RADIOCARBON DATES—(*Continued*)

Our No.	Sample	Age (years)
606	*Waterton*: Western Alberta, Canada, glacial forest bed in northwest quarter, Sec. 8, T. 2R. 29 at Waterton (*cf.* Waterton Lakes topographic map). Stratigraphy: Topsoil, 1′; gravel, 12′; lacustrine clay, 6″; gravel, 2′; sandy silt with invertebrate fossils, 2′; forest bed, 2′; dark-brown Keewatin drift, 9′. This sample was wood. L. R. Wilson, University of Massachusetts, says it is black-and-white spruce. The ecology is similar to that at edge of tundra now. Submitted by Leland Horberg, University of Chicago.	3261 ± 250
607	*Waterton Peat*: Same as 606, except peat instead of spruce wood.	3327 ± 320
629	*Seeds*: Ancient Manchurian lotus seeds, still fertile. Collected by Ichiro Ohga in the Pulantien Basin of South Manchuria in a peat layer presumably of Pleistocene age; uplift and erosion had exposed the layer on the walls of the Pulantien River valley. Ohga germinated several hundred seeds, either filing the thick outer shell or soaking seeds in concentrated sulfuric acid for 1–5 hr. Genus *Nelumbo*, similar to the Indian lotus *N. nucifera*. Submitted by R. W. Chaney, University of California, Berkeley.	1040 ± 210

RADIOCARBON DATES—(*Continued*)

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Technical Papers

Some Properties of an Ascorbic Acid Oxidation Inhibitor in Vegetables[1]

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During the course of previous work in this laboratory, it was observed that the ascorbic acid content of frozen spinach decreased more rapidly during freezer storage than did the ascorbic acid content of frozen snap beans (*1*). Variations in the processing method (scalding, cooling, or type of package) did not appreciably affect this rate of loss. However, several workers (*2–8*) have shown that some vegetables have an inhibiting effect on the oxidation of ascorbic acid. Spinach has some inhibitory effect (*3, 7*) but its action is very slight as compared to the effect of vegetables such as snap beans, cauliflower, and cabbage. The purpose of this study was to investigate some of the chemical and physical properties of the inhibitor of ascorbic acid autoxidation in frozen snap beans.

In order to eliminate as many variables as possible, and also to accelerate the rate of autoxidation, a method was devised in which the ascorbic acid solutions were maintained under constant oxygen pressure and at a constant temperature. Gas bottles holding the samples were kept in a water bath at 35° C. Oxygen was supplied to the samples from an oxygen tank through a glass manifold with 12 side outlets to which the gas bottles were attached. In order to facilitate keeping the pressure constant from experiment to experiment, a mercury manometer (15-in. CODC Standard Cleanout Manometer) was connected to the other end of the manifold.

Solutions of snap-bean extract to be tested for their effect on ascorbic acid autoxidation were pipetted into duplicate gas bottles. Fifty ml of ascorbic acid solution, containing 2 mg ascorbic acid, was added to each gas bottle, the mixture shaken, and a 5-ml aliquot removed and combined with an equal amount of 2% *m*-phosphoric acid solution preparatory to reduced ascorbic acid determinations.

When the 12 gas bottles were thus set up, they were placed in the water bath and attached to the manifold. In order to saturate the solutions with oxygen, the pure gas was bubbled through them for 6 min at a rate of 8 liters/min, as determined by an oxygen flow meter (Linde Oxygen Therapy Flow Meter Type L-14). The system was then closed, and, on the basis of the barometric reading, the oxygen pressure was increased until equivalent to 69.86 cm Hg and was

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TABLE 1

PROPERTIES OF AN ASCORBIC ACID OXIDATION INHIBITOR IN FROZEN SNAP BEANS

Experiment	Ascorbic acid retention (%)
I. *Concentration of inhibitor*	
Control*	38
Control + 5 ml snap-bean extract	86
" + 5 " " " " diluted 10:100	90
" + 5 " " " " " 5:100	86
" + 5 " " " " " 2:100	63
" + 5 " " " " " 1:100	60
II. *Stability of inhibitor*	
Control*	33
Control + 5 ml snap-bean extract	93
" + 5 " " " " stored at 0° C, 1 week	93
" + 5 " " " " " " −20° C, 1 "	94
" + 5 " " " " " " −20° C, 2 weeks	93
" + 5 " " " " " " −20° C, 3 "	94
III. *Volatility of inhibitor*	
A. Control*	33
Control + 5 ml snap-bean extract (pH 6.4)	86
" + 5 " " " distillation residue (pH 6.1)	75
" + 5 " " " distillate†	83
1. (pH 7.5)	88
2. (pH 8.1)	51
3. (pH 8.5)	
B. Control*	
Control + 5 ml snap-bean extract (pH 6.4)	43
" + 5 " " " (pH 8.2)‡	94
" + 5 " " " distillation residue (pH 7.7)	48
" + 5 " " " distillate	43
1. (pH 9.2)	88
2. (pH 9.0)	95
3. (pH 5.9)	78

* 50 ml, 2 mg, ascorbic acid.
† Distillate was collected in three 30-ml fractions.
‡ Snap-bean extract brought to pH 8.2 with .2 *M* bisodium phosphate.

maintained at this level. This pressure had been previously found to give the desired rate of oxidation in the control samples.

Following a total oxygenation period of 45 min, the system was opened, each sample mixed, and a 5-ml aliquot removed from each bottle and combined with 5 ml of 2% *m*-phosphoric acid. The system was then put under oxygen pressure for another 45-min period, including the 6-min flushing time, after which the final aliquots of the 12 samples were removed and ascorbic acid determinations again made. The reduced ascorbic acid was measured by the method of Loeffler and Ponting (*9*).

In order to minimize the possibility of mineral contamination, glass-redistilled water was used for preparing all solutions. The snap-bean extract was prepared by mixing frozen snap beans with water, in a ratio of 1:3, for 10 min in a Waring Blendor. The blended material was then filtered, using coarse (No. 226 Reeve Angel) filter paper. The controls were pure solutions of ascorbic acid. Percentages reported as retained (Table 1) are those found after the second period of autoxidation under oxygen pressure, or after 1½ hr oxygenation. The pH of the test solutions ranged from 6.0 to 6.4. The protection exhibited by 5 ml of freshly prepared frozen snap-bean extract was used as the standard of comparison in evaluating the protection exhibited by fractions of the extract. The average amount retained by this standard was 88% of the amount initially present as compared with 35% retained by the control.

Typical data are presented in Table 1. Dilutions of the snap-bean extract (Table 1, Expt I) were made by diluting 10, 5, 2, and 1 ml of the original extract to a total volume of 100 ml with redistilled water. Five-ml aliquots of the respective dilutions were compared with 5 ml of undiluted extract in their ability to inhibit ascorbic acid autoxidation. It was found that the snap-bean extract diluted to 5% of the original concentration was as effective as the undiluted standard. More dilute extracts gave less protection to the ascorbic acid.

To test the stability of the inhibitor (Table 1, Expt II), samples of snap-bean extract were kept at room (20°–27° C), refrigerated (0° C), and freezer storage (−22° C) temperatures for periods of 1, 2, and 3 weeks. Those samples kept in freezer storage were as effective as the fresh snap-bean extract in inhibiting ascorbic acid autoxidation, but samples stored at temperatures above that of freezer storage varied in this respect.

Takahashi (*8*) reported that steam distillates of garden radishes, onions, and mustard were effective in inhibiting copper-catalyzed oxidation of ascorbic acid. To test the volatility of the autoxidation-inhibiting factor in the snap-bean extract (Table 1, Expt III),

a 100-ml sample of extract was distilled at atmospheric pressure. The distillate was collected in three 30-ml fractions, and the residue brought back to the original volume with redistilled water. Some of the distillates inhibited autoxidation, but, since it was not always possible to detect the inhibitory factor(s) in the distillates, it was felt that volatility of the factor(s) must depend on some unknown condition. Since the pH of the distillates was in the alkaline range (7.5–8.5), additional distillations of snap-bean extract were carried out, using alkaline snap-bean extracts that had been prepared by blending snap beans with solutions of dibasic sodium phosphate instead of water. It was found that as the pH of the snap-bean extract increased, the volatility and stability, as shown by storage studies, of the inhibitory factor(s) apparently increased, though at the high pH values (above pH 8), the extract itself did not show its usual strong inhibition of ascorbic acid autoxidation.

These results strengthen the existing evidence that vegetables contain an inhibitor(s) of ascorbic acid oxidation. Some of the properties of the inhibitor(s) in snap beans are also indicated. Further work is in progress to define more definitely the nature of the inhibitor(s).

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Prolongation of Clotting Time in Dormant Estivating Mammals[1]

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During an investigation of the seasonal changes in blood volumes of ground squirrels of two species (*Citellus columbianus* and *C. parryii ablusis*), it was discovered that a prolongation of the clotting time of the blood normally occurs in these animals when they are in a dormant state.

The blood volumes were determined by Cartland and Koch's (*1*) micromodification of the Keith-Rowntree-Geraghty (*2*) plasma dye dilution method. Because of the relatively small size of the animals and the lack of large superficial blood vessels, sufficient blood could not be taken readily from the ears or tails as is ordinarily done in other experimental animals. Hence heart punctures, using a #23 hypodermic needle, were resorted to. By this method adequate amounts of blood could be secured, and blood volumes satisfactorily determined while the animals were still in an active state. When the animals became dormant either during estivation or hibernation, this method proved unsatisfactory. The primary disadvantage was that the animals usually died shortly after the blood samples were taken. It was revealed upon post-mortem examinations that death was caused by internal hemorrhages, the pericardial cavity being completely filled with blood. When a finer-gauge needle (#26) was substituted, blood samples could be taken without the accompanying pericarditis and resultant death.

It was at first assumed that the internal hemorrhages were due to faulty technique and that death was caused by a mechanical injury to the heart or adjacent blood vessels. However, when samples of the blood of the dormant animals were exposed to the air, they did not clot normally even after an exposure of several days. Hence death was not due to faulty technique but to a hemophilic condition of the blood.

Comparative studies on the clotting time of the blood of both active and dormant ground squirrels were then made. Three regular techniques, capillary tube, Lee and White's, and Howell's, were used. These methods of determining clotting time differ from each other essentially in the diameter of the tubes. The capillary tubes have the narrowest lumen, those used in Howell's technique the widest. As can be seen from Table 1, all produced similar results.

TABLE 1

No. animals tested	No. blood samples	Clotting time (min and sec) Minimum	Maximum	Av
		Capillary tube method		
16 active	30	0′ 33″	12′ 0″	4′ 34″
11 dormant	15	10′ 51″	51′ 45″	20′ 6″
		Lee and White's method		
10 active	10	3′ 41″	29′ 30″	14′ 36″
7 dormant	9	16′ 43″	39′ 0″	26′ 25″
		Howell's method		
5 active	5	4′ 30″	13′ 20″	8′ 12″
5 dormant	5	35′ 23″	68′ 30″	48′ 43″

The term active refers to all animals that were not in a truly dormant state and includes those that were drowsy. Some squirrels remained in the mid-state of drowsiness, neither completely active or completely dormant. The longest periods on clotting times for active squirrels were obtained from these drowsy animals.

In the really active ground squirrels a complete clot occurred and determined the end point of clot formation, whereas in the dormant animals a complete clot never did form. Only a partial clot formed even if the blood was exposed for several days.

The prolongation of blood-clotting time may be considered a remarkable adaptation of estivating

[1] Acknowledgment is made to Army Air Force contract A.F. 33 (038) 18509 for aid and assistance in conducting this investigation.