LIII. SULPHURETTED HYDROGEN AS A FACTOR IN THE DETERMINATION OF FREE AND BOUND AMYLASE IN UNGERMINATED CEREALS.

BY TADEUSZ CHRZĄSZCZ AND JÓZEF JANICKI.

From the Laboratory of Agricultural Technology, Poznań University.

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THE question of the valuation of cereals in regard to total amylase content, even in the period before their germination, is very important both from the theoretical and the practical points of view. It is well known that apart from free amylase, which can be extracted by the use of water from ungerminated cereals, there are also large quantities of bound amylase which are inactive and which cannot be extracted, but which are freed during the process of germination, excluding of course from consideration the possibility of the formation of new quantities of amylase during this process. In order to determine the content of bound amylase, papain is usually added [Ford and Guthrie, 1908]. Our researches have shown that the action of papain can be partially replaced by that of eluants [Chrząszcz and Janicki, 1933], especially in the case of malt. It has further been shown that apart from papain, there are other proteolytic enzymes which can be used, trypsin being a special case in point [Chrząszcz and Janicki, 1934, 1, 2]. The present authors have also shown [1935] that chymosin (rennin) can likewise be used for the determination of bound amylase content in ungerminated cereals as this, especially with some cereals, has a greater influence upon increases in the active amylase than papain. In addition, it has been demonstrated in the papers referred to above that the decomposition of the proteins of the various cereals depends on the type of enzyme used, this having been shown particularly in the case of the decomposition of the protein of oats by rennin [1935]. Following this line of approach we were able to extend the observations of Blagovestshensky [1934] on the specific action of the proteolytic enzymes. If it be accepted that amylase is really bound by the protein of the ungerminated cereal, and that the protein of a given type of cereal is decomposed most strongly by the proteolytic enzyme of the same origin, then it might be expected that by using these enzymes the greatest quantity of bound amylase would be extracted. The researches of Mendel and Blood [1910], Willstätter and Grassmann [1924; 1925], and Grassmann and Dyckerhoff [1928] have demonstrated that the plant proteolytic enzymes become active under the influence of sulphuretted hydrogen. The researches of the present authors upon the influence of H_2S on the quantity of amylase in ungerminated cereals have shown that this gas must have a specific action on amylase [1935-36]. On this basis it proved possible to elaborate a method of determining the amylase content by the use of H_2S .

EXPERIMENTAL.

In the experiments, four samples of barley (Nos. 1-4), two of wheat and one of rye were taken, Merck's soluble starch (Erg. B 5) and papain (1:100) were used.

Preparation of aqueous extracts. 25 g. of ground cereal + 150 ml. of H_2O + 4 ml. of toluene were left at room temperature (c. 18°) for 15 hours and shaken from time to time, or mechanically shaken at 60 r.p.m.

Preparation of extracts with addition of papain. The process followed was the same as above but with the addition of 3 g. of papain.

The H_2S method. 25 g. of the ground cereal + 150 ml. of H_2O + 4 ml. toluene were placed in a flask. The air present in this flask was driven out by sulphuretted hydrogen produced by a Kipp's apparatus, after which the extract was saturated with H_2S under pressure for 20 min., during which period it was well mixed about every 5 min. and then left under pressure for 15 hours and shaken as above.

The extracts were then filtered and their amylolytic power ascertained by determinations of the saccharifying, dextrinising and liquefying powers in accordance with the methods described in previous papers [Chrzyszcz and Janicki, 1935]. In view of the fact that the dextrinising and liquefying powers under the influence of papain and H_2S underwent very slight fluctuations, their values have not been given in the tables. The saccharifying power was determined in extracts diluted in the proportion of 10:100, and expressed in ml. *N*/20 iodine, corresponding to the amount of sugar produced under the given conditions by the amount of extract equivalent to 1 g. of the cereal. Blank experiments were made when determining the saccharifying power, taking into consideration the reducing power of the extracts themselves and of the starch solution. Using this method of applying H_2S it is necessary to carry out the determination of the saccharifying power immediately after filtration and dilution, as otherwise the H_2S will escape or be oxidised by the air and so a weaker saccharifying power will be recorded.

RESULTS.

Barley. In order to compare the influence of papain and of H_2S on the quantity of active amylase obtained from ungerminated barley, extracts were prepared from four samples. The extracts of barley Nos. 1-3 were prepared by shaking from time to time, whilst sample No. 4 was in addition mechanically shaken.

Table I. *Saccharifying power of barley.*

Extract	Extracts shaken from time to time						Extracts shaken mechanically			
	Sample No. 1		Sample No. 2		Sample No. 3		Sample No. 4			
	<i>N</i> /20 iodine ml.	In- crease %	<i>N</i> /20 iodine ml.	In- crease %	<i>N</i> /20 iodine ml.	In- crease %	<i>N</i> /20 iodine ml.	In- crease %	<i>N</i> /20 iodine ml.	In- crease %
Aqueous	296.0	—	405	—	278	—	223.6	—	132.6	—
H_2S added	884.0	198	863.2	113.1	559	101	681.2	205	374.4	182
Papain added	740.0	150	754	86	465	67	556.4	149	312.0	135

From the above table it can be seen that the extracts prepared with the addition of H_2S show a considerably greater increase in the active amylase than those of samples by the papain method. Further, it can be seen that the extracts prepared by shaking occasionally have a greater saccharifying power than those shaken mechanically.

Wheat and rye. The results of the action of H_2S in comparison with the influence of papain on the activation of bound amylase in the cases of wheat and rye are given in Table II.

Table II. *Saccharifying power of wheat and rye.*

Extract	Extracts shaken from time to time		Extracts shaken mechanically		Extracts shaken from time to time			
	Sample wheat No. 1		Sample wheat No. 1		Sample wheat No. 2		Sample rye	
	N/20 iodine ml.	In-crease %	N/20 iodine ml.	In-crease %	N/20 iodine ml.	In-crease %	N/20 iodine ml.	In-crease %
Aqueous	390.0	—	278.2	—	317.2	—	413.2	—
H ₂ S added	681.2	74.6	509.7	83.2	494.0	55.8	530.4	28.3
Papain added	645	65.4	577.2	107.1	450.0	41.8	512.2	24.0

It will be noted that both these types of cereal give similar results, *viz.* a greater increase in the amount of active amylase under the influence of H₂S than under that of papain. It also transpires that the extracts of these cereals possess greater amylolytic power when they are not shaken mechanically during their preparation. It therefore appears best to shake the extracts only from time to time and not continuously.

SUMMARY.

1. A method is described for the determination of bound amylase in ungerminated cereals by the use of H₂S.
2. The increase in the amount of active amylase, *i.e.* the extraction of the bound amylase, is greater in presence of H₂S than when the papain method is used.
3. The extracts should only be shaken from time to time during preparation.

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