Acid Retention. Mean total ascorbic acid retention is shown in table 4. Okra cooked in low amount of water for ten minutes had the highest retention (64.5%) and okra cooked in high amount of water for fifteen minutes had the least total retention (41.3%). For

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[1] Gardner Automatic Color Difference meter (model AC-2A, Series 200), Gardner Laboratory Inc., Bethesda 14, Maryland.

TABLE 3

Mean ascorbic acid content of frozen okra before cooking

TREATMENT	ASCORBIC ACID
	mg/100 g
1A	13.3
1B	14.3
1C	12.1
2A	15.1
2B	17.6
2C	10.9

1, 2 designate 10 and 15 minutes cooking time, respectively

A, B, C designate low, medium and high amounts of cooking water, respectively

TABLE 4

Mean ascorbic acid retention of frozen okra cooked by boiling

FACTOR	1A	1B	1C	2A	2B	2C	F-Value	LSD
% retention in cooked okra	45.2	35.6	22.0	35.5	25.5	11.4	60.71**	4.11
% recovery from cooking water	19.3	19.7	24.4	25.1	27.3	30.0	150.63**	2.8
Total % retention	64.5	55.3	46.4	60.6	52.8	41.3	60.57**	3.4

1, 2 designate 10 and 15 minutes cooking time, respectively

A, B, C designate low, medium and high amounts of cooking water, respectively

** $P < 0.01$

a given cooking time total ascorbic acid retention decreased with increasing amount of cooking water ($P < 0.01$). Also for the different amounts of cooking water, total ascorbic acid retention decreased with increasing cooking time. Results of this study indicate that amount of cooking water and length of cooking time affect total ascorbic acid retention in frozen okra.

Ascorbic Acid Retention in Cooked Okra. Mean ascorbic acid retention in cooked okra ranged from 45.2% in the low amount of cooking water and short cooking time to 11.3% in the high amount of cooking water and long cooking time (table 4). For each cooking time the ascorbic acid retention in the cooked okra decreased ($P < 0.01$) with increasing amount of water, indicating an increasing rate of leaching of the vitamin from the okra pod. Also for the three different amounts of cooking water ascorbic acid retention decreased ($P < 0.01$) with increasing cooking time.

Ascorbic Acid Recovery from Cooking Water. The high solubility of ascorbic acid caused considerable ascorbic acid to be transferred to the cooking water. Acid medium favors stabilization of ascorbic acid while an alkaline medium indicates destruction of the vitamin (25). The pH of the cooking water for all treatments was 10.5, therefore the alkaline pH probably brought about some ascorbic acid destruction.

The recovery of ascorbic acid in the cooking water increased ($P < 0.01$) with increasing cooking time and amount of cooking water. The highest amount (30.0%) was recovered from okra cooked in high amount of water for fifteen minutes (table 4). This is not in agreement

with the work of Noble (31) who reported that ascorbic acid recovered from the cooking water did not increase with increased cooking period.

In Nigeria, okra is usually consumed with the cooking water; therefore, ascorbic acid leached into the cooking water is not lost. In places where okra is drained before eating, ascorbic acid in the cooking water may constitute a significant loss

Palatability Factors. The average of the mean palatability scores are shown in table 5 and detailed data are given in the appendix (tables 8-11). In general, there was a day to day variation in the judges' scores for all palatability factors, however the mean scores showed differences due to treatments. The results of this study indicated that length of cooking time rather than the amount of cooking water affected the various palatability factors of frozen okra.

Color. Taste panel members did not find much difference between okra samples cooked for the same length of time but scores were significantly ($P < 0.01$) higher for okra samples cooked ten minutes than for those cooked fifteen minutes. The scores decreased with increasing amount of cooking water for each cooking time (table 5).

Flavor. Panelists could not find any significant difference in the flavor of okra samples cooked by the six treatments (table 5).

Texture. Okra samples cooked for ten minutes were given significantly ($P < 0.05$) higher scores than those cooked for fifteen minutes (table 5). Judges did not detect any significant differences in okra samples cooked in different amounts of water. Okra samples cooked for fifteen minutes had a mushy texture.

TABLE 5

Average of mean palatability scores for cooked okra

FACTORS	1A	1B	1C	2A	2B	2C	F-value	LSD
Color	3.8	3.5	3.4	2.6	2.4	2.3	11.50**	0.65
Flavor	3.7	3.5	3.3	3.3	3.3	3.3	0.66 ns	-
Texture	2.8	3.3	3.2	2.4	2.6	2.3	3.79*	0.69
Over-all acceptability	3.6	3.6	3.6	2.9	3.0	2.8	3.95*	0.60

1, 2 designate 10 and 15 minutes cooking time, respectively.

A, B, C designate low, medium and high amounts of cooking water, respectively.

** $P < 0.01$

* $P < 0.05$

ns not significant

Over-all acceptability. The judges' scores indicated a preference for okra pods cooked for ten minutes and scores were significantly ($P < 0.05$) higher than okra pods cooked for fifteen minutes (table 5). No differences were found in okra pods cooked in varying amounts of water for the same time.

Color Difference Values. Table 6 shows mean Rd, $\bar{a}$ and b values for the six treatments. Rd measures the darkness of the okra sample and the higher this value compared to the standard tile, the darker the samples. Okra samples cooked for ten minutes were darker ($P< 0.01$) than those cooked for fifteen minutes. There were no significant differences in okra samples cooked in varying amounts of water for the same time. A negative value of $\bar{a}$ indicates greeness. Okra pods cooked for ten minutes had significantly ($P < 0.01$) higher values of $\bar{a}$ and were greener than okra samples cooked for fifteen minutes. A positive value of b indicates yellowness. No significant differences were observed in the six treatments.

TABLE 6

Mean Rd, $\bar{a}$ and b values for color differences of cooked okra

FACTORS	1A	1B	1C	2A	2B	2C	F-value	LSD
Rd	29.3	30.1	29.1	28.4	28.0	28.3	97.4**	1.0
$\bar{a}$	11.3	11.5	11.5	9.4	9.5	9.3	82.07**	1.0
b	26.2	27.4	26.6	26.4	25.6	26.7	0.14 ns	-

1, 2 designate 10 and 15 minutes cooking time

A, B, C designate low, medium and high amount of cooking water

Values for standard tile: Rd = 9.6

$\bar{a}$ = -2.2

b = +20.8

** $P < 0.01$

ns non-significant

SUMMARY

The effect of variation in the amount of cooking water and length of cooking time on ascorbic acid retention and palatability of frozen okra was studied. A balanced incomplete block design with five replications of each treatment was used for collection and analysis of data. The taste panel evaluated cooked okra samples for color, flavor, texture and over-all acceptability.

Total ascorbic acid retention was significantly ($P < 0.01$) affected by variation in amount of cooking water and length of cooking time. Maximum ascorbic acid (64.5%) was retained when okra pods were boiled in the low amount of water for ten minutes and minimum ascorbic acid (41.3%) was retained when okra pods were boiled in the high amount of water for fifteen minutes. Ascorbic acid in the cooked okra decreased with increasing amount of cooking water and length of cooking time; but ascorbic acid increased in the cooking water with increasing amount of cooking water and length of cooking time.

Okra samples cooked for ten minutes had higher scores ($P < 0.01$) for color, texture and over-all acceptability than okra samples cooked for fifteen minutes. Treatments did not have any significant effect on the flavor of the okra samples. Palatability scores did not reveal any differences in okra samples cooked in varying amounts of water for the same time. Okra samples cooked for ten minutes were darker and greener ($P < 0.01$) as measured with a color difference meter than okra samples cooked for fifteen minutes.

It was concluded that okra cooked for 10 minutes in the low amount of water gave the most acceptable product with the greatest ascorbic acid retention.

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APPENDIX

METHOD OF STANDARDIZATION OF L-ASCORBIC ACID

One hundred mg of l-ascorbic acid were weighed accurately, placed in a 100 ml volumetric flask and diluted to volume with 4% trichloroacetic acid solution. Two ml of this solution were diluted to 100 ml with 4% trichloroacetic acid. One ml of the solution was equivalent to 20 µg of l-ascorbic acid.

Twenty-five ml of the standard l-ascorbic acid were shaken vigorously with ½ teaspoon of Nort for one minute. The solution was filtered through Whatman No. 42 filter paper. The following portions; 0.0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml of the filtrate were added to cuvettes and diluted each to 4 ml with 4% trichloroacetic acid which has also been shaken with Norit and filtered. One drop of thiourea and 1 ml of 2,4-dinitrophenylhydrazine solution were added and the tubes were placed in a boiling water bath for exactly ten minutes. At the end of this time the tubes were placed in crushed ice. Five ml of 85% sulfuric acid were added slowly drop by drop and mixed by twirling. The tubes were allowed to stand ten minutes and then read in a colorimeter against a blank (prepared in exactly the same manner, except for the omission of the 2,4-dinitrophenylhydrazine until after the addition of 85% sulfuric acid). The blank was set at 100% transmission with 500-570 nm wavelength.

Corresponding percent transmittance values for readings obtained from the colorimeter were obtained from the Klett-Summerson handbook. A graph of percent transmittance against micrograms of l-ascorbic acid in sample was plotted and this was used in the main experiment for calculation of concentration of ascorbic acid in okra samples.

TABLE 7

Total ascorbic acid values (mg/100 g) of frozen okra before cooking

TREATMENTS	REPLICATIONS 1	2	3	4	5	MEAN
1A	14.1	13.8	10.9	16.6	11.2	13.3
1B	12.2	14.8	13.1	14.9	16.9	14.3
1C	12.2	10.2	8.4	15.6	14.4	12.1
2A	14.4	15.7	22.2	8.8	14.4	15.1
2B	17.0	15.8	20.9	12.5	22.2	17.6
2C	9.4	11.3	11.6	12.5	9.7	10.9

1, 2 designate 10 and 15 minutes cooking time, respectively

A, B, C designate low, medium and high amounts of cooking water, respectively

SAMPLES	COLOR[1]	FLAVOR	TEXTURE	OVER-ALL ACCEPTABILITY
1				
2				
3				

COLOR		FLAVOR	
5	Bright green	5	Fresh, not bitter
4		4	
3	Green	3	Fairly fresh
2		2	
1	Greenish brown	1	Off flavor or bitter

TEXTURE		OVER-ALL ACCEPTABILITY	
5	Firm but tender	5	Very desirable
4		4	Slightly desirable
3	Fairly firm	3	Acceptable
2		2	Slightly acceptable
1	Soft and mushy	1	Undesirable

[1] Please score sample for color under Macbeth skylight.

Fig. 1 Score card for cooked okra

TABLE 8

Color scores for cooked okra

TREATMENTS	REPLICATIONS 1	2	3	4	5	MEAN
1A	3.0	3.2	4.0	4.6	4.2	3.8
1B	3.4	3.4	4.1	3.6	3.0	3.5
1C	3.2	3.0	3.3	3.8	4.0	3.4
2A	2.8	2.1	2.0	3.1	2.0	2.4
2B	3.6	2.0	2.3	3.5	1.8	2.6
2C	2.6	1.8	2.0	2.5	2.6	2.3

1, 2 designate 10 and 15 minutes cooking time, respectively

A, B, C designate low, medium and high amounts of cooking water, respectively

TABLE 9

Flavor scores for cooked okra

TREATMENTS	REPLICATIONS 1	2	3	4	5	MEAN
1A	3.4	3.8	3.6	4.3	3.6	3.7
1B	3.4	3.4	3.6	3.8	3.5	3.5
1C	3.0	3.2	3.8	3.6	4.3	3.5
2A	4.1	3.6	3.2	3.0	2.6	3.3
2B	4.0	4.0	2.8	3.3	2.6	3.3
2C	3.6	3.4	2.8	3.8	3.0	3.3

1, 2 designate 10 and 15 minutes cooking time, respectively

A, B, C designate low, medium and high amounts of cooking water, respectively

TABLE 10

Texture scores for cooked okra

TREATMENTS	REPLICATIONS 1	2	3	4	5	MEAN
1A	2.8	2.6	3.0	3.3	2.6	2.8
1B	3.6	3.6	3.6	3.5	2.6	3.3
1C	3.4	3.6	2.0	3.3	4.1	3.2
2A	3.3	2.5	2.4	1.6	2.3	2.4
2B	3.1	3.0	2.0	2.6	2.3	2.6
2C	2.6	2.2	1.8	2.6	2.3	2.3

1, 2 designate 10 and 15 minutes cooking time, respectively

A, B, C designate low, medium and high amounts of cooking water, respectively

TABLE 11

Over-all acceptability scores for cooked okra

TREATMENTS	REPLICATIONS 1	2	3	4	5	MEAN
1A	3.2	3.4	3.8	4.1	3.6	3.6
1B	3.4	4.0	4.1	3.8	3.1	3.6
1C	3.2	3.8	3.0	3.8	4.3	3.6
2A	3.9	3.1	3.0	2.5	2.6	2.9
2B	3.6	3.2	2.6	3.1	2.8	3.0
2C	3.3	2.4	2.2	3.5	2.8	2.8

1, 2 designate 10 and 15 minutes cooking time, respectively

A, B, C designate low, medium and high amounts of cooking water, respectively

EFFECT OF COOKING ON ASCORBIC ACID RETENTION AND PALATABILITY OF FROZEN OKRA

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Total ascorbic acid retention was significantly ($P < 0.01$) affected by variation in amount of cooking water and length of cooking time. Maximum ascorbic acid (64.5%) was retained when okra pods were boiled in low amount of water for ten minutes and minimum amount (41.3%) was retained when okra pods were boiled in high amount of water for fifteen minutes. Ascorbic acid in the cooked okra decreased with increasing amount of cooking water and length of cooking time, however, ascorbic acid increased in the cooking water with increasing amount of cooking water and length of cooking time.

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