USE OF SALTS OF 6-ACYL ESTERS OF L-ASCORBIC AND D-ISOASCORBIC ACIDS IN BREADMAKING[1,2]

R. C. HOSENEY, P. A. SEIB, and C. W. DEYOE, Kansas State University, Manhattan, KS 66506

ABSTRACT

Cereal Chem. 54(5) 1062-1069

Salts of 6-acyl esters of L-ascorbic and D-isoascorbic acids have been found to be particularly useful in the production of yeast-leavened baked products. They give a pronounced dough-conditioning effect; thus, the dough makes up easier and can tolerate more water than if the compounds are not used. The compounds are effective antistaling agents. In doughs containing a normal level of shortening, they are as effective as monoglycerides. However, when the shortening in the dough is replaced by the esters, they are significantly more effective than monoglycerides. Bread baked from dough containing 0.5% sodium 6-palmitoyl-L-ascorbate was found by high-pressure liquid chromatography to retain 81% of the additive. Because the 6-acyl esters of L-ascorbic acid are equivalent on a molar basis to L-ascorbic acid in vitamin C activity, bread can now be fortified with vitamin C added at the dough stage.

Surfactants are used in breadmaking to strengthen dough and to soften bread crumb. In 1958, Ofelt and his colleagues (1) found that L-ascorbyl 6-palmitate (AP) and D-isoascorbyl 6-palmitate (IAP) softened bread crumb as effectively as did monoglycerides (Fig. 1). However, they reported that, at levels of 0.4% based on flour, those compounds (AP and IAP) also darkened the crumb of bread. They did not comment on dough-conditioning effects of the ascorbic acid esters.

"Ascorbyl palmitate" is on the list of chemical preservatives in Section 121.101 of the Federal Code; no limit has been set (2) on its level of usage in foods. Ascorbyl palmitate[3] is currently being produced commercially for use as an antioxidant in high-fat foods (2).

We report here our results on the use and stability of salts (SPA and SPIA) of 6-acyl esters of ascorbic acid in breadmaking.

MATERIALS AND METHODS

Breadmaking

Pup loaves (100 g of flour) were baked from a regional standard blend of hard winter wheat flours using the straight-dough procedure (4). Salts of the esters of ascorbic acid were added directly as dry solids to the mixing bowl, whereas the free-acid forms of the surfactants were ground (Stein Laboratory Mill, Fred Stein Laboratories, Inc., Atchison, Kans.) intimately with flour for 30 sec prior to dough mixing. Dough strengthening was determined by the handling characteristics of the dough during punching and molding, and by the loaf-volume effect on soy-fortified breads. Antifirming action of an additive was determined on loaves of optimum volume stored in plastic bags at 25°C. A 1.75-in. cube of bread was cut from the center of a loaf, and a gelometer was used to

[1]Contribution No. 912-J. Kansas Agricultural Experiment Station, Manhattan, KS 66506.

[2]Presented at the 60th Annual Meeting, Kansas City, MO, Oct. 1975.

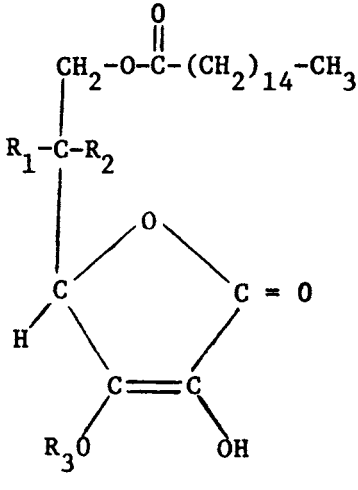
[3]Commercial ascorbyl palmitate is predominantly L-ascorbyl 6-palmitate as determined by thin-layer chromatography (3).

measure the compressibility of the crumb. Compressibility was measured along the three main axes of the cube and the three readings were averaged.

Acyl Esters of L-Ascorbic and D-Isoascorbic Acids and their Salts

L-Ascorbyl 2-palmitate was prepared by a modification (3) of a procedure (5) in which 5,6-*O*-isopropylidene-L-ascorbic acid (6) is reacted at room temperature with palmitoyl chloride in pyridine. After isolation of 5,6-*O*-isopropylidene-2-*O*-palmitoyl-L-ascorbic acid (mp 96°−104°C), the latter compound was deacetonated in 0.1 *M* methanolic hydrogen chloride to give 2-*O*-palmitoyl-L-ascorbic acid, mp 105°C. The same procedure was used to prepare 2-*O*-oleoyl-L-ascorbic acid (semi-solid).

6-Acyl esters of L-ascorbic and D-isoascorbic acids were prepared in yields of up to 85% using a modification (3) of the procedure of Swern *et al.* (7). The 6-



AP: $R_1 = R_3 = H$; $R_2 = OH$

SPA: $R_1 = H$; $R_2 = OH$; $R_3 = Na$

IAP: $R_2 = R_3 = H$; $R_1 = OH$

SPIA: $R_2 = H$; $R_1 = OH$; $R_3 = Na$

Fig. 1. Structures of 6-acyl esters of L-ascorbic and D-isoascorbic acid and their salts.

esters were purified by recrystallization to constant melting point.

The structures assigned to the 2- and 6-esters of L-ascorbic and D-isoascorbic acids were verified by ^{13}C-nuclear magnetic resonance spectroscopy (3). The melting points of the esters of L-ascorbic acid were: 6-valerate, 92°–93° C; 6-caprate, 98°–102° C; 6-laurate, 106°–107° C; 6-myristate, 111°–112° C; 6-palmitate, 116°–117° C; and 6-stearate, 118° C. 6-*O*-Sebacoyl-L-ascorbic acid was an amorphous solid. The melting point of D-isoascorbic acid 6-palmitate was 89°–90° C; and of 6-myristate, 84°–85° C.

Salts of the 6-acyl esters of the ascorbic acids were prepared by two methods. The following is an example of the first method used to produce the sodium, potassium, or magnesium salts. To an acetone (60 ml) solution of 6-*O*-palmitoyl-L-ascorbic acid (5 g, 12 mmol) was added slowly while stirring 10–12 mmol of sodium methoxide in methanol (1*M*). The mixture was evaporated to dryness under reduced pressure below 50° C. The residue was further dried at 60° C over anhydrous calcium sulfate, then ground to a fine powder; yield, 4.9 g (93%).

The second method was used to produce calcium 6-*O*-acyl-L-ascorbates. 6-*O*-Palmitoyl-L-ascorbic acid (5 g, 12 mmol) and one equivalent of calcium propionate were dissolved in absolute ethanol (50 ml). Ethanol was removed by evaporation under reduced pressure, and the residue was subjected to reduced pressure over sodium hydroxide until the odor of propionic acid disappeared. The sodium or magnesium salts can also be made by this method using sodium propionate and magnesium acetate.

Recovery of 6-*O*-Palmitoyl-L-Ascorbic Acid from Bread

High-pressure liquid chromatography was used to determine the amount of sodium 6-*O*-palmitoyl-L-ascorbate (SPA) that survived breadmaking

TABLE I
Dependence of Shortening-Sparing and Dough-Conditioning Effects on the Manner in which 6-*O*-Palmitoyl-L-Ascorbic Acid (AP) is Added to Dough

Amount[a] of AP g	Manner of Addition	Absorption g	Loaf Volume cc	Dough Conditioning[b]
None	...	65	905	−2
None (shortening control)	...	63	990	0
0.5	Directly to mixer	66	925[c]	+2
0.5	Ethanol solution, 2 ml	66	900[d]	+1
0.5	Ground in flour	66	985	+3
0.5 (Sodium salt)[e]	SPA added directly to mixer	66	990	+3

[a]Quantity of all ingredients given in amount per 100 g of flour. All doughs contained 20 ppm of potassium bromate and no shortening except the control loaf (3 g of shortening).

[b]Doughs with plus values felt drier and stronger at the molder; absorption was also increased.

[c]The crust surface of the loaf contained numerous black specks.

[d]A loaf containing 2.0 ml of ethanol and 0.5% sodium 6-*O*-palmitoyl-L-ascorbate gave optimum loaf volume (990 cc).

[e]Sodium 6-*O*-palmitoyl-L-ascorbate.

conditions. Details of the procedure will be published (8). Bread baked from dough containing 0.5 g of SPA/100 g flour was freeze-dried, ground, and the ground crumb digested with α-amylase. An aliquot was injected into a high-pressure liquid chromatograph equipped with a 2.3 × 1000-mm stainless-steel column packed with Micro-Bondapak-C_{18} on Porasil (Waters Associates, Inc., Milford, Mass.). The reverse-phase column was developed using a mixture of methanol-water, and the column eluent was monitored by its uv absorbance (254 nm). A standard curve was used to determine that 81% of the amount of SPA added to the dough was recovered from the crumb of duplicate loaves.

RESULTS AND DISCUSSION

Salts of 6-*O*-acyl-L-ascorbic and D-isoascorbic acids were prepared to improve the dispersibility of the esters in bread dough. Data in Table I show that 6-*O*-palmitoyl-L-ascorbic acid (AP) was not fully effective in replacing shortening or in strengthening dough when AP was added directly to the mixer

TABLE II
Effect of Sodium 6-*O*-Palmitoyl-L-Ascorbate (SPA) on Dough Conditioning using a No-Shortening Bread Formula

Amount[a] of SPA g	Absorption g	Loaf Volume cc	Dough Conditioning
None	65	905	−2
None (shortening control)	63	990	0
0.25	65	885	−2
0.38	65	945	+1
0.50	66	990	+3
0.75[b]	66	995	+2
1.0[b]	67	975	+1

[a]Footnote a, Table I.
[b]Potassium bromate at 30 ppm.

TABLE III
Effect of Sodium 6-*O*-Myristoyl-L-Ascorbate (SMA) on Bread Containing Soy Flour

Amount of SMA g	Shortening g	Soy Flour[a] g	Absorption g	Loaf Volume cc
0	3	0	63.5	980
0	0	8	72.5	662
0.5	0	8	74.5	882
0	3	8	72.5	888
0.5	3	8	74.5	995

[a]Ardex 550, sold by Archer Daniels Midland Company, Decatur, Ill.

bowl in either dry powder form or in ethanolic solution. The numerous black specks on the crusts of bread so prepared are due to charring of the nondispersed fraction of AP. On the other hand, sodium 6-*O*-palmitoyl-L-ascorbate (SPA) was fully functional and has normal crust color. Full functionality was also imparted to AP by grinding it intimately with the flour (Table I).

Dough conditioning by sodium 6-*O*-palmitoyl-L-ascorbate was first detected between 0.25 and 0.38%; the optimum level was approximately 0.5% (Table II). Another expression of the dough-conditioning properties of the 6-acyl esters of ascorbic acid: the ability to carry soy protein in bread is shown in Table III.

In contrast with the 6-acyl esters of L-ascorbic acid, the 2-acyl esters showed no desirable dough-conditioning effects (Table IV). In fact, the 2-esters were detrimental to loaf volume, because they "tightened" dough structure.

The functional 6-acyl esters of L-ascorbic acid have a structure required for effectiveness: a minimum chain length of 12-carbons (lauric acid) in the acyl group (Table IV). Dough-conditioning and shortening-sparing effects were maximum for the lauryl acyl group, but the effect suddenly disappeared when the acyl group contained 10 or fewer carbons. Table IV shows that the sodium salts of L-ascorbyl 6-valerate, 6-caprate, and 6-sebacate were not only ineffective but detrimental to loaf volume in no-shortening breads.

An ester of D-isoascorbic acid (IAP) behaved similar to those of L-ascorbic acid in breadmaking (Table IV), probably because AP and IAP are closely related in structure (C-5 epimers). A greater change in functionality might be expected for esters of D-ascorbic or L-isoascorbic acids, in which the side chain in the lactone ring is positioned to the opposite side of the lactone ring. (The synthesis and baking properties of esters of D-ascorbic or L-isoascorbic acids

TABLE IV
Effect of Structure of L-Ascorbic Acid Esters on Dough Conditioning in a No-Shortening Bread Formula[a]

Additive	Absorption g	Volume cc	Dough Conditioning
None	65	905	−2
None (shortening control)	63	998	0
6-valerate[b]	65	740	−2
6-sebacate	64	720	−2
6-caprate	65	740	−2
6-laurate	66	1015	+4
6-myristate	66	995	+3
6-palmitate	66	997	+3
6-stearate	66	968	+2
2-palmitate[c]	65	740	−2
2-oleate[c]	66	793	−2
6-palmitate (iso)[d]	66	975	+3

[a]Footnote a, Table I. All additives tested at 0.5 g/100 g flour.
[b]All C-6 esters tested as sodium salts.
[c]Free acid form of ester ground with flour before dough mixing. Potassium bromate 30 ppm.
[d]Sodium 6-*O*-palmitoyl-D-isoascorbate.

will be examined in the near future.)

The sodium salt of the 6-palmitoyl ester of L-ascorbic acid was equal to monoglyceride for crumb softening when shortening was present in bread (Table V). But when the shortening was left out of the bread formula, the ascorbate ester was a better crumb softener (Table V). It is doubtful that all crumb softeners are more effective without shortening. For example, in our laboratory we found sodium stearoyl 2-lactylate did not soften bread without shortening more effectively than it did with shortening.

Optimum crumb softening by a 6-fatty acid ester of L-ascorbic acid apparently occurs when the C-6 acyl group is a palmitoyl moiety (Table VI). On the other hand, we obtained optimum dough conditioning with the 6-laurate ester (Table IV). The 6-myristate ester therefore probably represents a compromise structure for both dough conditioning and crumb softening. The optimum crumb-softening level of sodium 6-*O*-myristoyl-L-ascorbate was 0.75% (Table VII). Sodium 6-*O*-myristoyl D-isoascorbate (at all levels) gave the same magnitude of crumb softening as did the L-ascorbate isomer.

TABLE V
Crumb-Softening by Sodium 6-*O*-Palmitoyl-L-Ascorbate

Additive[a]	Shortening g	Gelometer Readings[b] g		
		Day 1	Day 3	Day 5
None	3.0	108	179	275
Monoglyceride[c]	3.0	99	147	251
6-palmitate	3.0	86	162	248
6-palmitate	0	78	132	206

[a]All additives used at 0.5 g/100 g of flour.
[b]A reading in the table is the average determined for duplicate loaves.
[c]"Myverol," Distillation Products Industries, Rochester, N.Y.

TABLE VI
Crumb Softening by Salts of Esters of L-Ascorbic Acid

Additive[a]	Shortening g	Gelometer Readings[b] g		
		Day 1	Day 3	Day 5
None	3	99	173	281
Monoglyceride[c]	3	103	164	233
6-laurate	0	93	159	212
6-myristate	0	97	148	202
6-palmitate	0	79	121	175
6-stearate	0	90	145	205

[a]Tested at a level of 0.5 g/100 g of flour.
[b]See footnote b, Table V.
[c]See footnote c, Table V.

Data in Tables V and VI show that bread with 0.5% sodium 6-*O*-palmitoyl-L-ascorbate can be stored approximately 1.5 days longer than bread softened with 0.5% monoglycerides and 3% shortening. It should be mentioned that no oxidant effect (such as that obtained from L-ascorbic acid) was observed when the salts of 6-acyl esters of L-ascorbic or D-isoascorbic acids were added to a dough.

Surprisingly, 81% of sodium 6-*O*-palmitoyl-L-ascorbate added to dough at a level of 0.5% survived breadmaking conditions. The bread at 37% moisture was found to contain 0.25% of SPA. Previously we showed (9) that only ~ 25% of L-ascorbic acid survived when added to dough at a practically equivalent molar level (0.21% based on flour).

Although the data are not presented here, animal-feeding experiments using young guinea pigs verified the vitamin C potency of bread baked from dough containing the sodium salts of L-ascorbyl 6-laurate, 6-palmitate, or 6-stearate. It has been well established (10) that fatty acid esters of L-ascorbic acid are physiologically equivalent to L-ascorbic acid as a source of vitamin C. Two slices of bread containing 0.25% of SPA would provide an adult with more than his daily recommended dietary allowance of 45 mg of L-ascorbic acid. Perhaps vitamin C fortified bread or rolls could be used in school breakfast or lunch programs or in countries where bread is a staple food.

In summary, salts of 6-fatty acid esters of L-ascorbic and D-isoascorbic acids are dispersed easily in bread doughs. They are effective dough conditioners when the fatty acid chain length is 12–18 carbons. They may be used to replace shortening in bread and, most importantly, they are the most effective crumb softeners reported to date. In addition, they provide a way to fortify bread with vitamin C at the dough stage.

Sodium 6-*O*-palmitoyl-L-ascorbate (SPA) very effectively stabilizes aqueous dispersions of carotenoid pigments (H. Klaui, Wiss. Ver. Duetchen Gesellshalt for Ernaehrung, 9(1963) 390). Klaui reported a convenient method of producing SPA wherein ascorbyl palmitate is dissolved in chloroform, and the resulting solution added to water containing an equivalent amount of sodium bicarbonate. The mixture is evaporated to dryness under reduced pressure to give solid SPA.

TABLE VII
Crumb Softening at Various Levels of Sodium 6-*O*-Myristoyl-L-Ascorbate

Level[a] g	Shortening g	Gelometer Readings[b] g		
		Day 1	Day 3	Day 5
0	3	124	184	270
0.5[c]	3	111	166	246
0.25	0	115	190	240
0.50	0	97	148	202
0.75	0	88	123	175
1.00	0	82	124	206

[a]All levels given in g/100 g of flour.
[b]Footnote b, Table V.
[c]Monoglyceride, see footnote c, Table V.

Literature Cited

1. OFELT, C. W., MEHLTRETTER, C. L., MacMASTERS, M. M., OTEY, F. H., and SENTI, F. Effect on crumb firmness. II. Action of additives in relation to their chemical structure. Cereal Chem. 35: 142 (1958).
2. CORT, W. M. Antioxidant activity of tocopherols, ascorbyl palmitate, and ascorbic acid and their mode of action. J. Amer. Oil Chem. Soc. 51: 321 (1974).
3. COUSINS, R. C., SEIB, P. A., and HOSENEY, R. C. Synthesis of fatty acid esters of L-ascorbic acid. (Abstr. No. 94) Cereal Foods World 20: 456 (1975).
4. FINNEY, K. F., and BARMORE, M. A. Varietal responses to certain baking ingredients essential in evaluating the protein quality of hard winter wheats. Cereal Chem. 22: 225 (1945).
5. TANAKA, H., and YAMAMOTO, R. Pharmaceutical studies on ascorbic acid derivatives. I. Synthesis of esters of ascorbic acid and their physiochemical properties. Yakugaku Zasshi 86(5): 376 (1966).
6. JACKSON, K. G. A., and JONES, J. K. N. Synthesis of 3-hexuloses. Can. J. Chem. 47: 2498 (1969).
7. SWERN, D., STIRTON, A. J., TURER, J., and WELLS, P. A. Fatty acids monoesters of L-ascorbic acid and D-isoascorbic acid. Oil Soap 20: 224 (1943).
8. MAURO, D. J., HOSENEY, R. C., SEIB, P. A., and WETZEL, D. L. The extraction and determination of L-ascorbyl 6-palmitate from breads by use of high-pressure liquid chromatography. (Abstr. No. 110) Cereal Foods World 20: 458 (1975).
9. QUADRI, S. F., LIANG, Y. T., SEIB, P. A., DEYOE, C. W., and HOSENEY, R. C. Stability of L-ascorbate 2-sulfate and L-ascorbic acid in wheat foods and milk. J. Food Sci. 40: 837 (1975).
10. INAGAKI, C., ARAKAWA, N., SUZUKI, N., SAGO, Y., and NOGAMI, K. Metabolism of fatty acid esters of L-ascorbic acid. III. Biochemical activity of various L-ascorbic acid derivatives. Bitamin 37: 152 (1968).

[Received February 19, 1976. Accepted March 19, 1976]