A NEW PROTEIN IN POTATOES

by

E. H. GROOT, L. W. JANSSEN, A. KENTIE, H. K. OOSTERHUIS, AND H. J. L. TRAP

Nederlandsch Instituut voor Volksvoeding, Amsterdam; Staats Veeartsenijkundig Onderzoekingsinstituut, Amsterdam, and W. A. Scholten's Aardappelmeelfabrieken N.V., Hoogezand-Foxhol (Netherlands)

It is generally believed that the protein present in potatoes is a single substance, usually spoken of as "the potato protein" or "tuberin". Evidence has been accumulating in recent years that this conclusion is incorrect and that actually there are two distinct proteins present in potatoes. Our first evidence in this direction was obtained by two of the present authors (A.K. and H.K.O.) in 1942 when carrying out experiments in which a careful heat coagulation of the proteins present in potato press juice was performed in the following way: potato juice was first acidified to p_H 2.9 with dilute sulphuric acid, then gradually heated on a water-bath to a temperature of 45° C. The liquid was stirred mechanically and maintained at this temperature for one hour; the protein which flocculated during this time was centrifuged off. The clear supernatant fluid was then further heated, and when the temperature reached 55–57° a new flocculation of protein began. Between 45° and 55° no flocculation occurred. Similar observations of two different ranges of flocculation of protein have also been made at other p_H values, for instance at 4.0 and 4.9.

Earlier observations

OSBORNE and CAMPBELL (1896) performed salting-out experiments with potato juice and found that the protein of the potato is mainly a globulin named tuberin, but that there is in addition "a very small amount" of a proteose present. Presumably it is the latter remark that has formed the basis of the opinion that potato proteins other than tuberin may be neglected. The observation of OSBORNE and CAMPBELL is quoted for instance in PAROW's "Handbuch der Stärkefabrikation" (1928). KIESEL and coworkers (1924) compared the amino acid analyses of the proteins of the potato leaf with the analyses of the proteins of the tuber. On isolating protein of the tuber they obtained two fractions. One part was soluble in water and the other was not. The amino acid analyses of the two fractions showed only insignificant differences. These authors were therefore not led to suspect that two different proteins were present. The most obvious explanation of these observations is that part of the protein has been denatured during the isolation process. While this remains a possibility (native potato protein is rather labile in solution), it is remarkable, however, that these investigators isolated from a quantity of potato press juice 8 grams of protein soluble in water and 3.3 grams of insoluble protein. This proportion (71:29) is almost exactly the same as we have found in the experiments described below.

References p. 414.

EXPERIMENTAL

The question was further investigated by us in two ways; by means of electrophoretic analysis and by means of the so-called invert soap method. Although both methods were carried out only once, their results agreed so well with the above facts that our view was confirmed, and the proportions of the two components could be estimated. Moreover, we wish to stress that the electrophoresis and invert soap experiments were carried out quite independently of each other, the former by JANSSEN and GROOT in Amsterdam and the latter by TRAP in Hoogezand.

Electrophoresis of potato press juice

For this investigation the TISELIUS apparatus (1938) was used with a modified PHILPOT-SVENSSON optical system (SVENSSON, 1939, 1940). Two protein components

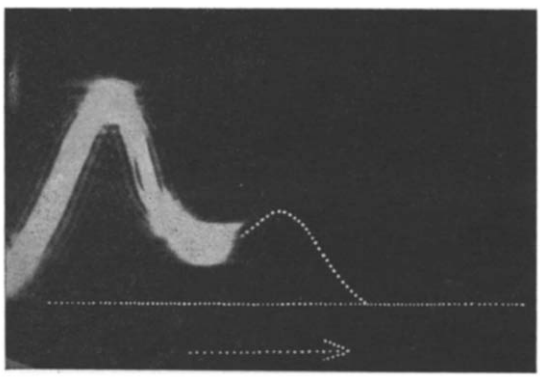

Fig. 1. Protein distribution in the negative part of the electrophoresis tube. For the sake of clearness the centre of the baseline and the maximum at the right are indicated by a dotted line. The arrow indicates the direction of movement. — The tuberin is clear and colourless and transmits the light well, while the other component is of a dark colour and shows turbidity, so that light is to a great extent absorbed

were clearly indicated (Fig. 1); the main fraction showed a slower-moving protein in the electric field and therefore must be assumed to possess a smaller negative charge than the second protein. The areas of the two surfaces enclosed by the abscissa and the two separate curves were estimated to be in the ratio of about 71:29, which indicates the approximate proportions of the two components.

The main fraction was clear and colourless, as is indicated by the good transmission of light in the left half of the photograph; the second protein was turbid and carried coloured substances, so that a considerable proportion of the incident light was absorbed (right side of the photograph). It seems reasonable to identify the main protein (insoluble in pure water) with the tuberin of OSBORNE and CAMPBELL, while the second fraction may correspond with their proteose and may also be identical with the watersoluble protein fraction of KIESEL *et al.* Our smaller fraction is, however, coagulable by heat, like tuberin but unlike the proteoses. It is our opinion that this substance is a true protein. It possesses more hydrophylic properties than tuberin.

In order to obtain good photographs in the electrophoretic procedure it is essential for the concentration of protein to be about 1 % and the liquid to be as clear as possible and only slightly coloured. The potato juice was therefore prepared by grating potatoes of the "Triumph" strain into a diluted solution of sodium bisulphite to avoid the formation of a red colour (GROOT, 1942). In order to obtain a sufficiently high concentration of protein the juice was concentrated in a desiccator, and it was further stabilised with a little sodium chloride and dialysed against a phosphate buffer containing sodium bisulphite and sodium chloride.

210 grams of potatoes were grated, the grated matter falling immediately into 30 ml of 1 % sodium bisulphite solution. The pulp was pressed out and the juice filtered to remove the starch. To

References p. 414.

100 ml filtrate, 19 ml 0.1 M. phosphate buffer (p_H 6.8) were added together with 171 mg sodium chloride and 3.2 ml 0.5 N NaOH. (The p_H of the mixture was then 6.8). The liquid was concentrated in a vacuum desiccator (containing silica gel as a drying agent) until its volume was reduced to two-thirds. It was then dialysed for ten hours in a cellophane bag against a 60-fold quantity of a solution containing the salts mentioned above in the same concentrations as in the concentrated juice. The outer solution was permanently stirred during dialysis.

After dialysis the protein content was determined refractometrically and was found to be 1.24 %. The juice was then centrifuged for 25 minutes at 5000 rpm, yielding a solution which was only slightly turbid. Electrophoresis was carried out for two hours.

The potatoes used were rather old and have sprouted a little since the experiment was carried out in May.

Fractional precipitation of the proteins of potato press juice with a quaternary ammonium salt ("Invert soap") solution

SCHMIDT (1943) has described the properties of salts of quaternary ammonium bases. They possess the property of precipitating proteins; fractions can be obtained by gradually increasing or decreasing the acidity of the solution. Such experiments may be described by plotting the amount of acid added to a series of solutions against the amounts of nitrogen in the precipitate formed in each sample of solution. In order to reduce the concentration of electrolytes which, according to SCHMIDT, have a disturbing effect, it was desirable to dialyse the juice, though the dialysis could not be continued too long because of the danger of part of the protein being flocculated.

New potatoes of the strain "Noordeling", dug in November, were used. After being grated, the pulp was squeezed out and the juice allowed to stand for five hours, after which it was decanted from a solid residue. The starch-free solution was preserved by covering it by a thin layer of toluene and xylene. Three and a half hours dialysis proved to be a suitable time for the removal of the greater part of salts without causing flocculation of the proteins. Dialysis was carried out in a parchment bag rotating in flowing water. It was next necessary to determine the proper proportions of "invert soap" (salt of the quaternary ammonium base) and potato juice. The invert soap used was "Sapamin-KW" from CIBA, Basle.

Experiments were carried out with solutions of the following concentrations: 0.5, 1.0, 1.5 and 2.0 %. To a number of centrifuge tubes, each containing 5 ml of dialysed potato juice, ware added respectively:

0.5 ml of 0.5 % KW-solution and 9.5 ml of water,
1.0 ml of 0.5 % KW-solution and 9.0 ml of water,
1.5 ml of 0.5 % KW-solution and 8.5 ml of water, etc.

Similar ranges of reaction mixtures were prepared with the other KW-concentrations. After standing at room temperature for 18 hours, the reaction-mixtures were centrifuged and nitrogen determined in the supernatant fluid by the KJELDAHL method. The results of the analyses, carried out four times and calculated as amounts of nitrogen precipitated, are recorded in figure 2. Each point represents the average of the four determinations. The maximal difference between duplicates was 0.25 mg nitrogen. It should be noted that corrections were made for the nitrogen content of the Sapamin itself (2.27 %). The curves show that complete precipitation is only obtained by adding 2 % Sapamin solution; 5 ml is the smallest amount producing this effect.

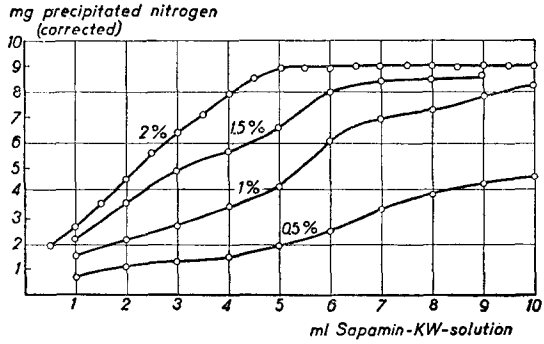

Fig. 2. Precipitation of proteins from 5 ml partly dialysed potato juice by 0.5, 1.0, 1.5 and 2.0 % Sapamin-KW solutions

The final experiment was carried out as follows: 5 ml starch-free and partly dialysed potato juice, 5 ml 2 % KW-solution and 5 ml acetate buffer (0.2. M., p_H 4.2) were pipetted into a number of centrifuge tubes. Then *a* ml 0.1 N HCl and (0.9 — *a*) ml of water were added to each tube. The tubes were closed and allowed to stand for 24 hours; then the contents

References p. 414.

were centrifuged and nitrogen estimated in the supernatant fluids. The maximal difference between duplicates was again 0.25 mg. Corrections for the nitrogen of the reagent were made.

The results are recorded in figure 3, in which the amount of nitrogen precipitated is plotted against the added amount of 0.1 NHCl. This figure again shows very clearly the presence of two proteins, and it even seems possible that the main fraction is composed of two subfractions. As, however, electrophoresis did not give any such indication, no definite conclusion is possible on this point.

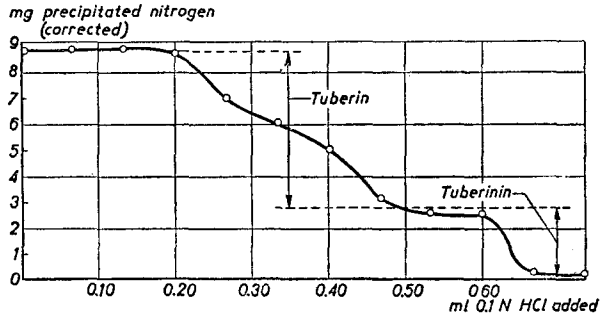

Fig. 3. Precipitation of the proteins present in 5 ml partly dialyzed and starch-free potato juice supplemented with 5 ml 2 % Sapamin-KW solution, on treating with different amounts of 0.1 N HCl

On adding acid to the solution obtained from the potato press juice, the main protein fraction precipitates first and the other only after the addition of more acid. The isoelectric point of the latter is therefore certainly lower, agreeing with the electrophoresis. We think the main fraction should be considered as the globulin tuberin, the second fraction corresponding to the protein of higher electrophoretic mobility.

We propose the name „tuberinin" for this new protein, which was suggested to us by Prof. JANSEN. Figure 3 shows that the ratio of amounts of tuberin and tuberinin is about 67:33, a result that agrees satisfactorily with the data obtained by electrophoresis. The agreement is the more remarkable when it is remembered that in the two kinds of experiments different strains of potatoes were used.

SUMMARY

Evidence is given for the presence of two proteins in potatoes. The protein occurring in the greater quantity is the well-known globulin tuberin; the other, for which we propose the name tuberinin, is more hydrophylic and its isoelectric point is situated at a lower p_H than that of tuberin. The ratio of the amounts present is about 7 : 3. Both tuberinin and tuberin are coagulated by heat.

RÉSUMÉ

Démonstration de la présence de deux protéines dans les pommes de terre. La protéine la plus abondante est une globuline, la tubérine; l'autre, pour laquelle est proposé le nom de tubérinine, est plus hydrophile et possède un point isoélectrique situé à un p_H plus bas que celui de la tubérine. Ces deux protéines sont présentes dans un rapport d'environ 7:3. La tubérinine comme la tubérine, est coagulée par la chaleur.

ZUSAMMENFASSUNG

Beweise für die Anwesenheit von zwei Eiweisstoffen in Kartoffeln werden gegeben. Das Eiweiss, das in der grösseren Menge vorkommt, ist das wohlbekannte Globulin Tuberin; das andere, für welches wir den Namen Tuberinin vorschlagen, ist stärker hydrophil, und sein isoelektrischer Punkt liegt bei einem niedrigeren p_H als der von Tuberin. Das Verhältnis der anwesenden Mengen ist ungefähr 7 : 3. Sowohl Tuberinin wie Tuberin werden durch Hitze koaguliert.

References p. 414.

We are much indebted to Prof. Dr B. C. P. JANSEN and Prof. Dr H. G. K. WESTENBRINK for their interest and advice and to Dr H. S. FRENKEL for the hospitality in his laboratory.

REFERENCES

E. H. GROOT, *Arch. néerl. de Physiol.*, 26 (1942) 472.
A. KIESEL, A. BELOZERSKY, P. AGATOW, N. BIWSCHICH, AND M. PAWLOWA, *Z. Physiol. Chem.*, 226 (1924) 73.
T. B. OSBORNE AND G. F. CAMPBELL, *J. Amer. Chem. Soc.*, 18 (1896) 575.
E. PAROW, *Handb. d. Stärkefabrikation*, Berlin (1928) 297.
K. H. SCHMIDT, *Z. Physiol. Chem.*, 277 (1943) 117.
H. SVENSSON, *Kolloid Z.*, 87 (1939) 181.
H. SVENSSON, *Kolloid Z.*, 90 (1940) 141.
A. TISELIUS, *Kolloid Z.*, 85 (1938) 129.

Received April 18th, 1947