

Origins of the Poor Filtration Characteristics of Wheat Starch Hydrolysates

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ABSTRACT

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The effects of wheat starch components on the filtration characteristics of wheat starch hydrolysates were investigated with a model-based approach. The filtration rate was not affected by the removal of the pentosans or by altering the conformation of the protein. On the other hand, the filtration rate increased when a hydrolysate was defatted with chloroform or butanol. Some commercially available enzymes also increased the filtration rate. The filtration rate of potato starch hydrolysates decreased when gluten, pentosans, solubles, or propanol extract from defatted wheat starch were added. The latter had by far the largest effect. The composition of this extract was 65% lipid and 11% protein. The main lipid in wheat starch is lysophosphatidylcholine (LPC). This single-chain lipid

forms micelles above a concentration of 0.025 g/kg. The filtration rate decreased when LPC was added to potato starch hydrolysates or glucose solutions at concentrations above the critical micelle concentration. This effect of LPC on glucose solutions proves that the filtration characteristics are not related to the formation of amylose-lipid complexes. Therefore, micelle formation must be responsible for the effect of LPC on the filtration rate. The critical micelle concentration is only 2.5% of the amount of lysophospholipids in wheat starch hydrolysates. Thus, almost all of these lipids have to be removed from wheat starch hydrolysates to increase the filtration rate.

In Europe, wheat starch is often used as raw material for the preparation of hydrolysis products. In an enzymatic process, wheat starch is heated and converted into a hydrolysate. Subsequently, the hydrolysate is filtered to remove the insoluble impurities. Activated carbon and ion-exchange resins are used for further refinement of the hydrolysates (Schenck and Hebeda 1992).

In a separate article (Matser and Steeneken 1998), we described differences between the filtration characteristics of wheat and maize starch hydrolysates. Wheat starch hydrolysates have very poor filtration characteristics: low filtration rates, almost no removal of the undesired components, and obstruction of the filter cake. On the other hand, maize starch hydrolysates show good filtration characteristics. It is possible to remove the largest part of the undesired components of maize starch hydrolysates in a relatively short time (due to the high filtration rate). Because hydrolysis products are the main end use of wheat starch, the objective of this study was to investigate the main reasons for the poor filtration characteristics of wheat starch hydrolysates.

There are many theories about the factors that influence the filtration rate of wheat starch hydrolysates adversely. The following components are supposed to have a negative effect on the filtration rate of wheat starch hydrolysates: nongelatinized starch (Bowler and Towersey 1985), pentosans (Bowler and Towersey 1985, Derez 1987, Ducroo 1987, Konieczny-Janda and Richter 1991), amylose-lipid complexes (Hebeda and Leach 1974), and complexes between lysophospholipids and amylose or proteins (Konieczny-Janda and Richter 1991).

In this article, we describe a model-based approach to the influence of the wheat starch components on the filtration characteristics of the hydrolysates. First, we removed components from wheat starch hydrolysates and evaluated the effects on the filtration rate. Second, we decreased the amounts of nonstarch components in wheat starch. The hydrolysates made of these starches were examined for their filtration characteristics. The components removed from wheat starch were collected and added one-by-one to potato starch. Because potato starch has very good filtration characteristics, the effects of the added components on the hydrolysates' filtration rate are an indication of the influence on the filtration characteristics of wheat starch. In this manner, the effects of the individual components of wheat starch on the filtration characteristics were studied.

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MATERIALS AND METHODS

Materials

Wheat starch was a gift from Cerestar (Sas van Gent, The Netherlands), and potato starch and potato dextrose were gifts from Avebe (Veendam, The Netherlands). Enzyme products used included: Termamyl 120 L, Palatase M, Neutrase, SP 348, Pulpzyme HB, Energex, Viscozym (Novo Nordisk, Denmark), Amigase, Spezyme, and Maxazyme (Genencor, Delft, The Netherlands). Lysophosphatidylcholine (LPC) was purchased from Sigma (Zwijndrecht, The Netherlands), and bran was obtained from a local grocery.

Preparation and Filtration of Hydrolysates

The hydrolysates were prepared according to an enzymatic process with two heating steps in a laboratory-scale jet cooker. We used a 30% suspension of starch in water with 300 μ L of Termamyl/kg of suspension and 400 mg of CaCl₂·2H₂O/kg of suspension. Termamyl 120 L is a heat-stable α -amylase. The first heating step was performed at 105°C, and the second was performed at 140°C. Incubation was performed at 95°C. After the second conversion, the enzyme was inactivated due to the high temperature. Therefore, a second Termamyl dosage (300 μ L/kg of suspension) was added.

The filtration rate was measured with a laboratory-scale vacuum filter with a filter aid (Dicalite, Grefco). Large-scale filtration was imitated by a filtration cycle of: 40 sec of immersion of the filter in the hydrolysate under vacuum, 40 sec of vacuum suction of the filter while the filter was not in the hydrolysate, and 40 sec during which 50 μ m of the top layer was cut off under vacuum to remove the insoluble particles and to expose a fresh surface. This filtration process is called a discontinuous filtration. The continuous filtration rate was measured by immersion of the filter in the hydrolysate under vacuum and weighing the amount of filtrate after a certain time.

This process and the filtration of the hydrolysates are described in more detail in a separate article (Matser and Steeneken 1998). The analytical methods used were described in the same article.

All filtration experiments were done in duplicate. The values in the tables are the means of the duplicates. The standard deviation of these experiments is 10%. The standard deviation of the lipid and protein concentration in the hydrolysates is 5%.

Critical Micelle Concentration

The surface tension was measured as function of the concentration with a Lauda TVT surface tensiometer at 25°C. We measured the surface tension of each concentration in tenfold. The critical micelle concentration (CMC) is defined as the concentration at which the surface tension drops sharply.

RESULTS AND DISCUSSION

Physicochemical Treatment of Wheat Starch Hydrolysates

We changed the composition of wheat starch hydrolysates with different methods and evaluated the effects on the filtration rate. Gruppen et al (1989) explained that pentosans could be removed from wheat starch by sieving through a sieve with pores <50 μm . We sieved wheat starch hydrolysates through sieves with pores of 45 or 25 μm and measured the filtration rate (Table I). The concentration of pentosans was not measured before and after sieving. Evaluating the effectiveness of the removal was not possible. The standard deviation of the filtration experiments was $\approx 10\%$. From Table I, we conclude that the filtration rate is not changed by sieving the wheat starch hydrolysates.

We did not remove protein from the hydrolysates. The conformation of the proteins was changed by alteration of the pH or by addition of some complexing agents. Table I shows that the filtration rate was not changed substantially by these treatments. The filtration rate of the hydrolysate with pH 11 was increased. However, the difference with the nontreated hydrolysate was small when compared to the filtration rate of butanol-extracted hydrolysates. The protein and lipid contents of the hydrolysates and filtrates were determined. A measure for the effectiveness of the filtration is the difference between the lipid or protein content of the hydrolysate and that of the filtrate. The alterations of the conformation of the proteins did not influence these differences, which were quite small anyhow. Hence, these treatments did not change the filtration rate or the filtration characteristics.

Lipids were removed from wheat starch hydrolysates with several solvents. The filtration rate was determined according to the discontinuous method described in elsewhere (Matser and Steenen, 1998). Some experiments were repeated. The filtration rates of these hydrolysates were measured with a continuous filtration. Comparison of continuous and discontinuous filtration was not possible, so we applied both filtration methods on a nontreated hydrolysate. From Table I, we conclude that extraction with butanol increased the filtration rate substantially. Extraction with refined petrol increased the filtration rate in the continuous

filtration but only to a minor extent. Extraction with chloroform resulted in an increase of the filtration rate of the continuous filtration but not of the discontinuous filtration. The lipid and protein concentrations of the hydrolysates are given in Table II. Extraction of hydrolysates with butanol or chloroform decreased the lipid and protein contents. The other treatments only had a small influence on the composition of the hydrolysates. We compared the filtration rates with the protein and lipid concentrations. The results show that the filtration rate increased substantially when the lipid content decreases from 0.12 g/kg to 0.09 g/kg. This suggests an upper limit for satisfactory filtration of 0.1 g of lipid/kg of hydrolysate. This may explain the large difference in filtration rates of chloroform-extracted hydrolysates in discontinuous and continuous filtration. The chloroform-extracted hydrolysate used for discontinuous filtration had a lipid content that was slightly higher than the threshold (Table II).

The main effect of the extraction of hydrolysates with butanol or chloroform appears to be a reduction of the lipid content. Since the extraction resulted also in a reduction of protein, it cannot be ruled out that protein also has a negative influence on the filtration rate.

Effect of Enzymes on Filtration Rate

We investigated the influence of different enzymes on the filtration rate of wheat starch hydrolysates. Table III lists the enzyme preparations used, their main activities, and the filtration rates after treatment. All incubations were done at the pH, temperature, and dosage recommended by the manufacturers. Besides the main activities mentioned in Table III, most enzyme mixtures contained additional enzymes at lower activity levels.

We concluded that several enzyme preparations increased the filtration rate: the glucoamylase mixtures (Amigase and Spezyme) and the mixtures with more than six enzymes (Energex and Viscozym). All enzyme preparations were tested at one concentration only. Because the activities of the different enzymes present in these preparations (and the identity of some of them) were unknown, it is difficult to decide which of these enzymes had a real effect on the filtration rate and, hence, which components in the hydrolysates determine the filtration characteristics.

Removal of Components from Wheat Starch

We investigated the effects of removing certain components from wheat starch on the filtration rate of hydrolysates made of

TABLE I
Filtration Rates of Wheat Starch Hydrolysates After Physicochemical Alteration of the Components

Component	Treatment	Filtration Rate (g sec ⁻¹ m ⁻²)	
		Discontinuous	Continuous
None		29.5	259.7
Pentosans	45- μm sieved	33.9	...
	25- μm sieved	27.9	...
Protein	HCl, pH \approx 0	21.1	...
	H ₂ SO ₄ , pH \approx 0	28.1	...
	NaOH, pH 11	41.0	...
	1% CuSO ₄	27.5	...
	EDTA, ^a pH 4.4	36.8	...
	Lipid	Refined petrol extraction	36.6
	Butanol extraction	808.3	845.5
	Chloroform extraction	38.8	768.2
	Dichloromethane extraction	30.9	...

^a Ethylenediaminetetraacetic acid.

TABLE II
Lipid and Protein Content in Hydrolysates (g/kg) After Extraction with Solvents

Extraction Solvent	Filtration	Lipid	Protein
None	Discontinuous	0.93	0.58
Refined petrol	Discontinuous	0.84	0.56
Butanol	Discontinuous	0.09	0.30
Chloroform	Discontinuous	0.12	0.22
Chloroform	Continuous	0.02	0.17
Dichloromethane	Discontinuous	0.78	0.46

TABLE III
Enzyme Mixtures and Filtration Rates of Hydrolysates

Mixture	Enzymes	Rate (g sec ⁻¹ m ⁻²)
Control	None	30.3
Amigase	Glucoamylase	450.2
Spezyme	Glucoamylase	466.0
Neutraxe	Protease	25.5
Maxazyme	Xylanase	33.7
Pulpzyme HB	Xylanase	30.3
Palatase M	Lipase	24.6
SP348	β -glucanase, pentosanase	20.3
Energex	Additional enzymes ^a	138.8
Viscozym L	Additional enzymes ^a	190.1

^a Arabinase, xylanase, β -glucanase, hemicellulase, pectinase, and cellulase.

TABLE IV
Protein and Lipid Contents of Wheat Starches^a

Component Removed	Lipid	Protein	Rate ^b
None	0.48	0.23	32.5
Pentosans	0.50	0.22	34.9
Protein, 0.16M NH ₃	0.46	0.18	32.8
Protein, pH 10–10.5	0.43	0.19	32.4
Lipid	0.12	0.15	43.2

^a Components were removed and filtration rates of the hydrolysates were made from these starches. Concentrations in g/100 g of dry starch.

^b Filtration rate in g sec⁻¹m⁻².

these starches. Insoluble pentosans were removed by sieving wheat starch through a sieve with pores of 45 μm . Protein was removed by suspending wheat starch in 0.16M NH_3 solution or a solution of NaOH in water (pH 10–10.5). These suspensions were centrifuged, followed by removal of the brown layer that had precipitated separately. Lipid was removed by refluxing starch with 75% aqueous propanol according to Morrison and Coventry (1985).

Table IV shows the protein and lipid contents of the starches and the filtration rates of the hydrolysates made of these starches. None of these treatments increased the filtration rates of wheat starch hydrolysates. However, the procedures to remove protein did not cause a reduction of the protein content of wheat starch. Refluxing with propanol caused a reduction of the lipid content. Lipids ($\approx 25\%$ of the original amount) were still present in the starch. In view of our previous discussion on the possible effects of lipids, it is probable that this remaining amount also needs to be removed for an increase of the filtration rate.

Addition of Components to Potato Starch

Hydrolysate made of potato starch had a filtration rate of 671 $\text{g sec}^{-1}\text{m}^{-2}$. This is remarkably higher than the filtration rate of hydrolysates made of maize starch (211 $\text{g sec}^{-1}\text{m}^{-2}$) or wheat starch (33 $\text{g sec}^{-1}\text{m}^{-2}$). We investigated the effect of components of wheat starch on the filtration rate of starch hydrolysates by adding these components to potato starch. Using a wet-milling process, we fractionated Minaret wheat into starch, gluten, pentosans, germ, bran, and solubles (Meuser et al 1989). The solubles consisted of the process water remaining after centrifugation. Germ and bran were separated by sedimentation of the combined germ-bran mass. These components were added to potato starch in a concentration of 1% on dry matter basis except for germ and solubles. The germ was used in a concentration of 0.1%. The solubles were added by using centrifuged process water instead of water. Table V shows the concentration of protein and lipid in the hydrolysates and the filtration rate of the hydrolysates made from potato starch with added components. Germ, bran, and wheat starch did not influence the filtration rate of potato starch hydrolysates at the concentration used in these experiments. Gluten, pentosans, and solubles decreased the filtration rate of potato starch hydrolysates. The filtration rates of hydrolysates made with these components were lower than that of potato starch alone. However, the rates were considerably higher than those of wheat starch hydrolysates. The protein and lipid contents were altered by the addition of wheat components. There was a negative relation between the filtration rate and the protein or lipid content of the hydrolysate. This suggests that protein or lipid has a negative influence on the filtration rate of hydrolysates. However, there was also a strong correlation between the protein and the lipid concentration of the hydrolysates. The effects of both components on the filtration rate could not be separated.

We also added the wheat starch lipid fraction obtained by propanol extraction to potato starch and evaluated the effect on the

filtration rate of the hydrolysates. The filtration rates of potato starch hydrolysates decreased to the values found for wheat starch hydrolysates. Even the addition of only 10% of the amount of the lipids present in wheat starch caused a large reduction of the filtration rate (Table V). It is remarkable that in the latter case the lipid content is lower than the presumed threshold value of 0.10 g/kg .

We have described a model-based approach to characterize the origins of the poor filtration characteristics of wheat starch hydrolysates. From the results of these experiments, we conclude that the filtration rate of wheat starch hydrolysate was not affected by the removal of the pentosans by sieving or by altering the conformation of the protein. The filtration rate increased when a wheat starch hydrolysate was defatted with butanol or chloroform. This suggests that lipids cause the low filtration rate of wheat starch hydrolysates. However, these extractions with butanol or chloroform also removed some protein. Some commercially available enzyme mixtures increased the filtration rate of wheat starch hydrolysates. Because these mixtures are combinations of enzymes, it is not possible to conclude which enzymes improved the filtration rate. Removal of (part of) the pentosans, protein, or lipid from wheat starch did not alter the hydrolysate filtration rate. It cannot be ruled out that removing a still larger part of these components will increase the filtration rate. The filtration rate of potato starch hydrolysates decreased by adding gluten, pentosans, solubles, or the propanol extract of wheat starch to potato starch. The propanol extract caused the largest reduction of the filtration rate of potato starch hydrolysates. The composition of this extract was 34.6% lipids, measured as fatty acids, and 11% protein. The results of the experiments suggest that the presence of lipids in wheat starch is the main factor in disturbing the filtration process. However, we were not able to use pure components in these experiments. It cannot be ruled out that problems in hydrolysate filtration are enhanced by combination of impurities.

Effect of Lysophospholipids

According to Morrison (1988), wheat starch contains 773–1,171 $\text{mg}/100 \text{ g}$ of lipids. Of these, lysophospholipids are by far the most important. The most abundant single lipid is LPC (499–684 $\text{mg}/100 \text{ g}$). On the basis of these data, we estimate that the lipid concentration in the propanol extract is $\approx 65\%$ on a dry matter basis. The structure of LPC is given in Fig. 1. Lysophospholipids contain just one fatty acid residue and can form micelles. We propose that these micelles can obstruct the filter cake during the filtration of wheat starch hydrolysates. These components may also alter the filtration characteristics by forming amylose-lipid complexes.

The formation of micelles can be studied by measuring the surface tension as a function of the concentration as shown in Fig. 2. At first, the surface tension did not change when the concentration of the propanol extract was increased. At higher concentrations, the surface tension decreased because of the adsorption of molecules at the surface. At a certain concentration, a stable value of the surface tension was achieved. Increasing the concentration of extract did not lower the surface tension further. This is caused by the formation of micelles above the CMC. For the propanol extract, the CMC was $\approx 0.04 \text{ mg}$ of extract/ g of water. This corresponds to 0.014 g of fatty acid/ kg of water. Above this concentration, micelles are formed that can obstruct the filter cake. This concentration is very low when compared with the concentration of fatty acids in a wheat starch hydrolysate (0.6 g/kg of hydrolysate).

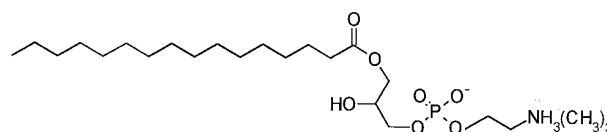


Fig. 1. Structure of lysophosphatidylcholine.

TABLE V
Protein and Lipid Contents^a and Filtration Rates of Hydrolysates from Potato Starch with Added Components

Added Component	Lipid	Protein	Rate ^b
None	0.02	0.13	671.3
Pentosans	0.04	0.47	459.7
Solubles	0.08	1.09	358.3
Germ	0.02	0.17	661.6
Gluten	0.05	0.68	318.4
Bran	0.04	0.29	607.9
Wheat starch	0.03	0.15	729.2
100% propanol extract	0.44	0.37	43.5
10% propanol extract	0.04	0.20	55.3

^a Concentrations in $\text{g}/100 \text{ g}$ of dry starch.

^b Filtration rate in $\text{g sec}^{-1}\text{m}^{-2}$.

Figure 3 shows the filtration rate as a function of the fatty acid content of the hydrolysates. Low concentrations of fatty acids resulted in a very high filtration rate, while high concentrations resulted in a very low filtration rate. The turning point between a high and a low filtration rate is not the same for all experiments. This transition lays between 0.02 and 0.04 g/kg for potato starch hydrolysates with added propanol extract and between 0.09 and 0.12 g/kg for defatted wheat starch hydrolysates. Both are in reasonable agreement with the CMC of the propanol extract.

With these results, it is possible to explain why defatting wheat starch did not affect the filtration. The residual concentration of fatty acids was still 0.20 g/kg in the defatted hydrolysate. This is much higher than the CMC. There was no relationship between the filtration rate and the protein concentration (data not shown). Although the extract we used also contained 11% protein, we conclude that occurrence of lysophospholipids above the CMC is the main origin of the poor filtration characteristics of wheat starch hydrolysates. To examine the effects of lysophospholipids in more detail, we conducted some experiments with pure LPC.

The CMC of LPC was determined in water at 25°C. The CMC was 0.025 mg/g. The LPC used contains mainly C₁₆ and C₁₈ fatty acid residues and an average molecular mass of 500. The CMC is $5 \times 10^{-5}M$. This agrees with the CMC of diacylphosphatidylcholine of $10^{-4.5}M$ (Kabalnov et al 1995). The CMC was measured at 25°C although the filtration experiments were conducted at different temperatures. Generally, the CMC increases at higher temperatures. However, this increase is limited to a few percent (Evans and Wennerström 1994). We added several concentrations of LPC

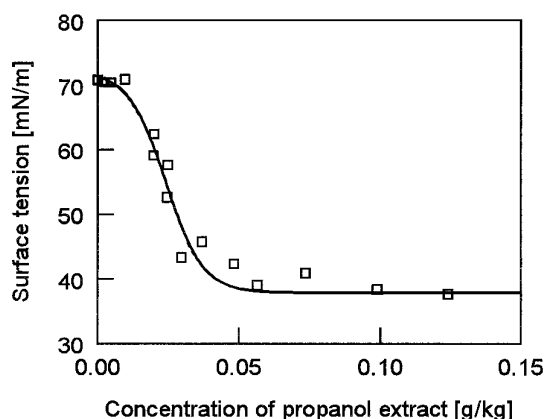


Fig. 2. Surface tension as a function of the concentration of propanol extract from wheat starch.

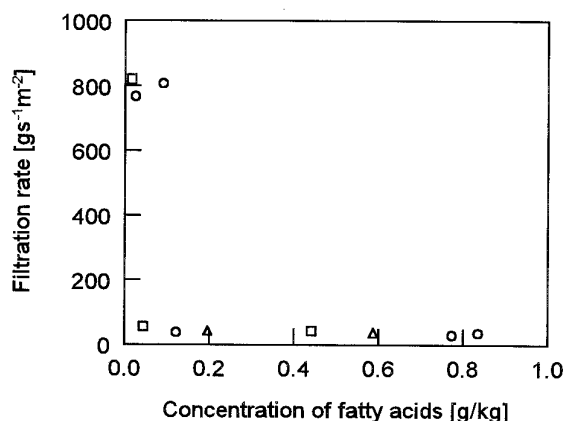


Fig. 3. Filtration rate as a function of the concentration of fatty acids in hydrolysates of wheat starch or defatted wheat starch (Δ), potato starch hydrolysates with added propanol extract (\square), or defatted wheat starch hydrolysates (\circ).

to potato starch and measured the filtration rates of hydrolysates made from it (Table VI). LPC strongly decreased the filtration rates of potato starch hydrolysates. Up to the CMC, the filtration rate was not affected by the addition of LPC. The filtration rate decreased above the CMC. It is worth noting that a second filtration through a used filter cake resulted in a considerably lower filtration rate (Experiments D2 and E2). A reason for this could be that obstruction of the filter cake only occurs after a certain number of micelles have been filtered. Because the reduction of the filtration rate started above the CMC, it is reasonable to assume that the formation of micelles is the explanation for the influence of LPC on the filtration rate. However, the formation of amylose-lipid complexes could also have an impact on filtration characteristics. Therefore, we added LPC to a solution of 25% glucose in water and measured the filtration rate. The results of these experiments are shown in Table VII. The overall behavior of the glucose solution and potato starch hydrolysates with added LPC were quite similar. The filtration rate strongly decreased when LPC was added at concentrations above the CMC. In a glucose solution, amylose-lipid complexes cannot be formed. These results suggest that the formation of micelles of LPC is the main cause for the poor filtration characteristics of wheat starch hydrolysates.

CONCLUSION

This article describes the influences of removing components from wheat starch (hydrolysates) or adding wheat components to potato starch on hydrolysate filtration characteristics. Some alterations affected the filtration process. An efficiently defatted wheat starch hydrolysate had a higher filtration rate than a nondefatted hydrolysate. The filtration rate of potato starch hydrolysates decreased after adding gluten, pentosans, solubles, or the propanol extract of the defatted wheat starch. These results suggest that lipids exert an important influence on the filtration process.

TABLE VI
Filtration Rate and Concentration of Lysophosphatidylcholine (LPC) of Hydrolysates from Potato Starch with Added LPC

Hydrolysate ^a	LPC ^b	Rate ^c
Potato starch	...	729.6
B	0.024	826.0
C	0.024	738.2
D1	0.060	309.9
D2 ^d	0.060	91.1
E1	0.114	96.5
E2 ^e	0.114	91.8

^a Letters B through E2 represent experimental batches.

^b Concentration in g/kg.

^c Filtration rate in $g \text{ sec}^{-1}m^{-2}$.

^d Same filtercake as D1.

^e Same filtercake as E1.

TABLE VII
Filtration Rate and Concentration of Lysophosphatidylcholine (LPC) in Solutions of 25% Glucose with Added LPC

Solution ^a	LPC ^b	Rate ^c
Glucose	...	502.6
B	0.015	432.4
C	0.015	391.8
D1	0.050	236.9
D2 ^d	0.050	116.2
E1	0.100	156.3
E2 ^e	0.100	68.2

^a Letters B through E2 represent experimental batches.

^b Concentration in g/kg.

^c Filtration rate in $g \text{ sec}^{-1}m^{-2}$.

^d Same filtercake as D1.

^e Same filtercake as E1.

LITERATURE CITED

- Lipids in wheat starch are mainly lysophospholipids. LPC is the most abundant lipid. These lipids form micelles above a concentration of 0.025 g/kg. When LPC was added to potato starch or glucose solutions, the filtration rate decreased until values were reached that were equal to those of wheat starch hydrolysates. The filtration rate decreased only above the CMC (0.10 g/kg). In combination with the reduction of the filtration rate after adding LPC to glucose solutions, the formation of micelles is the most probable mechanism for the effect of LPC on the filtration rate. Formation of amylose-lipid complexes is not plausible as an explanation.
- The concentration of fatty acids in wheat starch hydrolysates is 0.6 g/kg. This is equivalent to 1 g of lysophospholipids/kg and 40× higher than the CMC. Thus, nearly all of the lipids have to be removed from wheat starch (hydrolysate) to obtain an acceptable filtration rate.
- Therefore, we conclude that lysophospholipids are the main origin for the poor filtration characteristics of wheat starch hydrolysates, due to the formation of micelles that obstruct the filter cake. Filtration improvement by using so-called filtration enzymes must be due to the presence of sufficient lysophospholipase activity in these preparations. Only when the concentration of these lipids is <2.5% of the original amount in wheat starch hydrolysates do the lysophospholipids no longer affect the filtration process. It is puzzling that these filtration problems do not occur with hydrolysates of maize starch, where lysophospholipids account for ≈25% of the starch lipids. Presumably the lysophospholipids form a single phase with the water-insoluble free fatty acids that are the predominant lipids in wheat starch.
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