

Influence of Added Enzymes on the Rheological Properties of a Wheat Flour Dough

L. LINDAHL and A.-C. ELIASSON¹

ABSTRACT

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The changes in rheological properties of a wheat flour dough due to the addition of enzymes were measured. The dough, made of flour of standard baking quality, was characterized by oscillatory measurements at room temperature and after storage at 40°C. Different commercially available α -amylases and mixtures of α -amylases and proteases were added. The elastic modulus, G' , increased slowly with time at room temperature, whereas during the same time (2 hr) at 40°C, a maximum value followed by a continuous decrease in G' was observed. The phase angle, δ , increased slightly with time at 40°C. The rheological properties of

the wheat flour dough were clearly influenced both by the type of α -amylase and by mixing protease with the α -amylase. The presence of α -amylases caused a decrease in G' after a shorter period of time, and lower G' values were obtained. A time and temperature dependency of G' was evident. The change in G' related to the amount of damaged starch was also studied, and it was found that G' increased as the level of starch damage increased. This increase in G' could be compensated for by addition of more water.

Wheat flour contains enzymes, including α - and β -amylases, proteases, lipases, phosphatases, and oxidases (Reed and Thorn 1971). If the wheat is ungerminated, the enzymes are present in low levels, and they remain inactive during storage if moisture and temperature are kept low. The characterization of enzymes in wheat is dependent on the source from which the enzymes derive. Three groups of enzymes can be distinguished: the indigenous enzymes (normal constituents of food material at harvest), e.g., amylases; the endogenous enzymes produced in situ by microorganisms either present as contaminants or added as cultures; and the exogenous or added enzymes (Fox and Mulvihill 1982).

Whether the enzymes are normal constituents or added, the amylases in wheat flour are very important during baking. α -Amylase has been intensely studied, since small amounts of α -amylases caused by preharvest sprouting can lower bread quality. This can be avoided by harvesting when amylase activity is low (Hill and MacGregor 1988). Supplementation of α -amylase can therefore be necessary, providing benefits such as improved gas retention and increased loaf volume (Cauvain and Chamberlain 1988). This makes optimizing the amount of amylase valuable to produce a high-quality end-product (Kruger and Reed 1988). Most of the added amylases used in bakeries are from bacterial or fungal sources. These exogenous enzymes have different physical properties, such as thermal stability. Bacterial α -amylase has a higher degree of heat tolerance than cereal α -amylase, whereas fungal amylase has a lower tolerance.

The proteases in wheat flour are mainly of the endogenous type and are thought to be of little importance to the flour proteins during baking (Ewart 1977). Addition of proteases to obtain weaker and more flexible doughs from strong wheats is normally not necessary in breadmaking, but proteases can be used in flours for cookies or wafers.

The aim of the present investigation was to study the effect of added enzyme preparations (principally α -amylases) on rheological properties of dough. A second objective was to investigate whether the enzymes commercially available to the baker affect the rheological properties of dough in the same way. The study concentrated on rheological changes occurring during a time and temperature protocol corresponding to fermentation. The high proportion of starch in a wheat flour and the amount of water present, together with amylases, indicate the possibility of rheological changes with time in a wheat dough. The results from such studies might be expected to depend on the enzymes present and their properties. Enzymes from different microbial sources vary depending on type and strain of the microorganism, growth conditions, and purification methods (Himmelstein 1984). The enzyme preparations used in this study were all of commercial

quality, and the purity in such enzyme batches may differ from one preparation to another. Thus, it is valuable to know the actual behavior of the commercial enzyme preparation on a pure substrate. The activity of the added enzyme preparations was therefore studied on a gelatinized starch suspension in a viscometric test. The rheological properties of the wheat flour doughs were characterized by oscillatory measurements. Oscillation tests were chosen because it is possible to work with small deformations and small shear rates without any orientation effects, since direction of deformation changes. In oscillatory measurements, viscoelastic properties of a wheat dough can be divided into two components, the dynamic shear storage modulus, G' , and the dynamic shear loss modulus, G'' . G' is the dominating factor when small strains are applied, i.e., at strains below 0.1 (Funt Bar-David and Lerchenthal 1975). A more complete study of dynamic shear moduli in wheat flour doughs and their dependence on amplitude and frequency was published by Smith et al (1970).

MATERIALS AND METHODS

Flour

The wheat flour used was of standard baking quality provided by Skånemöllan, Tågarp, Sweden. The composition was, on a 15% moisture basis, 10.5% protein, 1.5% lipids, and 73% carbohydrates. The damaged starch content was 9.3% (also on a 15% moisture basis). The flour was treated in dry-milling equipment, consisting of a stainless steel tube with three stainless steel balls, to obtain higher values of damaged starch. The damaged starch was analyzed according to AACC Method 76-30A (AACC 1983). The falling number of the wheat flour was 270.

Enzymes

The amylase preparations used were of commercial quality from Röhm GmbH, Darmstadt, Germany, and available under the trade name Veron (Table I). They contain α -amylase from fungal sources, and some of the batches also have protease activity. Fungal amylases also were supplied by Grindsted Products A/S, Brabrand, Denmark. These amylase preparations are available under the trade name Grindamyl (Table I). All of the doughs and starch gels in this study were made with tap water containing 1.5 mM Ca^{2+} ions, which is a sufficient quantity for maintaining the stability of the α -amylase (Marchylo et al 1976).

Starch Gel

A wheat starch of commercial quality (AB Juvel, Stockholm, Sweden) was used. The starch gels were made at a concentration of 4% dry weight. The starch suspension was gelatinized while it was gently stirred in a water bath at 95°C for 60 min. The starch gel then was equilibrated for 30 min in a water bath at 25°C before use. All of the analyses were performed on a mixture of gelatinized starch solution (25 ml) and enzyme preparation. The enzyme preparations were added after gelatinization of the

¹Department of Food Technology, University of Lund, Lund, Sweden.

TABLE I
Commercial Enzyme Preparations Used

Trade Name	Type of Enzyme	Recommended Dosage (mg/10 g of flour)	Used Dosage (mg/10 g of flour)	Activity SKB/g
Röhm				
Veron AV	α -Amylase	0.9–1.5	1.3	1,500
Veron AC ^a	α -Amylase	0.3–0.5	0.4	4,500
Veron AF	α -Amylase + protease	1.0–2.0	1.9	1,500
Veron AP ^b	α -Amylase + protease	1.0–2.0	1.9	1,500
Grindsted				
Grindamyl A 1000	α -Amylase	\approx 0.60	0.65	NM ^c
Grindamyl S 100	α -Amylase	\approx 2.0	2.2	NM

^a Veron AC has a threefold greater α -amylase activity compared with the other Röhm enzymes.

^b Veron AP has greater protease activity than Veron AF.

^c Not measured.

starch as powder, and the enzymatic activity was followed as a decreasing viscosity during a constant shear rate of 146.7 sec^{-1} for 10 min.

Dough Mixing

Ten grams of flour was mixed with 5.5 ml of tap water for 8 min in a farinograph (Do-Corder, C. W. Brabender, Hackensack, NJ). The temperature was 25°C during mixing. When enzyme preparations were added, they were premixed with the flour before water addition. No other additives were used. One gram of the dough was tested immediately at 25°C in oscillatory measurements. The rest of the dough was wrapped in plastic film and stored either at room temperature or in a heat cabinet at 40°C for at least 2 hr. About every 15 min, 1 g of dough was tested. The dough-mixing procedure was repeated at least two to three times for every analyzed combination. The variation of the initial G' values was 10% or less.

Rheological Measurements

The oscillation and viscometry analyses were performed on a Bohlin rheometer system (Bohlin Reologi, Lund, Sweden). The viscometry test was performed between concentric cylinders, test volume 14 ml, with a fixed shear rate of 146.7 sec^{-1} during 10 min. The measuring system was thermostated to 25°C . In oscillation, a cone-and-plate system, with a gap of $150 \mu\text{m}$ between the tip of the cone and the plate, was used when wheat doughs were examined. One gram of dough was placed between the 30-mm-wide bottom plate and the 5.4° angled cone. A complete measurement was performed within 3 min at a temperature of 25°C . The relaxation for a wheat flour dough is in the magnitude of 1 sec (Bohlin and Carlson 1981), and, to avoid influence of earlier treatment of the dough, different delay periods before and during oscillation were tested. It was found that an initial delay of 60 sec and 10 sec between frequencies gave reproducible results. The frequencies varied from 0.02 to 5.0 Hz with a strain value of 1.8×10^{-2} . The strain must be below 2×10^{-3} for a wheat dough to reach the linear region (Faubion et al 1987). The oscillation test and parameters used have been described elsewhere (Lindahl and Eliasson 1986).

In the present investigation, the rheological measurements were repeated with a new piece of dough from the same initially mixed wheat flour dough after different periods of time. This solved the problem of keeping both temperature and humidity constant to avoid drying of the dough surface during extended experiments. Coating the dough surface with Vaseline or silicon oil is possible, but that introduces undesired delays in preparation when a time-dependent function is studied.

RESULTS

The rheological behavior of a wheat flour dough without any additives was analyzed in oscillation tests. The temperature of 40°C was chosen because of its similarity to the proofing step in a bakery. The elastic modulus, G' , at a frequency of 5.0 Hz was followed during 2 hr and compared with the initial value

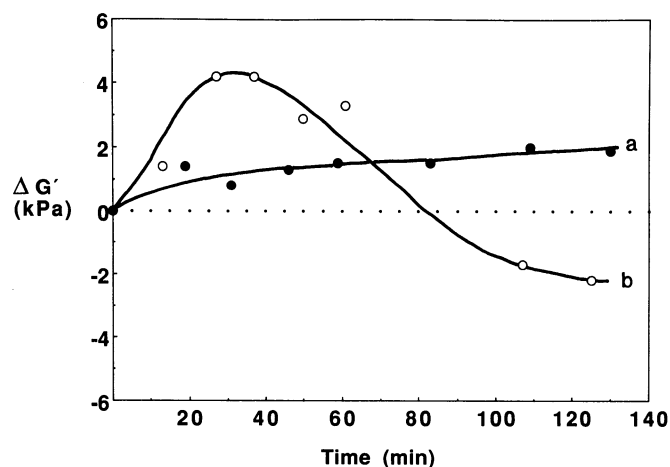


Fig. 1. The change in the elastic modulus, G' , where $\Delta G'$ is defined as $G'(t) - G'(t = 0)$, in a wheat flour dough versus time. a = Storage at room temperature ($22.5 \pm 1^\circ\text{C}$), b = storage at 40°C .

of G' . This led to a positive or negative difference, called $\Delta G'$, in relation to the G' value directly after mixing (Fig. 1). The increase in G' was 10–12% after 2-hr storage of the dough at room temperature. The $\Delta G'$ for the dough stored for the same time at a higher temperature was negative. The dough at 40°C reached the maximum increase in G' , about 25%, 30 min after mixing. The $\Delta G'$ for the wheat dough stored at the raised temperature returned to zero about 90 min after mixing.

The experimental values are marked in Figure 1 together with a curve adjusted to these values. In the other figures, where more than two experiments are shown, only the curves are drawn. The same curve representing the wheat dough stored at 40°C in Figure 1 is shown in Figures 2–5.

The values of the phase angle, δ , for the doughs at room temperature and at 40°C were almost identical during the time course of the experiment. The δ value was $33.5 \pm 1^\circ$, with a slight increase with time at 40°C .

Addition of enzymes to the dough changed the rheological parameters. The changes in G' at 40°C are shown in Figures 2–4. The results in Figures 2 and 3 show the effects of recommended dosages of amylases. The difference in G' returned to zero after about 30 min (compared with 90 min without added amylases). After the peak in $\Delta G'$ was reached, the different amylases behaved in two different ways. Either G' decreased continuously, or after a certain period of time, G' reached a stable value. When both amylases and proteases were added, the zero difference was reached around 60 min after dough mixing (Fig. 4). Oscillation tests on wheat dough with added amylases also were performed after storage below 40°C . As expected, the lower temperature gave a curve between the native wheat dough stored at 40°C and the curve representing wheat dough plus added enzymes at 40°C (not shown). When enzymes were added, δ had a somewhat higher value, around $35.5 \pm 1^\circ$, compared with 33.5

$\pm 1^\circ$ in the untreated dough. With enzymes present, δ also tended to increase with time.

Wheat flour normally contains 5–9% damaged starch (Drapron and Godon 1987). As long as the degree of damaged starch is of the same order from one flour to another, their influence can

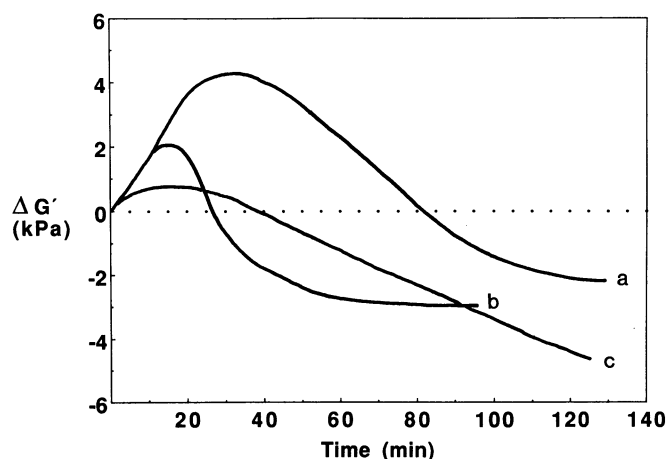


Fig. 2. The change in the elastic modulus ($\Delta G'$) for wheat flour doughs stored at 40°C without added enzymes (a), with added α -amylase, Veron AV (b), and with added α -amylase, Veron AC (c).

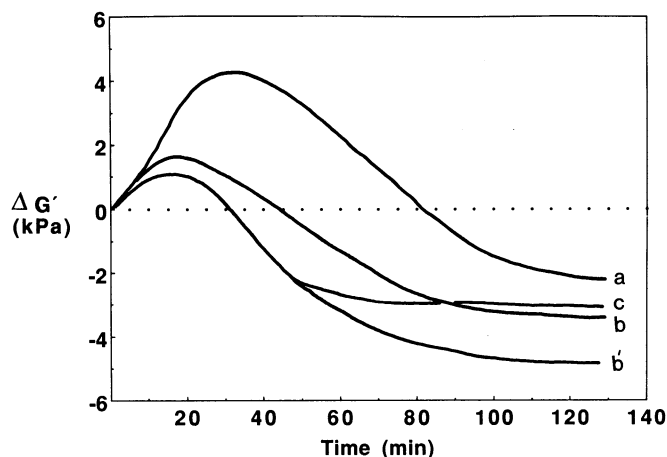


Fig. 3. The change in the elastic modulus ($\Delta G'$) for wheat flour doughs stored at 40°C without added enzymes (a), with added α -amylase, Grindamyl S 100 (two different batches, b and b'), and with added α -amylase, Grindamyl A 1000 (c).

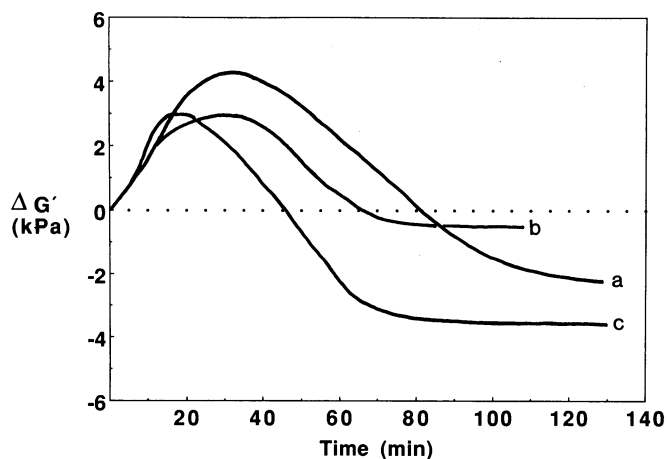


Fig. 4. The change in the elastic modulus ($\Delta G'$) for wheat flour doughs stored at 40°C without added enzymes (a), with added α -amylase and protease, Veron AP (b), and with added α -amylase and protease, Veron AF (c).

be neglected. If, for some reason, the amount of damaged starch should increase, the water-binding capacity of the flour, the accessibility of the naturally occurring α -amylase to the starch, and the rheological values would change (Fig. 5 and Table II). The amount of damaged starch and added water greatly affected G' as well as δ . The values in Table II and the curves in Figure 5 indicate increased water absorption because of damaged starch granules. Addition of more water reduced the increase in G' . The results in Figure 5 could be due to faster α -amylase digestion of the damaged starch granules.

The activity of the enzymes used was tested in a 4% (w/w) gelatinized starch suspension. The viscosity was followed during a constant shear rate of 146.7 sec^{-1} (Fig. 6). According to Evans et al (1986), there is little effect on the kinetics in the shear rate range of 42–571 sec^{-1} . The reliability of the viscometry test was verified with different amounts of the same enzyme, Grindamyl A 1000 (Fig. 6). Other enzymes are compared 3 min after addition in dosages corresponding to the recommended addition in wheat flour in Table III.

DISCUSSION

The curves in Figure 1 show a rheological change in a wheat flour dough related to temperature. It is known that damaged starch can swell at room temperature (Pomeranz 1971) and that farinograph absorption is influenced by the level of starch damage (Holas and Tipples 1978). The $\Delta G'$ value representing wheat dough at room temperature showed a very slow increase with time. If the slope of these curves represents the kinetics of "cold swelling," it is natural that increased values occur at raised temperatures. At 40°C, $\Delta G'$ reached a maximum after 30–45 min. After this period, the enzymatic breakdown may be the dominating factor; the curve, consequently, turned downward. After 90–120 min, no difference from the initial value was observed. Whether the decrease in G' continues or levels off depends on available sub-

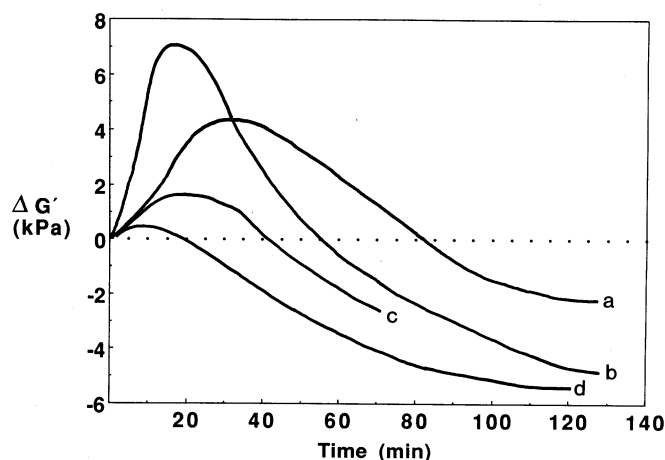


Fig. 5. The effects of increased damaged starch content on the elastic modulus ($\Delta G'$) of wheat flour doughs stored at 40°C. Doughs were made with 9.3% damaged starch and 5.5 ml of water (the same curve as in Figs. 1–4) (a), 20.9% damaged starch and 5.5 ml of water (b), 20.9% damaged starch and 6.5 ml of water (c), and 32.4% damaged starch and 9.0 ml of water (d).

TABLE II
Influence of the Level of Starch Damage on the Elastic Modulus, G' , and Phase Angle, δ , at a Frequency of 5.0 Hz Immediately After Dough Mixing

Damaged Starch (%)	Water Added (ml)	Water Increase (%)	Phase Angle δ°	G' (kPa)
9.3	5.5	...	32.9	12.4
20.9	5.5	...	21.5	46.2
	6.5	18.2	25.7	23.0
	7.0	27.3	25.3	14.9
32.4	9.0	63.6	19.0	19.4

strate and the amount of indigenous α -amylase. Further support for the interpretation that the increase in G' with time is due to cold swelling of starch was drawn from the changes in rheological behavior when the amount of damaged starch was increased (Fig. 5). When the increased amount of damaged starch was not compensated for by addition of extra water, ΔG increased considerably. The results in Table II show that an increase in damaged starch from 9.3 to 20.9% required an increase of 27% water to maintain a similar G' of the wheat dough. On the other hand, this also led to a faster digestion of the available starch and, thus, to a weaker dough. $\Delta G'$ decreased to zero almost 1 hr earlier than in the untreated wheat dough.

Because of the complexity of wheat dough, there are problems in achieving a homogeneous heat transfer at elevated temperatures. This might be one explanation for the scatter in the results (Fig. 1). Dough can be described as a foam with high density, where air cells are surrounded by a continuous phase consisting of a hydrated gluten-starch mixture. The model indicates great variation in heat coefficients in a wheat dough. Values between 0.3 and 0.6 W/(m · K) have been reported (Tschubik and Maslow 1973, Neznanova et al 1978) for the thermal conductivity of wheat dough. The highest value, 0.6, agrees with the coefficient for pure water.

The results in Figures 2 and 3, in which exogenous or added enzymes were used, can be interpreted on the basis of increased enzymatic activity. It seems as though α -amylases effectively "chop off" the rheological effects of cold swelling of starch and start the digestion immediately. The enzyme preparation with the greatest activity (Table I), Veron AC, continued the breakdown of starch after 120 min, which illustrates the importance of achieving the right dosage of amylase during long proofing times. Table III shows the recommended dosages of the commercial enzymes used on a 4% gelatinized starch solution. The small differences

in viscosity between starch gels 3 min after mixing with enzymes were almost insignificant. The initial viscosity of the starch gel at a shear rate of 146.7 sec⁻¹ without any enzymes present was 115 mPa·sec. The enzymes compared in Figures 2 and 3 are thought to be of α -amylase character; the discussion is based on this assumption. Therefore, it could be of interest to use enzymes with functions other than starch digestion.

The experiments in Figure 4 were performed with a mixture of α -amylases and proteases at 40°C. When the mixed enzymes (Veron AF and Veron AP) were used, the reactions between α -amylase and starch seemed to be blocked. But in the viscometry test (Table III), the enzyme mixture was as effective as α -amylase in digestion of starch. The result could be explained by the diluted system used, where the proteases and the α -amylases could be rapidly separated. The reduced effects in dough may be because of protease attack on the α -amylase, which could be more pronounced in the concentrated dough system. This explanation is supported by the fact that the sample with the highest protease activity (according to the supplier) produced a curve closest to the native dough (curve b in Fig. 4). A combined effect where proteases and amylases are attacking protein and starch, respectively, also is possible. However, such a combined effect would be expected to give a more distinct decrease in G' . Protease from the mixed enzymes was not available for complementary experiments to study the effect of protease alone. Whatever the reason, addition of a mixture of α -amylase and protease to the dough caused only minor changes in the rheological behavior of the dough. Other flours with stronger gluten might show greater response to added proteases. Addition of α -amylases to a wheat flour resulted in characteristic changes in rheological behavior of the dough. From a practical point of view, it is very important to be aware of rheological effects based on the amount of damaged starch and α -amylase content. The interactions of the starch and the enzymes in relation to available water are very time- and temperature-dependent.

ACKNOWLEDGMENT

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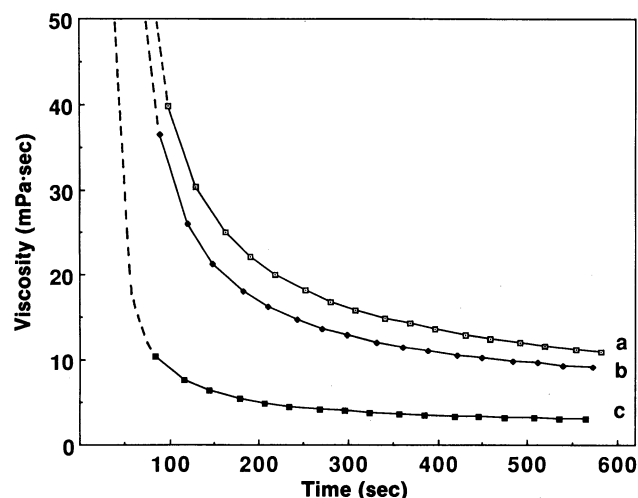


Fig. 6. The viscosity, η , as a function of time when 7.0, 7.8, and 9.1 mg (for a-c, respectively) of α -amylase, Grindamyl A 1000, were added to a 4% gelatinized starch suspension.

TABLE III
Viscosity, η , of a 4% Gelatinized Starch Solution 3 Min After Addition of Enzymes

Enzyme Trade Name	Viscosity ^a (mPa·sec)	Dosage ^b (mg)
Veron AV	18.3	16
Veron AP	19.5	20
Grindamyl S 100 (a) ^c	11.8	27
Grindamyl S 100 (b) ^c	11.5	27
Grindamyl A 1000	16.2	8

^a Measured at 146.7 sec⁻¹ 180 sec after mixture reaches 25°C. Based on recommended dosages in Table I.

^b Mixed in 25 ml of gelatinized starch.

^c Two different batches are indicated by (a) and (b).

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