

Stability of Protein Water Concentrates From Potato Starch Factory Waste Effluents

Edward S. DellaMonica, Charles N. Huhtanen and Eugene O. Strolle

US Department of Agriculture, ARS Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, Pennsylvania 19118

The microbial activity and chemical composition of concentrates prepared from potato starch factory waste were studied. Concentrates stored at room temperature and 35 °C showed gas formation and caramelisation; this study showed that these were not caused by indigenous aerobic or anaerobic bacteria. Chemical composition data indicate that these visible signs of instability were due to non-enzymatic browning. Results also reveal that enzymic proteolysis is occurring. Potential use of this product, e.g. as an animal feed, is very limited and will require a final drying.

1. Introduction

Potato starch manufacture consists essentially of physically separating the starch from the pulp, skin and the soluble solids found in the juice. While the pulp and skin can be dried and used as a cattle feed, the solubles are discharged in the waste effluent and present a serious pollution problem. Research efforts at the Eastern Regional Research Center have been seeking ways to recover these solubles profitably so as to pay for part or all of the now mandatory waste treatment cost.

Processes have been developed at the Eastern Center for recovering proteins,¹ amino acids and potassium,² and organic acids and phosphates.³ However, a cost analysis⁴ showed that individual recovery of the solubles is not economically feasible and that the most economically viable method is to concentrate the entire effluent by thermal evaporation and use the concentrates in poultry rations.

We observed, however, that these concentrates, when stored at room temperature, showed marked colour changes along with considerable gas formation suggesting active microbial activity and (hence) gross instability of these products. In order to study these changes a series of concentrates ranging from 46 to 65% total solids was prepared and stored at several temperatures. Both aerobic and anaerobic methods were used to measure microbial populations. Also reported are the results of concurrent studies on the chemical composition of these concentrates.

2. Experimental

2.1. Preparation of potato protein water (PPW)

2.1.1. Preparation of protein water

Idaho Russet potatoes were washed, ground and sulphited (2 g NaHSO₃/kg fresh potatoes), and the slurry pumped to a solid-bowl continuous centrifuge to produce

full-strength juice. Additional solubles were recovered by rewetting and regrinding the cake and centrifuging again. The liquids were combined to give PPW containing 3.8–4.5% solids.

2.1.2. Concentration

Small batches (2–4 litres) were prepared in a Mojonier^a stainless steel, falling-film evaporator whose heat transfer section consisted of 10 tubes, 24 in (60.96 cm) long, 7/8 in (2.22 cm) i.d., approximately 4.6 ft² (0.428 m²) heating surface. A vacuum of 700–725 mm (*ca* 32–42 °C) was used, the heating medium was vacuum steam, and the solids content was determined periodically during the evaporation with a hand refractometer. The true concentration was obtained from a previously determined calibration curve for Idaho Russet concentrates. These concentrates were then distributed into a series of screw cap jars and stored at room temperature (22–25 °C) and 37 °C. At each time interval during the storage study the contents of individual jars were analysed.

2.2. Microbiological methods

Aerobic plate counts were made using plate count agar (PC Difco), potato dextrose agar (PD Difco) and tryptone glucose extract agar (TGE Difco) with incubation for two days at room temperature (22–25 °C), 30 °C or 35 °C. Anaerobic plate counts were made in anaerobic agar (AN Bioquest) with incubation at 30 °C for two days in a Torbal (Torsion Balance Co., Clifton, New Jersey) jar using an atmosphere of H₂, CO₂ and N₂ (10:10:80). The Torbal jars were evacuated twice and the atmosphere replaced with this gas mixture.

2.3. Chemical methods

Total nitrogen was determined directly on a weighed portion of concentrated PPW by the semi-micro Kjeldahl method.⁵ Protein nitrogen content was determined in the following manner. A weighed aliquot of concentrate was reconstituted to an original juice strength of approximately 5% total solids and the protein was precipitated by adding 15% trichloroacetic acid containing a few drops of 0.1% sodium tungstate. After centrifuging the supernatant was discarded and the protein pellet washed once with 2% trichloroacetic acid. The washing was discarded and the acid precipitated protein pellet was transferred to a Kjeldahl flask for nitrogen determination. The non-protein nitrogen was calculated as the difference between total and the trichloroacetic acid precipitable protein nitrogen.

The sulphur dioxide concentrations were determined as described by Nury *et al.*⁶ Sulphamic acid was added to the sodium tetrachloromercurate solution to eliminate possible interference in the determination of sulphur dioxide due to nitrogen dioxide.⁷

Titrateable acidity was determined using Glass Electrode Method⁸ for highly coloured solutions. Acidity is reported as per cent citric acid.

Total solids of the PPW concentrates were determined on a weighed sample which had been evaporated to near dryness on a steam bath. Final drying was done in a vacuum oven set at 70 °C, > 25 mm vacuum, for 16 h.

^a Use of a company or product names does not imply approval or recommendation of the product to the exclusion of others which may also be suitable.

Gas formation was observed after opening of the jars as an overflow or increase in volume of the contents. Caramelisation was noted as a colour change of the concentrate from its original light grayish-tan to a darker brown.

3. Results

3.1. Microbiological studies

Several preliminary studies on a PPW concentrate were done to select a suitable culture medium and incubation temperature for subsequent studies. Table 1 shows the results obtained using PC, PD, TGE, AN and mixtures of PD + PC and PD + TGE. The counts were all about the same except PD which, when used by itself, gave lower counts. Incubation was for a total of seven days, although there were no changes in counts after the second day.

Table 1. Growth of bacteria from potato protein water concentrates in different media

Agar medium	Aerobic	Anaerobic
	Counts $\times 10^{-6}/g$	
Plate count	16.0	—
Potato dextrose	2.5	—
Plate count and potato dextrose (50:50)	12.0	—
Tryptone glucose extract	13.0	—
Tryptone glucose extract and potato dextrose (50:50)	12.0	—
Anaerobic	16.0	14.0

Another experiment comparing room temperature and 35 °C incubation was made using TGE agar with PPW before and after concentration in the Mojonnier evaporator at 711 mmHg vacuum and 36.7 °C. The PPW before concentration had counts of 5 and 2×10^6 per ml for room temperature and 35 °C respectively, while the concentrates had counts of 4.8 and 4.5×10^3 per ml. It was apparent from this experiment that most of the original bacteria were killed during the Mojonnier concentration.

Table 2. Effect of inoculation on bacteria in potato protein water concentrate after one week storage at room temperature

Concentrate	Plate count		
	Initial (counts $\times 10^{-5}/g$) ^a	After 1 week (counts $\times 10^{-5}/g$) ^a	Increase (%)
PPW-14	3.9	5.8	49
PPW-15	27.4	41.0	49
PPW-14 + 1.5% PPW-15	3.9	7.2	85

^a Counts are average of two, incubation under anaerobic conditions in AN agar, 2 days at 30 °C.

Inoculation of a low bacterial count concentrate (PPW-14) with 1.5% of a high count concentrate (PPW-15) gave somewhat higher counts after one week of storage (Table 2). The counts, however, did not reach the levels of the higher bacterial count concentrate. Both high and low bacterial concentrates showed about 50% increase in counts after one week at room temperature while the inoculated PPW increased 85%. These preliminary experiments which showed less than a doubling of the original bacterial count in a week indicated that the PPW concentrates did not support active growth. We then planned a more comprehensive study using a series of PPW concentrates of different solids content stored for extended periods of time at several temperatures.

Table 3. Effect of prolonged storage on gas formation, colour and bacterial counts of potato protein water concentrates

Concentrate no.	Storage time (days)	Storage temperature (°C)	Colour formation ^a	Gas formation ^b	Bacterial counts (× 10 ⁻⁵ /g)	
					Aerobic	Anaerobic
PPW-18 (65.6%) ^c	0		—	—	160	155
	3	22-25	0	0	180	172
	7		0	0	95	112
	35		P	0	20	26
	3	37	0	0	13	40
	7		P	0	11	33
	35		P	0	8	18
PPW-19 (55.6%)	0		—	—	4.7	9.5
	3	22-25	0	(×) ^d	2.2	3.2
	7		0	(×)	1.8	0.30
	35		S	0	1.2	0.52
	3	37	0	+++	1.6	2.2
	7		0	+++	1.3	0.34
	35		P	++++	1.7	0.39
PPW-20 (48.6%)	0		—	—	38	3.0
	3	22-25	0	+++	25	2.4
	7		0	++++	17	1.4
	35		0	+++	4.8	3.6
	3	37	0	++	1.8	0.5
	7		0	++	2.6	0.95
	35		P	++	2.1	0.93
PPW-21 (61.8%)	0		—	—	41	0.98
	3	22-25	0	0	16	0.81
	7		S	+	11	0.42
	35		P	0	12	2.5
	3	37	0	+	12	1.6
	7		0	0	8.2	0.41
	35		P	0	10	3.2
PPW-24 (56.1%)	0		—	—	10	9.4
	3	22-25	0	++	5.7	2.8
	7		0	+++	12	7.2
	35		0	++++	3.2	1.4

^a 0, no colour formation; S, slight; P, pronounced.

^b 0 to +++++, degree of gas production.

^c Total solids.

^d (×), increase in volume, no overt gas.

The results of the comprehensive study of the bacteriological changes of the PPW concentrate are shown in Table 3. Round screw cap jars were filled with the concentrates and examined at intervals for visual gas formation, colour changes (particularly the appearance of caramelisation) separation of the liquid from the solid phase, and aerobic and anaerobic bacterial counts. Four of the five concentrates stored at 37 °C showed caramelisation in 35 days and one in only 7 days. When they were stored at room temperature, two showed caramelisation at 35 days and one at 7 days. Four of the concentrates showed some gas production by the third day at both temperatures. PPW-19 at 3 and 7 days showed only volume changes on opening; this was probably the result of dissolved gas of insufficient quantity to cause overflow of the concentrate. PPW-20 having lowest solids content showed separation of the liquid and solid phase. This occurred in 3 days, was not evident at 7 days and recurred in 35 days in both the room temperature and 37 °C stored concentrates. None of the other samples showed this liquid separation.

The bacterial counts after the storage intervals are also shown in Table 3. There did not appear to be any correlation between the changes in bacterial counts and the appearance of either gas or caramelisation. The counts in general decreased on storage. There appeared to be a more rapid decline in numbers at the higher temperature. At zero time the aerobic and anaerobic counts were about the same in PPW-18 and PPW-24. The anaerobic counts were somewhat higher in PPW-19 and were lower in PPW-20 and PPW-21. These studies indicated that the chief visible signs of instability were not the result of microbial action.

3.2. Chemical studies

Six of the PPW concentrates stored at room temperature over a 91 day period were analysed to determine titratable acidity, sulphur dioxide levels, total nitrogen as well as protein nitrogen, pH changes and total solids. Results of this study are shown in Table 4.

The consistency of the various concentrates ranged from a syrupy solution to a semi-solid paste. Although the total solids varied from 48 to 65% there was no correlation between consistency and solids content. The initial pH of each sample varied only slightly (pH 6.08 to 6.25) and remained fairly constant for the first 35 days of storage. During this same storage interval the titratable acidity decreased in all but the first concentrate analysed. At the end of the storage period all but one concentrate showed an increase in titratable acidity over the 35 day value and a drop in pH.

Kjeldahl analyses indicated very little variation in total nitrogen content of the PPW concentrates over the entire storage period. Also, the protein nitrogen in all samples decreased slightly during the initial seven days at room temperature. However, at the end of the 35 day interval four of the concentrates showed a definite decrease in protein nitrogen. In the two concentrates showing minimal decrease in protein nitrogen at the early stages of this storage study, the sulphur dioxide content was found to be in excess of 700 parts/10⁶. When the sulphur dioxide level decreased significantly the protein nitrogen likewise decreased. After 91 days storage trichloroacetic acid (TCA) precipitable protein nitrogen of all samples was significantly less than at the beginning of the storage period indicating proteolytic activity.

Table 4. Effect of prolonged storage on the chemical composition of potato protein water concentrates^a

Concentrate no.	Storage time (days)	Total solids (%)	pH	Titrateable acidity (% MFB)	Total nitrogen (% MFB)	Protein nitrogen (% MFB)	Sulphur dioxide (parts/10 ⁶)
PPW-18	0	65.58	6.08	4.65	6.64	1.84	—
	7	—	6.18	5.37	7.15	1.80	—
	35	—	5.97	5.89	6.49	1.59	30.0
	91	—	5.70	6.76	6.70	1.48	—
PPW-19	0	55.60	6.13	6.33	7.42	2.19	—
	7	—	6.24	6.08	7.40	2.04	134
	35	—	6.19	5.69	7.16	1.56	—
	91	—	6.00	5.95	7.09	1.57	—
PPW-20	0	48.61	6.25	5.84	7.31	1.94	—
	7	—	6.29	5.42	6.58	1.67	131
	35	—	6.36	5.09	6.80	1.43	131
	91	—	6.05	5.01	6.60	1.15	20
PPW-21	0	61.78	6.11	5.77	6.48	1.63	532
	7	—	6.18	5.17	6.38	1.60	437
	35	—	6.08	5.55	6.46	1.46	—
	91	—	5.90	6.36	6.62	1.43	—
PPW-24	0	56.08	6.11	5.32	5.68	1.18	>700
	35	—	6.20	4.91	5.70	1.17	—
	56	—	6.09	5.15	5.77	1.00	33
	91	—	6.10	5.99	6.14	0.94	—
PPW-25	0	58.30	6.22	5.06	6.28	1.29	>700
	35	—	6.30	4.73	6.40	1.33	—
	56	—	6.10	5.30	6.08	1.25	26
	91	—	6.13	5.46	6.29	1.00	—

^a Storage temperature, 22–25 °C.

4. Discussion

The chief visible signs of instability in the PPW concentrates were the production of gas, caramelisation and liquid separation. The gas production was probably not the result of proliferation of microorganisms *per se* since the number of bacteria generally decreased during the storage period nor did it appear to be correlated with high initial bacterial levels. The concentrate with the highest initial bacterial count (PPW-18) showed no gas formation at 37 °C or room temperature. However, the concentrate with the lowest initial count (PPW-19) did show considerable gas formation at 37 °C and little gas at room temperature.

It is more likely that the gas formation was due to the production of carbon dioxide which Maillard⁹ observed in the latter stages of the reaction between glucose and glycine. Talley *et al.*¹⁰ have shown that potato contains ample free amino acids which readily react with reducing sugars. From experience we have found 25–40% (MFB) reducing sugars in PPW concentrates. Lewis *et al.*¹¹ reported a similar spontaneous formation

of carbon dioxide in canned foodstuffs stored at 50 and 100 °C. Using model systems, Cole¹² further studied the reaction between free amino acids and reducing sugars and found a significant relationship ($r = +0.90$) between carbon dioxide formation and the rate of browning. He also noted a one unit drop in pH after his model systems became very dark. A similar drop in pH was observed in the present study (Table 4) in all six PPW concentrates. In four of the concentrates colour formation was obvious after 35 days storage at 37 °C. In the two preparations containing excess sulphur dioxide (> 700 parts/10⁶) significant retardation of colour formation was observed. Lewis *et al.*¹³ reported a similar observation with model systems containing 2000 parts/10⁶ sulphur dioxide.

The results of this study also indicate that proteolytic activity is occurring concurrently with the non-enzymatic browning reaction. All concentrates showed a decrease in TCA precipitable protein nitrogen after only 7 days storage at room temperature. The only exceptions were the concentrates containing high levels of sulphur dioxide, a known inhibitor of enzymic activity. After 13 weeks the concentrates all showed a marked decrease in protein nitrogen (TCA precipitable). The presence of a proteolytic enzyme in the juice of potato tubers was demonstrated by Niemann.¹⁴ He later showed¹⁵ that the proteolytic enzyme could hydrolyse several proteins including potato protein. It appears that along with the physical instability due to non-enzymatic browning an enzymic hydrolysis of the potato protein also occurs.

5. Conclusions

The instability of potato protein water concentrates, namely, gas formation and caramelisation are the result of the non-enzymatic browning reaction. Hydrolysis of potato protein by a proteolytic enzyme present in potato juice is occurring simultaneously. The potential for commercial usage of potato protein water concentrates is limited because of its instability. A dry preparation may be more desirable, and research is being conducted at this laboratory to determine optimum conditions (cost and quality) for preparing a dry, stable, free flowing feed material.

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