Automated analysis of starch in cooked sausage

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Introduction

The cheap, but very popular types of Finnish cooked sausage contain an average of 8 % potato starch, a component that has a significant effect on the texture of the sausage (3). Determination of the starch content of sausage is thus important from the point of view of quality control.

The starch content of meat products can be determined gravimetrically (5), enzymatically (2) or by acid hydrolysis (1, 4, 6, 8). In the gravimetric method the starch is separated from the protein and fat components by treatment with an alcoholic solution of a base, precipitated with alcoholic acetic acid and then weighed (5). In the enzymatic method the starch is hydrolysed by amyloglucosidase to glucose, which is then determined enzymatically (2). In the acid hydrolysis methods the starch is hydrolysed using HCl, H_2SO_4 or other acid into glucose, which is then determined either titrimetically (1), enzymatically (4, 6) or colorimetrically (8). Of the three methods, it is the gravimetric one that is in routine use in Finland. In view of the fact that the method is slow (analysis time about 2 days), it was decided to try to develop an automatic method that would be faster, at least as accurate and simple to carry out.

The method developed was based on the dinitrosalicylate method of Sumner and Sisler (7), and consisted of determination of the native undecomposed starch content of the sausage. The starch is hydrolysed enzymatically to glucose, which is then determined using a colour reagent. The results were compared with those obtained with the gravimetric method.

Method

1. Principle

The potato starch is solubilizated from the sausage using acid. The content of reducing sugars in the sample is determined using dinitrosalicylate (DNSA) both before and after enzymatic hydrolysis. The starch content was calculated using the standard straight line drawn for commercial potato starch (Hämeen Peruna, Finland).

2. Preparation of the sample

1,5 g of minced sausage (0-130 mg starch) was weighed into a 100 ml volumetric flask and 25 ml of 1,4 N hydrochloric acid were added. The flask was shaken and sealed with a stopper. The flask was placed in a hot water bath at 70°C for 30 minutes and shaken occasionally. After cooling, the pH of the solution was adjusted to 4,9 - 5,1 using 20 ml of a solution that was 1,75 N with respect to sodium hydroxide and 0,13 M with respect to trisodium citrate. The solution was made up to the mark with distilled water, shaken and then filtered through fast filter paper. The filtrate was then used for the determination.

3. Determination using the Technicon UV spectrophotometer

The sample was first pumped through the hydrolysis bath (15 min, 60°C) with water (no enzyme), following which it was pumped together with the DNSA solution (2 g 2-hydroxy-3,5-dinitrobenzoic acid, 3,2 g sodium hydroxide and 60 g hydrated potassium sodium tartrate in 200 ml of water) into the colour reaction bath (5 min, 90°C). The resulting coloured solution was diluted with water before measurement on the Technicon UV spectrophotometer. The photometer measures the colour in the sample as an increase in absorbance (wavelength 540 nm). In the second stage, the same sample was pumped into the hydrolysis bath together with the enzyme (amyloglucosidase Merck 1330, concentration 3 mg/ml in a solution 0,12 M with respect to trisodium

citrate and 0,06 M with respect to citric acid, pH c. 5), from where it was pumped into the colour reaction bath as before, and the increase in absorbance shown on a recorder and a printer (Fig. 1). The wetting agent used in the DNSA solution was aerosol 22 (Technicon) at a concentration of 1 ml/l.

The starch content of the sample (% w/w) is calculated from the following formula:

$$\% \text{ starch} = \frac{36{,}87 \cdot (A_1 - A_2) + 11{,}17}{m} \times 100$$

where $36{,}87 \cdot (A_1 - A_2) + 11{,}17$ is the equation of the standard straight line for potato starch (mg starch/100 ml), A_1 is the absorbance of the sample with enzyme and A_2 the absorbance without enzyme, and m is the weight of the sample in milligrammes.

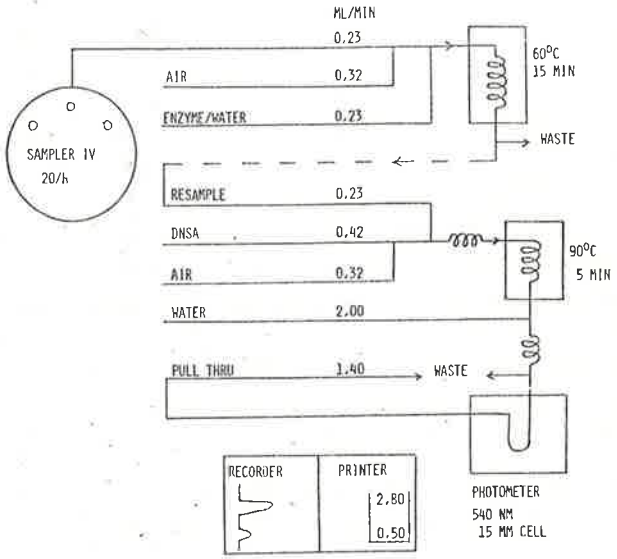

Figure 1. Manifold for AutoAnalyzer for analysis native starch in cooked sausages by dinitrosalicylate reaction.

Results and discussion

Commercial potato starch (Hämeen Peruna, Finland), with a starch content of 78 % (w/w) as determined by the reference method (5), was used for calibration. This starch was used to determine the standard straight line (mg starch/100 ml = 36,87 · (diff. in absorbance) + 11,17).

The determination was shown to be linear for starch contents below 130 mg/100 ml, which corresponds to 1,5 g of sausage containing 8,7 % starch. In the linear range of the determination the reproducibility (standard deviation) was 0,3 % (n = 14).

The method is based on the equilibrium state of the enzyme reaction, which is why the required amount of enzyme and reaction time were first sought. Equilibrium was reached with 3 mg of amyloglucosidase per ml and a reaction time of 15 min (pH 5,0, 60°C). At lower enzyme concentrations the reaction did not reach completion.

In order to establish the effect of any interfering factors, a test was carried out to see whether the lactose contained in dried milk added to the sausage (average 3 %) was decomposed into glucose and galactose. The effect of acid hydrolysis (6 % perchloric acid at 110°C for 90 minutes) on lactose was also studied. It was found that the decomposition of lactose had no significant effect on the result obtained with the automatic method, whereas when acid hydrolysis was used, the decomposition of lactose caused an error in the determination of + 1,4 %.

The starch contents obtained for the cooked sausages using the Technicon were compared with those given by the gravimetric method (5). The results are shown in Table 1. The results obtained for sausages with a low starch content (4 - 5,5 %) were about 0,8 % higher with the gravimetric method than with the Technicon. The results were about the same for higher starch contents (6 - 9 %). There was no significant difference between the methods ($p < 0{,}05$), and the correlation between them was highly significant ($p < 0{,}001$).

Table 1. Starch content of cooked sausages.

Test no.	Starch content by analysis (%)		Difference
	Technicon (1)	Gravimetric (2)	(1-2)
1	3,3	4,2	- 0,9
2	5,6	4,5	+ 1,1
3	4,8	5,5	- 0,7
4	6,2	6,0	+ 0,2
5	7,3	7,8	- 0,5
6	8,5	8,5	0,0
7	8,6	8,6	0,0

Six test samples, to which 0 - 12,5 % of starch was added (Table 2), were prepared to enable the methods to be compared. Potato starch was mixed with the sausage emulsion and the starch then gelatinized by heating for 30 min at 70°C. It can be seen from Table 2 that the content of starch as determined gravimetrically does not increase in the same proportion to that in which it is added, i.e. linearly. It can be seen that the results for low contents (0 - 2 %) are too high, while those for high contents (7,5 - 12,5 %) are too low. For this reason, the recovery with the gravimetric method is on average 14 % lower than that for the Technicon method.

Table 2. Recoveries for the two methods. Percentage recovery = 100 · (% starch content found - % starch content of test sample 1) : % of added starch.

Test no.	Added starch	Starch content found, %	
		Technicon	Gravimetric
1	0,0	0,9	1,5
2	2,5	2,6	3,6
3	5,0	5,0	4,8
4	7,5	7,9	5,4
5	10,0	9,8	8,6
6	12,5	12,7	11,6
Average percentage recovery		85	71

The six samples shown in the table above were used to establish the accuracy and reproducibility of the two methods. Table 3 shows that the accuracy (-0,23 and 0,33 % points) and reproducibility (0,10 and -0,23 % points) of the two methods were good for quality control methods. The enzymatic method proved to be more accurate than the gravimetric method (average 0,10 % points) and to have greater reproducibility (average 0,13 % points).

Table 3. Accuracy and reproducibility of the two methods. Accuracy = (added - analysed) starch %; reproducibility = difference between two parallel determinations. Test sample numbers as in Table 2.

Test no.	Technicon		Gravimetric	
	accuracy %	reproducibility %	accuracy %	reproducibility %
1	-0,9	0,1	-1,5	0,0
2	-0,1	-0,1	-1,1	0,1
3	0,0	0,3	0,2	0,2
4	-0,4	-0,6	2,1	0,0
5	0,2	-0,1	1,4	-1,1
6	-0,2	1,0	0,9	-0,6
Average	-0,23	0,10	0,33	-0,23

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