Appl Microbiol Biotechnol (1997) 48: 435–440

MINI-REVIEW

F. Mayer · J.-O. Hillebrandt

Potato pulp: microbiological characterization, physical modification, and application of this agricultural waste product

Received: 18 April 1997 / Received revision: 12 June 1997 / Accepted: 14 June 1997

Abstract Potato pulp, one of the agricultural waste products obtained in high quantities during starch production, contains starch, cellulose, hemicelluloses, pectin, proteins, free amino acids and salts. It exhibits physical and physicochemical properties of a typical colloid. It is mainly used, in a dried and pelleted form, as cattle feed. Its autochthonic microbial flora (bacteria, fungi) was identified and studied with a view towards the degradative potential of the microorganisms and ways of conserving the pulp for subsequent technical applications; 33 isolates (28 bacteria, 4 fungi, 1 yeast), belonging to 15 genera were characterized. Biological conservation was possible at very low oxygen pressure, brought about by the autochthonic anaerobic microorganisms causing acidification. Chemical conservation was achieved with sorbic aicd. By treatment with hot water vapour under pressure (autoclaving), followed by a pressure release procedure, intact cells in the pulp (both potato cells and microorganisms, not spores) were destroyed, and their contents and wall fragments were set free. This process resulted in low drying costs and was a prerequisite for the production of a powder that can be used as glue or as animal feed.

Introduction

During the production of starch from potatoes, huge amounts of waste products are produced. Remnants of cell walls of potato tuber, including the skin, and residual intact cells containing starch are mixed with potato fruit liquid. The potato fruit liquid can be separated from the particulate fraction. It is characterized by a high content of proteins, free amino acids and salts, whereas the particulate fraction, called potato pulp, besides remnants of fruit liquid and water in intact cells, contains starch, cellulose, hemicelluloses, pectin and proteins (Kempf 1980) (Table 1). Even after separation of the fruit liquid from the particulate fraction (pulp), the pulp contains up to 90% water; it is highly viscous and exhibits the properties of a colloid.

Potato fruit liquid is primarily used as a source for the enrichment of proteins and amino acids, and as a fertilizer because of its high nitrogen contents. Potato pulp is applied as cattle feed, as a substrate for cultivation of fungi, which may be used for degradation of soil contaminants, in enzymatically treated and condensed form as syrup for crisps and chips and for the production of cosmetics. However, all these applications only minimally reduce the amount of potato pulp piling up in years of high potato production; amounts close to 1×10^6 tonnes per year can be reached in Europe. Pulp not used up for the purposes mentioned above has to be returned to the soil; however, this may result in overfertilizing or damage to the soil and the subsoil water because of the high contents of mineral salts.

Therefore, processes and products should be established that convert potato pulp into valuable "bulk" technical products. An advantage of such an approach would be the low cost for the raw material; money could even be saved that would have to be payed to get rid of the hundreds of thousands of tonnes of pulp. A major drawback is the fact that removal of water from this colloid is very expensive. The water is not only tightly bound to fibres and pectin in the wall fragments, but it is also entrapped in the residual intact cells and would have to be transported across the cell membrane. Application of wall-degrading enzymes might be a solution, but this approach is not feasible because the amount of pulp to be treated is too high for such a process to be cost-effective when a bulk product is made.

F. Mayer (✉) · Jan-Otto Hillebrandt[1]
Institut für Mikrobiologie der Georg-August-Universität Göttingen, Grisebachstrasse 8, D-37077 Göttingen, Germany
Tel.: +49 551 39 3829;
Fax: +49 551 39 3793

Present address:
[1] Institut für Frucht- und Gemüse-Technologie, -Untersuchung und -Forschung, Faethe Labor GmbH, Postfach 15 09, D-33045 Paderborn, Germany

Table 1 Components of conventional wet potato pulp

Component	Relative to wet pulp (% w/w)	Relative to dry matter (% w/w)
Dry matter	13.0	–
Total organic matter	12.5	96
Ashes	0.5	4
Starch	4.9	37
Cellulose	2.2	17
Hemicellulose	1.8	14
Pectin	2.2	17
Fibre (unidentified)	0.9	7
Protein/amino acids	0.5	4

One of the preconditions for the technical use of potato pulp is that the pulp is available all year round in a state that allows its application. It is an intrinsic feature of the system that fresh potato pulp (and starch) are only produced during potato harvest and for following 5–6 months, and not during the remaining months of the year. There are two possible ways to solve this problem. One is to develop strategies for the conservation of fresh pulp, the other is to convert fresh pulp into a modified form, which can be stored at low cost. The first approach necessitates a detailed microbiological characterization of the autochthonic microorganisms degrading or conserving fresh pulp; the second approach includes removal of water, and the transformation of pulp into a form that will withstand contamination by microorganisms during long storage periods and is suitable for multiple applications. This second approach would, in addition, have the advantage that only the particulate fraction, not water, would have to be stored and transported to the site of application.

The present paper describes results of studies aimed at the microbiological characterization of fresh potato pulp, and it gives an outline of a newly developed technology for transforming fresh potato pulp into a powder that can be stored and used as animal feed (Mayer 1991) or as a glue (Mayer 1995, 1996).

Microbiological characterization of potato pulp

For the cultivation of bacterial reference organisms and isolates from potato pulp, growth media were used that are specifically designed for growth of the following bacteria:

Bacillus sp.: FP agar (containing trace element solution SL 8; Pfennig and Lippert 1966)

Lactobacillus sp.: MRS agar (De Man et al. 1960), Rogosa agar (Rogosa et al. 1951)

Flavobacterium sp., *Pseudomonas* sp., *Sphingobacterium* sp., *Sphingomonas* sp., *Xanthomonas* sp.: Nutrient agar (containing meat extract and peptone)

Pseudomonas sp., *Sphingobacterium* sp., *Sphingomonas* sp.: King agar B (King et al. 1954)

Pseudomonas sp.: Cetrimide agar (Brown and Lowbury 1965), *Pseudomonas* agar F (King et al. 1954), *Pseudomonas* agar P (King et al. 1954)

Pseudomonas sp., *Xanthomonas* sp.: GSP agar (Kielwein 1971a, b)

Clostridium sp.: *Clostridium* agar I (Hippe et al. 1992), *Clostridium* agar II (Skinner 1960), DRCM (Gibbs and Freame 1965)

Acinetobacter sp., *Micrococcus* sp.: *Corynebacterium* agar (containing peptone, yeast extract, glucose, NaCl)

Aerobic bacteria in general: standard II agar (Levetzow 1971)

Starch-, cellulose-, pectin-degrading aerobic bacteria: complex medium (Madi 1987) containing vitamin solution (Wolin et al. 1964) and trace element solution SL 4 (Pfennig and Lippert 1966).

Growth parameters were determined according to DIN techniques or with commercially available kits: water contents of soil (DIN/ISO 287, 1985), pH value (DIN 53124, 1988). Organic acids (lactic acid, acetic acid, butyric acid) were determined by using kits from Boehringer Mannheim, Germany (Boehringer Mannheim Gmbh 1989). Gases in potato pulp were determined gas-chromatographically with standardized gas mixtures for comparison (O_2, CO_2, CO, H_2, CH_4 mixtures from Scott Specialty Gases, Houston, Texas, USA). Starch was determined according to established procedures (Keppler and Decker 1974; Beutler 1978), triglycerides according to Eggstein and Kuhlmann (1974).

Special growth media for various microorganisms were also used as standarized kits purchased from bioMerieux, Nürtingen, Germany: API test systems 20E, 20NE, 20A, 50CHB, 50CHL, 20C AUX and 32 STAPH.

Bacteria were identified by analysis of morphology, motility, cell wall type (Gram staining), by the aminopeptidase test (Cerny 1976) and by the KOH test (Gregerson 1978). Ultrathin sections were used for confirmation of data. In addition, standardized test kits (bioMerieux, Nürtingen, Germany) were used for aerobic, anaerobic, and facultatively anaerobic bacteria, for gram-negative and for gram-positive bacteria. Enzyme activities (catalase, oxidase, polymer degradation) were determined qualitatively or semi-quantitatively (e.g., the Bactident oxidase test system 13 300; Merck, Darmstadt, Germany). Profiles of fatty acids were measured and compared with published data, and various additional biochemical and physiological tests (kind of substrate, related enzyme activity) were performed (reference data from DSMZ Braunschweig, Germany). The detailed determination of the relationships of isolated microorganisms to reference organisms was performed by partial sequencing of 16S rRNA (DSMZ Braunschweig, Germany) in cases where computer-supported numerical taxonomy (performed with data collected according the procedures described above) did not give satisfactory results.

Table 2 Bacteria isolated from potato pulp

Isolate	Similarity value (%) (comparison with reference organism)	Remarks
Gram-negative eubacteria		
Acinetobacter lwoffi	95.0	All aerobic mesophilic bacteria together: 10^7–10^8cells/g wet pulp (in upper layers)
Acinetobacter johnsonii	98.1	
Burkholderia cepacia	99.9	
Flavobacterium indologenes	99.8	
Pseudomonas aeruginosa	98.5	
Pseudomonas fluorescens	96.9	
Pseudomonas putida	98.4	
Pseudomonas stutzeri	99.9	
Sphingomonas multivorum	99.9	
Sphigomonas paucimobilis	98.1	
Xanthomonas maltophila	99.1	
Gram-positive eubacteria		
Bacillus amyloliquefaciens	98.9	
Bacillus cereus	99.9	
Bacillus circulans	99.9	
Bacillus macerans	99.3	
Bacillus polymyxa	99.9	
Bacillus pumilis	99.8	
Bacillus subtilis	98.9	
Clostridium butyricum/*C. beijerinckii*	99.9	10^2–10^4 cells/g wet pulp (in lower layers) (anaerobic)
Clostridium sporogenes	94.8	
Lactobacillus brevis	98.6	10^7–10^9 cells/g wet pulp (in lower layers) (anaerobic, but aerotolerant)
Lactobacillus casei	94.8	
Lactobacillus delbrueckii	80.6	
Lactobacillus helveticus	98.6	
Lactobacillus lactis	94.0	
Lactobacillus plantarum	99.9	
Micrococcus varians	99.9	

Table 2 presents a list of bacteria found to occur as autochthonic flora in potato pulp. Similarity values (%) are given, which indicate the degree of similarity of each isolate to the respective reference organism, based on the evaluation of computer-supported numerical taxonomy and, in part, on 16S rRNA analyses.

For the cultivation of fungi and yeasts (reference organisms and isolates), the following growth media were applied: potato-glucose/agar (Beever and Bollard 1970), Sabouraud glucose/maltose/agar (containing peptone), malt extract/agar (Reiss 1972), Ogy agar (Mossel et al. 1970).

Fungi were identified according to culture size, colour and shape, cell morphology and physiological properties. Commercially available test kits were used for the identification of yeasts, in combination with computer analysis (software Apilab plus, version A 1990/91, Apilab, Nürtingen/Germany).

Table 3 summarizes the fungi and a yeast isolate found in potato pulp, and data regarding their occurrence.

Degradation of potato pulp by microorganisms

Aerobically stored potato pulp exhibited degradation by autochthonic microorganisms in the uppermost layer. This was shown by measurement of the biological activity with a microcalorimeter. In this layer, biological activity gradually increased during storage. In the lower layers, the pulp turned into an anaerobic state, and biological activity gradually decreased to a value close to zero (see below: Conservation of potato pulp). Samples treated with sorbic acid lost any biological activity within a few days of aerobic storage; parallel determination of aerobic degradation of starch and

Table 3 Fungi and yeast isolated from potato pulp

Isolate	Remarks
Geotrichum candidum	Very common on patato pulp under aerobic conditions; medium growth at 25 °C; minor growth at other temperatures
Penicillium roqueforti	Only present in few samples; medium growth at 25 °C; good growth when acetate was present; no growth at 37 °C
Arthrinium phaeospermum	Not very common in potato pulp; good growth at 25 °C; no growth at 37 °C
Mucor circinelloides	Very common in potato pulp; good growth at 20 °C; very minor growth at 37 °C; mycel only few mm in height at 15 °C
Rhodotorula sp. (yeast)	Very minor growth under all conditions tested

triglycerides confirmed these results. The presence of enzyme activities (α-amylase, pullulanase, glycoamylase and cellulase) was shown. Pectinolytic activities could not be detected.

In addition, a study analysing the degradation of potato pulp in defined soil (''compost'') was performed. Standardized compost soil was used (EWEDRA, Dransfeld, Germany) and quantiative studies were performed by putting pulp samples, surrounded by a plastic ring, into the soil (containing a defined amount of water) and measuring the degradation of starch, triglycerides and organic polymers, with simultaneous detection of the presence of the respective enzymes (amylase, pullulanase, glucoamylase, cellulase, pectinase).

Macroscopic evaluation of the change of appearance of the potato pulp, both untreated and treated by physical modification (see below), revealed complete degradation of the pulp within a period of 90 days. The fastest degradation was observed for samples pretreated by physical modification, and under aerobic incubation in compost.

Conservation of potato pulp

The presence of autochthonic aerobic bacteria, lactic acid bacteria, clostridia (Table 2) and fungi was determined quantitatively during conservation tests. This was done by counting the viable cells (see above: Microbiological characterization of potato pulp) at various times after inoculation, and under various conditions [aerobic or anaerobic incubation, aerobic incubation after addition of 1% (w/w) sorbic acid, incubation after physical modification of the pulp]. Within 2–3 days after the beginning of the experiments, lactic acid and acetic acid concentrations increased drastically (by factors of 7–25) in the anaerobic area of stored potato pulp, i.e. several millimetres or more below the (aerobic) surface. Butyric acid increased by a factor of 6 in this area, whereas an increase by a factor of 21 was measured for this acid in the aerobic part of the stored pulp sample. These processes were accompanied by a gradual decrease of the pH down to 4.2 in the anaerobic part, and development of a characteristic smell (butyrate).

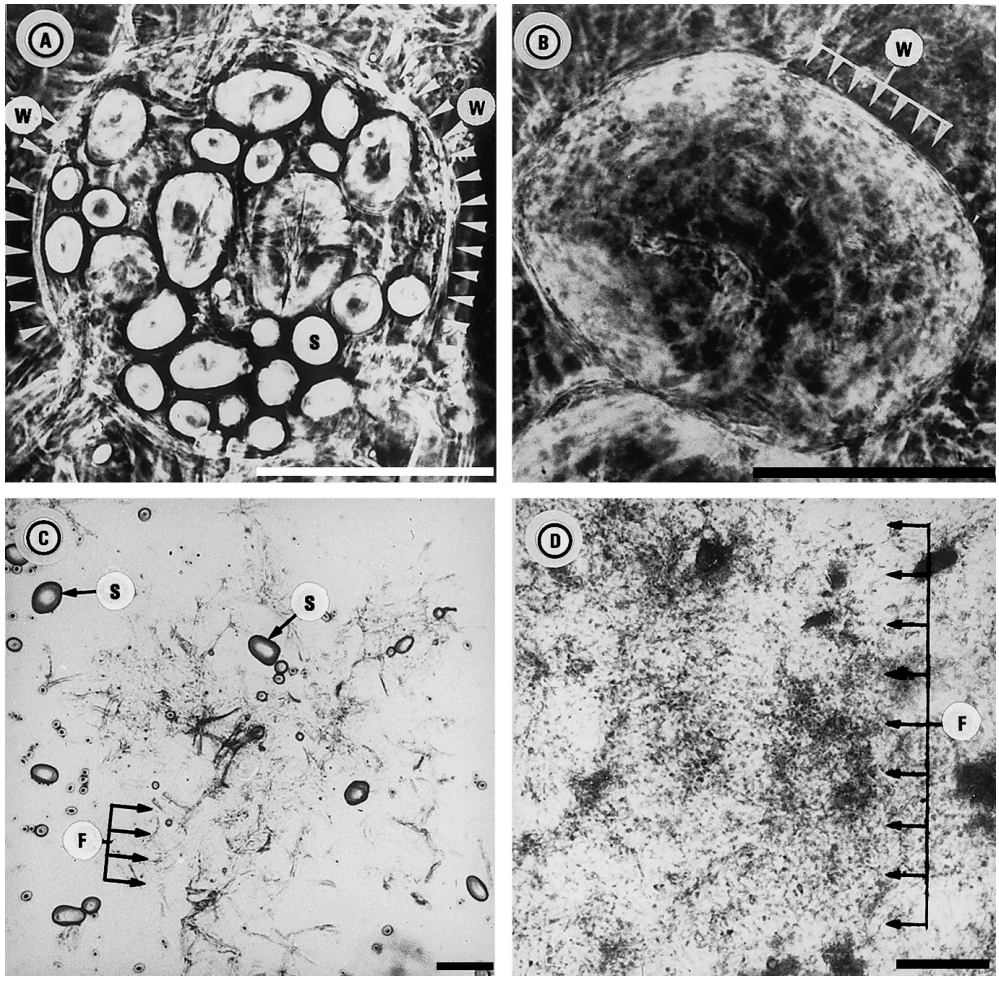

Fig. 1A–D Light micrographs of samples of potato pulp. **A** intact starch cell. Note starch granules (*S*) and intact cell wall. **B** as **A**, but after autoclaving. The cell wall (*W*) is preserved, the starch granules are no longer visible. **C** Result of a treatment of a pulp sample by pressure release, without preceding autoclaving. *F* destroyed cell walls, *S* preserved starch granules. **D** as **C**, but sample autoclaved prior to pressure release. Starch granules are no longer visible. *F* destroyed cell walls. *Magnification bars* 0.1 mm

Parallel measurements of gases in potato pulp revealed that, after 2–3 days of storage, the CO_2 concentration remained constant, with values around 30% under aerobic and anaerobic conditions, and significant reduction (to about 5%) when sorbic acid had been added. O_2 concentration decreased within 2–3 days to a constant value ranging between 0.3% and 0.5% both under aerobic and anaerobic conditions. When sorbic acid had been added, the O_2 concentration remained at around 19%. After equilibration of gas concentrations, fungi grew at the aerobic surface of the stored pulp. Under anaerobic conditions, no such growth of fungi could be observed even at the surface, nor did fungi grow when sorbic acid had been added.

Physical modification of potato pulp, and applications

Potato pulp was treated with hot water vapour under pressure (5 min at 121 °C, "autoclaving"), followed by a pressure-release process (various pressure values between 120 MPa and 170 MPa, performed with a French pressure cell). Potato pulp physically modified in this way was analysed by conventional light microscopy for cell integrity, size of wall fragments, and the size and state of the starch (Fig. 1), and by transmission electron microscopy for size and shape of the submicroscopic wall fragments. This material was also analysed with respect to biological degradation in defined soil (see above). Its complete mineralization within a period of less than half a year is not surprising; after all, it consists of remnants of potato tuber. The final state of the potato pulp after physical modification, i.e. a fine-grain powder, was obtained by spray-drying. It had a low water content (less than 10%), a weight of 350–400 kg/m^3, and could be stored, without any precautions, under room conditions. It retained its properties without contamination for several years (Fig. 2).

The powder is characterized by the small sizes of its components, the absence of intact cells, denaturation of starch and other macromolecular components, and a very low number of viable microorganisms (only spores).

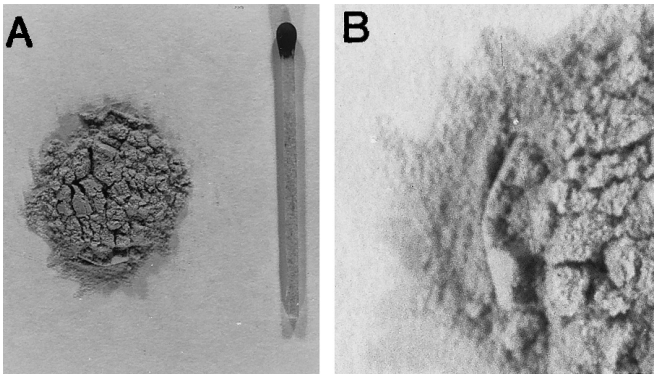

Fig. 2A,B Sample of physically modified, spray-dried potato pulp

When resuspended in water, it can be used for nutritional purposes, e.g. as animal feed, provided no clostridial spores have been preserved. Technical application as a glue was successfully demonstrated for the production of particle and fibre-boards, and for surfaces with minimal pore sizes which may subsequently be painted. The usual pressure, and temperatures above 140 °C should be applied in the process. This modified potato pulp can partially or completely replace any other glue usually applied in the process, it can replace other kinds of glue in the production of small containers such as plant pots etc., and it should be possible to use it, in a mixture with starch, for the production of small containers for the packaging of food etc. An initial first estimate of the costs of the amount of potato pulp glue needed for one tonne of fibre board (see above) is around DM 70–140, with a sales price of DM 1.00/kg glue powder.

The competitiveness of this new kind of glue has not yet been completely evaluated. When produced in high quantities, its cost of production should be equivalent to the cost of other glues, e.g. formaldehyde/urea, the competing glue, usually applied for similar purposes. Its major advantages are that the raw material is an agricultural waste product available at very low cost, and that the glue is completely biologically degradable. In these respects, it will be of advantage compared with conventional glues.

Acknowledgements The microbiological studies were financed by AVEBE, Foxhol, The Netherlands. The 16S rRNA studies were performed by the DSMZ Braunschweig, Germany. The valuable contributions provided by the coworkers of AVEBE New Business, Foxhol, The Netherlands, during the complete process of development are gratefully acknowledged. Application tests regarding high-density and medium-density fibre board production have been performed by HOMANIT, Herzberg, Germany.

References

Beever RE, Bollard EG (1970) The nature of the stimulation of fungal growth by potato extract. J Gen Microbiol 60: 273–279

Beutler HO (1978) Enzymatische Bestimmung von Stärke in Lebensmitteln mit Hilfe der Hexokinase-Methode. Starch/Stärke 30: 309–312

Boehringer Mannheim GmbH (1989) Methoden der biochemischen Analytik und Lebensmittelanalytik: Arbeitsanleitungen zur Analyse von Lebensmitteln, Chemikalien, Kosmetika, Pharmaka, Papier, Tabak, Wasser, Futtermitteln, Düngemitteln, biologischen Proben und anderen Probematerialien. Boehringer Mannheim, Mannheim

Brown VI, Lowbury EJL (1965) Use of improved cetrimide agar medium and other culture methods for *Pseudomonas aeruginosa*. J Clin Pathol 18: 752–756

Cerny G (1976) Method for distinction of gramnegative from grampositive bacteria. Eur J Appl Microbiol 3: 223–225

DeMan JC, Rogosa M, Sharpe ME (1960) A medium for the cultivation of Lactobacilli. J Appl Bacteriol 23: 130–135

DIN 53124 (1988) Bestimmung des pH-Wertes in wässrigen Extrakten. Deutsches Institut für Normung e.V. Beuth, Berlin

DIN/ISO 287 (1985) Bestimmung des Feuchtigkeitsgehaltes nach dem Wärmeschrankverfahren. Deutsches Institut für Normung e.V./International Organization for Standardization. Beuth, Berlin

Eggstein M, Kuhlmann E (1974) Triglyceride and glycerin. In: Bergmeyer HU (ed) Methoden der enzymatischen Analyse, 3rd edn, vol 2. Verlag Chemie, Weinheim, pp 1871–1877

Gibbs BM, Freame B (1965) Methods for the recovery of clostridia from foods. J Appl Bacteriol 28: 95–111

Gregerson T (1978) Rapid method for distinction of gram-negative from gram-positive bacteria. Eur J Appl Microbiol Biotechnol 5: 123–127

Hippe H, Andreesen JR, Gottschalk G (1992) The genus *Clostridium* – nonmedical. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes, 2nd edn, vol 2. Springer, New York, pp 1800–1866

Kempf W (1980) Beitrag zur mechanischen Entwässerung von Kartoffelpülpe. Starch/Stärke 32: 14–20

Keppler D, Decker K (1974) Glycogen: Bestimmung mit Amylglucosidase. In: Bergmeyer HU (ed) Methoden der enzymatischen Analyse, 3rd edn, vol 2. Verlag Chemie, Weinheim, pp 1171–1176

Kielwein G (1971a) Pseudomonaden und Aeromonaden in Trinkmilch: Ihr Nachweis und ihre Bewertung. Arch Lebensmittelhyg 22: 15–19

Kielwein G (1971b) Die Isolierung und Differenzierung von Pseudomonaden aus Lebensmitteln. Arch Lebensmittelhyg 22: 29–37

King EO, Ward MK, Raney DE (1954) Two simple media for the demonstration of pyocyanin and fluorescin. J Lab Clin Med 44: 301–307

Levetzow R (1971) Untersuchung auf Hemmstoffe im Rahmen der bakteriologischen Fleischuntersuchung. Bundesgesundheitsblatt 14: 211–213

Madi EA (1987) Characterization of an anaerobic, thermophilic bacterium and optimization of the conditions for the formation of its thermostable amylolytic enzymes. Dissertation, Universität Göttingen

Mayer F (1991) Nahrungsmittel, enthaltend Abfallstoffe aus der kartoffelverarbeitenden Industrie und Verfahren zu seiner Herstellung. Deutsches Patentamt, no. P 41 40 799.7

Mayer F (1995) Klebstoff auf der Basis von Kartoffelpülpe und Verwendung des Klebstoffs. Deutsches Patentamt, no. 1950 9633

Mayer F (1996) Klebstoff auf der Basis von Kartoffelpülpe sowie Herstellung und Verwendung des Klebstoffs. European Patent Office, no. 9610 3823.9

Mossel DAA, Kleynen-Semmeling AMC, Vincente HM (1970) Oxtetraglycine-glucose-yeast extract agar for selective enumeration of moulds and yeasts in foods and clinical material. J Appl Bacteriol 33: 454–457

Pfennig N, Lippert KD (1966) Über das Vitamin B_{12}-Bedürfnis phototropher Schwefelbakterien. Arch Mikrobiol 55: 245–256

Reiss J (1972) Ein selektives Kulturmedium für den Nachweis von *Aspergillus flavus* in verschimmeltem Brot. Zentralbl Bakteriol Mikrobiol Hyg I Abt Orig A 220: 564–566

Rogosa M, Mitchell JA, Wiseman RF (1951) A selective medium for the isolation and enumeration of oral and fecal lactobacilli. J Bacteriol 62: 132–133

Skinner FA (1960) The isolation of anaerobic cellulose-decomposing bacteria from soil. J Gen Microbiol 22: 539–554

Wolin EA, Wolfe RS, Wolin MJ (1964) Viologen dye inhibition of methane formation by *Methanobacillus omelianskii*. J Bacteriol. 87: 993–998