ice; 19.5 g. (0.280 mole) of c. p. sodium nitrite, dissolved in 200 cc. of water, was slowly added and the solution stirred constantly. The solution was then allowed to come slowly to room temperature. After standing overnight, the solution was distilled with steam. Nitrogen was evolved and a cream-colored crystalline material appeared in the distillate. When no more crystals came over, the distillate was filtered and the crystals carefully dried on a porous plate; yield 15.2 g. (96%). The crude material melted at 87–87.5°, and after two recrystallizations from alcohol it melted at 88.5–89.0° (corr.). Reinhard obtained a melting point of 89° for "x-chlororesorcinol." A mixed melting point determination, made on a portion of the material mixed with a sample of chlororesorcinol made by Reinhard's method, gave a value of 88.5–89.0°.

The bromine derivative, prepared according to Reinhard's method, melted at 103.5–104.0°, after one recrystallization from alcohol. Reinhard obtained a melting point of 105° for this compound.

The dibenzoyl derivative melted at 97° as compared with the value of 98° reported by Reinhard.

Analysis of the purified chlororesorcinol, as obtained by the above method, gave the following results: calcd., Cl, 24.53; found, Cl, 24.63. The method of Lemp and Broderson[4] was used in this analysis.

Conclusion

As a result of a synthetic method, in which chlororesorcinol was prepared from compounds of known structure, it has been shown that "x-chlororesorcinol" has the structure 1-chloro-2,4-dihydroxybenzene.

[4] Lemp and Broderson, THIS JOURNAL, **39**, 2069 (1917).

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Lipases of Wheat. I

BY B. SULLIVAN AND M. ALLISON HOWE

No studies of any of the lipases of wheat or its products have been reported in the literature. Furthermore, no references have been made concerning the importance of lipase in relation to the quality and storage of wheat products. Therefore considerable preliminary work is necessary in establishing the following fundamental facts (a) the most reliable method for the measurement of the lipase activity of wheat; (b) the best methods of extraction to increase the concentration of the enzyme from any given wheat product; (c) the distribution of the enzyme in the different milling separations of wheat; (d) the optimum conditions of time, temperature and *P*H for wheat lipase activity as well as the specificity of different buffer mixtures, the effect of various activators and inhibitors and the action of the enzyme on various substrates. These preliminary experiments described here are intended to establish some of the variables influencing the activity of the lipases of wheat and its products.

Experimental

An arbitrary method of measuring the lipase activity was first developed based on a number of different methods previously reported in the literature. Ground wheat was used as the source of the enzyme. The method used was as follows: 0.5 g. of finely ground wheat was incubated for twenty-four hours at 37.9° in a 250-cc. Erlenmeyer flask with 10 cc. of water, 2 cc. of toluene, the substrate (1 cc. of liquid or 1 g. of solid fat) and 10 cc. of buffer solution. At the end of the incubation period, 100 cc. of 3:1 acetone–ether mixture was added to the sample and also the blank which had been boiled previous to incubation; 0.1 N sodium hydroxide was used for the titration with 1% phenolphthalein as an indicator. The results obtained from various substrates are given in Table I. The action of the

TABLE I

ACTION OF LIPASE IN WHEAT PRODUCTS ON VARIOUS SUBSTRATES EXPRESSED IN CC. OF N/10 SODIUM HYDROXIDE

Clear																			
Triacetin		Tri-propionin		Tributyrin		Tri-*n*-valerin		Tricaproin		Tri-caprylin		Trimyristin		Tri-palmitin		Tristearin			
*P*H	Cc.	*P*H	Cc.	*P*H	Cc.	*P*H	Cc.	*P*H	Cc.	*P*H	Cc.	*P*H	Cc.	*P*H	Cc.	*P*H	Cc.		
7.6	31.4	7.4	13.7	7.1	1.8	7.6	0.4	7.3	0.3	7.6	0.2	7.6	0.2	7.6	0.5	7.6	0.2		
7.4	28.8	6.7	13.1	6.7	2.0	7.4	0.6	6.7	0.5	7.4	0.4	7.4	0.4	7.4	0.9	7.4	0.3		
5.2	14.7	4.7	8.4	4.7	2.6	5.2	1.6	4.7	1.4	5.2	0.7	5.2	1.4	5.2	1.6	5.2	1.2		

Clear						Wheat			
*P*H	Ethyl propionate	Ethyl butyrate	Ethyl stearate	Ethyl oleate	Olive oil	*P*H	Triacetin	Tri-stearin	Ethyl acetate
7.6	1.1	0.2	0.6	0.7	0.4	7.5	27.8	0.4	8.4
7.4	1.7	0.5	0.4	1.6	0.6	6.3	20.3	...	6.9
5.2	3.0	1.9	0.3	1.8	1.7	5.8	16.0	0.6	6.0
						5.2	12.6	1.3	5.9

enzyme was tried in the alkaline and also in the acid range on all the substrates used. It will be noted that in the case of the liquid triglycerides of the saturated fatty acids, the titration value decreases with an increase in molecular weight. Since a few of these substrates are solids, there would be less surface than in the case of the liquids and this would affect the rate of enzyme action. It is generally assumed that wheat germ which has the highest fat content of all the milling separations also has the highest lipolytic activity. However, in comparing all the milling separations, other fractions showed greater activity in the hydrolysis of certain neutral triglycerides. The *P*H of the boiled preparation including the sample, substrate, toluene and buffers was determined potentiometrically in all cases. Since phosphates are the natural buffers of wheat, it was desirable to use phosphate buffers wherever possible. Mixtures of primary potassium phosphate and secondary sodium phosphate were employed for one group of determinations, and primary potassium phosphate and borax for the more alkaline range. Graphs I and II show the results obtained. In Table II, the results are given of various methods tried in an effort to increase the activity of the enzyme. None of these methods was found to

give more than a very slight increase in the titration value over that obtained on the untreated and unextracted sample when using triacetin as the substrate. When germinated wheat was used with some of the higher

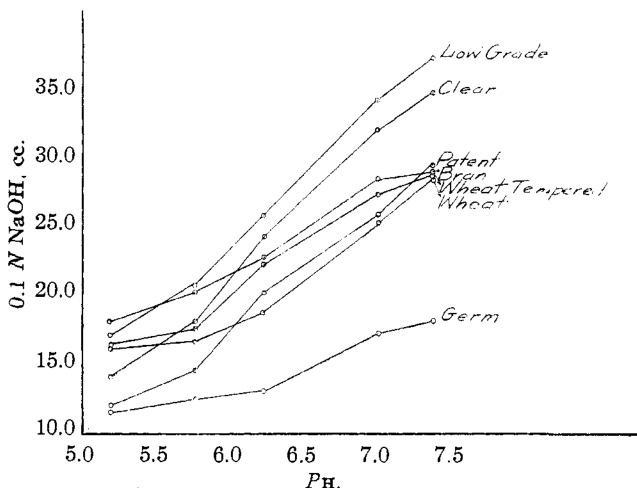

Fig. 1.—Buffers: KH_2PO_4 and $Na_2HPO_4 \cdot 12H_2O$.

triglycerides, an increased action was observed. As these higher triglycerides are the natural components of wheat, further work is to be done on the higher fats.

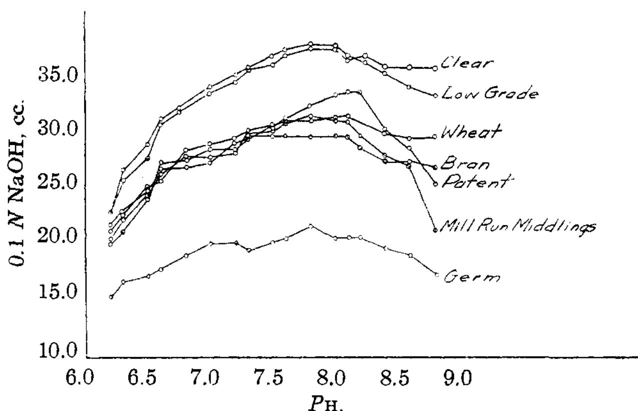

Fig. 2.—Buffers: 0.1 *M* KH_2PO_4 and 0.5 *M* $Na_2B_4O_7 \cdot 10H_4O$.

A comparison was made using *Ricinus* lipase and the lipase of soy bean prepared by exhaustive ether extraction of the beans. Neither of these preparations gave as high results as the ground untreated wheat when triacetin was used as a substrate.

A negative test for the free amino group was obtained by testing the in-

TABLE II

GROUND WHEAT EXTRACTED BY VARIOUS METHODS IN AN EFFORT TO INCREASE THE CONCENTRATION OF THE LIPASE WHICH ACTS ON TRIACETIN

Results Expressed in Cc. of $N/10$ Sodium Hydroxide

PH	Resting wheat not extracted	Exhaustive ether extraction	Exhaustive toluene extraction	Germinated	Glycerin extraction	Water extraction filtrate	Water extraction residue
5.8 (no buffers)	22.1	23.1	22.6	22.5	13.6	8.4	7.1
7.7	27.4	29.0	28.0	29.0	19.7	17.9	10.1
7.4	25.9	27.0	25.9	27.5	19.7	16.9	9.2
6.3	20.1	21.8	21.3	22.0	..	12.6	7.8
5.8	17.9	17.6	17.5	18.4	..	8.9	6.3
5.2	14.4	12.8	14.6	16.5	..	6.8	3.8

	Preliminary Digestion with Trypsin			Preliminary Digestion with Diastase	
PH	Residue extracted with ether	Residue not extracted with ether	Filtrate from tryptic digestion	Residue extracted with ether	Filtrate
5.8 (no buffers)	3.1	2.5	19.5	3.1	13.5
7.7	4.2	2.9	24.1	3.0	19.1
7.4	3.1	3.1	23.5	4.0	19.0
6.3	4.0	4.5	..	4.0	..
5.8	3.3	3.2	..	3.7	..
5.2	2.0	2.5	..	3.2	..

PH		Trimyristin	Tripalmitin	Tristearin	Wheat fat E. E.
	Unextracted wheat				
5.2	Germinated (three days)	3.2	2.6	1.8	2.2
5.2	Resting	1.5	0.7	0.4	0.7
	Ether extracted wheat				
5.2	Germinated previous to extraction	...	...	...	2.2
5.2	Resting	...	...	...	1.1

cubated products with ninhydrine, eliminating the possibility of any increase in titratable acidity being due to amino acids.

Discussion

Glycerides of the lower fatty acids, particularly triacetin and tripropionin, were acted on to the greatest extent, triacetin giving considerably higher results than any other substrate tried. Ethyl acetate gave a higher titration value than ethyl butyrate, which showed very little action, although neither of these esters was acted on to the extent of triacetin and tripropionin. This fat splitting enzyme of wheat acts on the glycerides of the fatty acids to a greater extent than on the corresponding esters. The higher titration values given by triacetin and ethyl acetate compared to the higher members of their series is, however, undoubtedly purely physical and is probably due to the relatively greater water solubility of these lower members. No triolein or glycerides of other unsaturated fatty acids were tried in these experiments, although olive oil was found to give a titration

value comparable to the higher saturated glycerides. With olive oil as a substrate, the enzyme has its optimum *P*H in the acid range. Many investigators in this field have measured the extent of lipase activity by the use of triacetin, tributyrin, ethyl butyrate and such compounds of the lower fatty acids as substrates. Since the higher fatty acids, such as stearic, palmitic and oleic, are those commonly present in plant and animal tissues, it would seem to the authors that lipase studies to be of any practical significance should be directed toward the use of such substrates as are present in greatest amounts in natural products.

This study is being continued in an effort to find the optimum conditions of time, temperature and *P*H under which the higher triglycerides, lecithin and other lipides naturally present in wheat are hydrolyzed. An effort is being made to obtain a more active enzyme preparation by other methods than those already described and to study the effect of various activators and inhibitors on such preparations.

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Summary

1. A method has been described for the measurement of the activity of the lipase of wheat products and it has been shown that with the time and temperature employed, and with triacetin as the substrate, the enzyme was most active on the alkaline side at *P*H 7.3 to 8.2 and that it has a wide optimum range. All wheat products show a marked action in cleaving triacetin, the highest values being given by low grade flour rather than by germ or bran. When the higher triglycerides of fatty acids naturally present in wheat were used as substrates, the enzyme showed an optimum on the acid side. Germination of wheat increased the activity of its lipase when the higher triglycerides were used as substrates, but made very little difference when triacetin and the glycerides of the lower fatty acids were employed.

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