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Improving the Quality of Ras Cheese Made Without Starter

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ABSTRACT

Trials were carried out to produce Ras cheese of good quality without the use of starter. Cheese was made from cows' milk, pasteurized and acidified with lactic acid or citric acid to pH 5·8; cheese was also made after adding 4·5 g glucono-δ-lactone per kg to the acidified curd. Control cheese was made by the traditional method.

The cheese had poor body and texture, weak flavour intensity, and low levels of soluble nitrogen compounds and free volatile fatty acids.

Incorporation into the cheese curd of mixtures containing Fromase 100 (fungal protease) and Piccantase B (fungal lipase) or Fromase 100 and Capalase K (animal lipase) enhanced flavour intensity and body characteristics and accelerated the formation of both soluble nitrogen compounds and free volatile fatty acids. The organoleptic properties of the experimental cheese with added enzymes were comparable to the control cheese.

INTRODUCTION

Direct acidification has been successfully practised in the manufacture of a number of unripened cheese varieties, such as cottage (Ernstrom & Kale, 1975), Mozzarella (Kosikowski, 1977) and the Egyptian skim milk soft cheese, kariesh (Wahba & El-Abbassy, 1982). Considerable progress has also been made on the production of Cheddar and Blue cheeses by the direct acidification technique. It would appear that acidification during manufacture poses few problems for most cheese varieties; the unsolved problems lie in the ripening stage. There appear to be two principal problems; controlling the growth of undesirable microorganisms, which the starter appears able to control during biological acidification, and the development of proper flavour (Mabbitt *et al.*, 1955; Breene *et al.*, 1964; Shehata & Olson, 1966; Green & Foster, 1974; O'Keeffe *et al.*, 1975).

Several investigators have shown that the addition of selected microbial proteases and lipases, preferably to the curd at salting, may have potential as a means of accelerating the ripening of cheese manufactured by conventional methods (Kosikowski and Iwaski, 1975; Abdel Salam *et al.*, 1979). However, this approach has not yet been applied to chemically acidified cheese.

Ras cheese is considered to be the main national hard type in Egypt. No research appears to have been carried out on the manufacture of Ras cheese by direct acidification. Therefore, the present work was instituted to assess information on the possibility of manufacturing Ras cheese by direct acidification. The effect on the quality of the resultant cheese of incorporating selected protease/lipase mixtures into the chemically acidified curd was also investigated.

MATERIALS AND METHODS

Milk

Cows' milk was obtained from the herd of Gimmeza Station, Institute of Animal Production Research, Ministry of Agriculture, Egypt.

Rennet

A rennet powder (1:100 000) was obtained from L. C. Glad Co. A/S, Copenhagen, Denmark.

Starter culture

A lactic culture of *Streptococcus lactis* CH-I was obtained from Chr. Hansen's Laboratorium, Denmark and used in the manufacture of control Ras cheese.

Acidulants

Lactic acid and citric acid (BDH Analar) were obtained from BDH Chemicals Ltd, England. Glucono-δ-lactone (GDL) was obtained from the Sigma Chemical Company, USA as a coarse powder containing essentially 100% GDL.

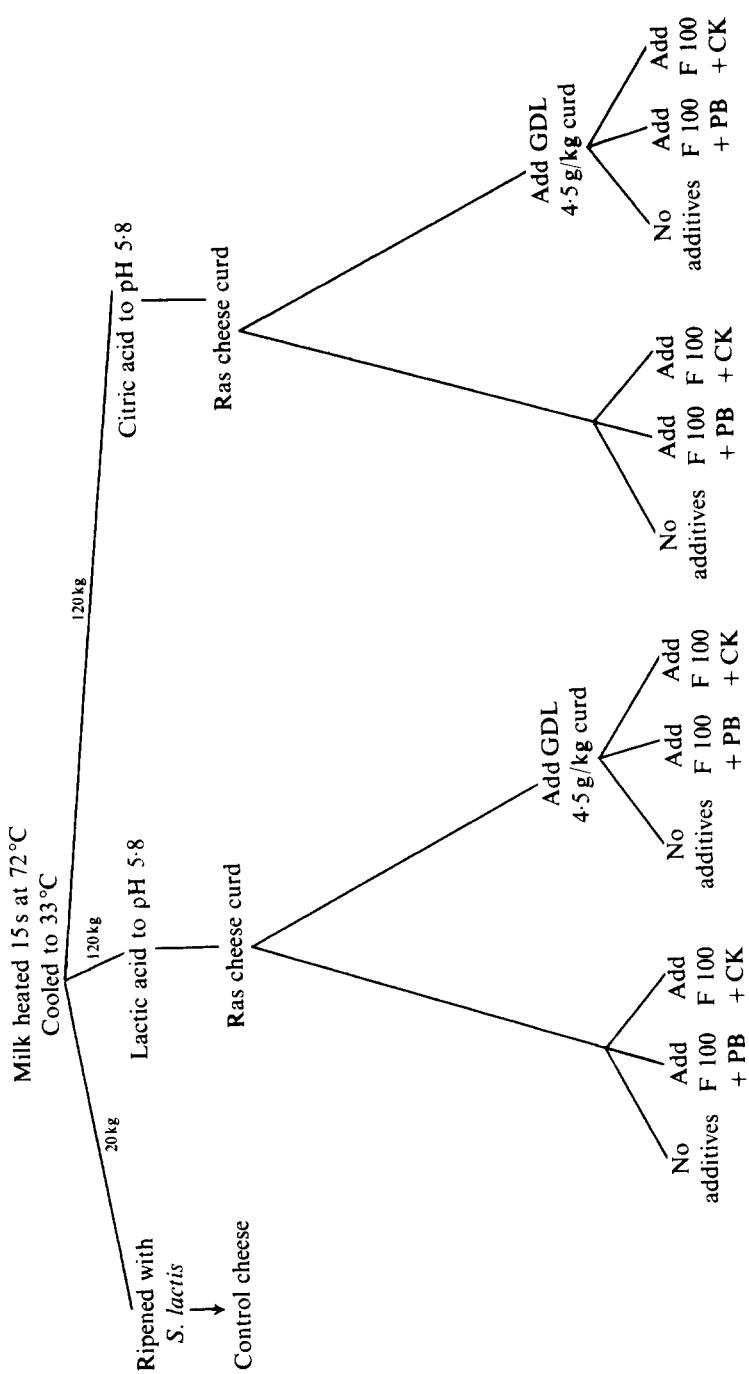
Proteolytic and lipolytic enzyme preparations

Fromase 100 (acid protease of *Mucor miehei*), Piccantase B (*Mucor miehei* esterase) and Capalase K (animal lipase) preparations were obtained from the Rapidase Co., Seclin, France. Blends containing Fromase 100 and Piccantase B or Fromase 100 and Capalase K (1:2) were prepared and used for incorporation into cheese curd.

Cheese making

Ras cheese for all treatments was manufactured as described by Abdel-Tawab (1963). Cheese milk was heated to 72 °C for 15 s and then cooled to 33 °C. Part of the cheese milk (20 kg) was ripened with a starter culture of *S. Lactis* and converted into the control Ras cheese. The rest of the milk was divided into two equal parts (120 kg each), acidified with either lactic or citric acid to pH 5·8 as described by Breene *et al.* (1964), and converted into Ras cheese curd. The resultant curd of each acidulant (lactic or citric acid) was divided into two parts. The first part was further divided into three portions. The first portion was moulded without additives, while the second and third portions were mixed with blends containing Fromase 100 and Piccantase B, and Fromase 100 and Capalase K, respectively, at a level of 10 g/100 kg curd.

The second part of the curd was mixed with GDL at a level of 4·5 g/kg curd for proper acid production during early ripening (Mabbit *et al.*, 1955). The curd containing GDL was divided into three portions and treated with enzymes for the first part. The cheese making process was



GDL, glucono-δ-lactone
F 100, Fromase 100
PB, Piccantase B
CK, Capalase K

Fig. 1

completed. All cheese was ripened at 12 ± 2 °C for 4 months. Trials were conducted in triplicate. Figure 1 summarizes the above treatments.

Chemical analysis

Cheese samples were analysed for moisture, fat, salt, acidity, total nitrogen (TN), soluble nitrogen (SN) and non-protein (NPN) as described by Ling (1963). The amino acid nitrogen (AN) was determined according to Stadhouders (1959). Free fatty acids (C_2, C_3, C_4 and C_5, and higher) were determined by the method of Harper (1953).

Organoleptic properties

The organoleptic properties of the cheese were examined by a test panel of five staff members, as described by Abdou *et al.* (1977) with maximum score points of 10, 50 and 40 for appearance, flavour, and body and texture, respectively.

RESULTS AND DISCUSSION

Gross chemical composition

Tables 1 and 2 show that the cheese made from milk acidified with lactic acid or citric acid alone, or coupled with the incorporation into the cheese curd of GDL, had higher moisture contents compared with control cheese made from milk ripened with a starter culture of *S. lactis*. This was associated with an increase in the salt content and a slight decrease in cheese fat. Titratable acidity of fresh Ras cheese from all treatments appeared to be nearly the same. Development of titratable acidity in cheese made from milk acidified with lactic acid or citric acid took place at a relatively slow rate compared with the control cheese. Incorporation into the cheese curd of GDL slightly increased the acid development during cheese ripening.

Addition of protease/lipase mixtures to the curd did not considerably affect the above properties of the chemically acidified cheeses. The results could be explained on the basis that direct acidification of cheese milk with lactic acid or citric acid appears to affect the physical structure of the curd or to shift the calcium equilibrium in milk and cheese (Askar *et al.*,

TABLE 1
Chemical Composition of Ras Cheese made from Milk Acidified with Lactic Acid as Affected by the Incorporation into the Curd of some Additives

Property	*Ripening period (months)*	*Control cheese (with starter)*	*Without GDL*[a]			*With GDL*[a]		
			A	*B*	*C*	*A*	*B*	*C*
Moisture (%)	Fresh	40·20	42·9	42·9	42·2	41·9	42·5	42·6
	2	35·70	41·0	40·2	40·8	40·1	39·1	40·8
	4	34·42	39·9	39·7	39·6	39·0	39·0	39·6
Fat (%) (DM)	Fresh	47·44	47·0	46·9	46·8	47·0	46·9	46·8
	2	49·62	48·9	47·8	47·8	49·0	47·8	47·9
	4	50·06	49·1	48·0	48·1	49·0	49·0	48·3
Salt (%) (DM)	Fresh	3·30	3·40	3·40	3·42	3·42	3·49	3·40
	2	3·81	4·06	4·15	4·20	4·04	4·20	4·11
	4	3·94	4·18	4·22	4·30	4·16	4·24	4·20
Acidity (% of lactic acid)	Fresh	0·30	0·18	0·20	0·20	0·22	0·22	0·21
	2	1·50	1·11	1·18	1·17	1·26	1·26	1·28
	4	1·82	1·32	1·47	1·46	1·50	1·52	1·50

[a] A, Cheese without enzymes.
B, Cheese with Fromase 100/Piccantase B mixture.
C, Cheese with Fromase 100/Capalase K mixture.

TABLE 2
Chemical Composition of Ras Cheese made from Milk Acidified with Citric Acid as Affected by the Incorporation into the Curd of some Additives

Property	*Ripening period (months)*	*Control cheese (with starter)*	*Without GDL*[a]			*With GDL*[a]		
			A	*B*	*C*	*A*	*B*	*C*
Moisture	Fresh	40·2	41·0	41·9	41·6	41·0	42·9	42·2
	2	35·7	38·6	38·9	39·1	39·0	40·0	39·2
	4	34·4	37·9	38·1	38·4	38·2	39·0	38·2
Fat (%) (DM)	Fresh	47·4	47·0	46·9	46·6	46·9	46·2	46·4
	2	49·6	47·8	47·1	47·8	47·8	47·6	47·0
	4	50·1	48·5	48·3	49·0	49·0	48·8	48·4
Salt (%) (DM)	Fresh	3·30	3·50	3·56	3·50	3·52	3·54	3·52
	2	3·81	4·00	4·00	4·05	3·95	4·00	4·00
	4	3·94	4·11	4·20	4·30	4·16	4·08	4·10
Acidity (% of lactic acid)	Fresh	0·30	0·18	0·18	0·16	0·20	0·20	0·22
	2	1·50	1·00	1·12	1·12	1·20	1·24	1·23
	4	1·82	1·28	1·28	1·26	1·40	1·46	1·44

[a] A, B and C, as for Table 1.

1982). These effects increase the capacity of the curd to retain more moisture. This in turn increases the curd ability to absorb more salt during the salting stage and decreases the fat contents (on dry basis). Development of acidity was different in the chemically modified cheese from that in a biologically acidified one, indicating the essential role of lactic acid bacteria in acid production. GDL slightly stimulated the changes in cheese acidity owing to its gradual hydrolysis to gluconic acid (Mabbitt *et al.*, 1955).

Ripening indices

Changes in soluble nitrogen (SN) non-protein nitrogen (NPN), amino acid nitrogen (AN) and free volatile fatty acids were considered as indices of cheese ripening.

Changes in SN, NPN and AN

Tables 3 and 4 shows that SN, NPN and AN of Ras cheese made from milk acidified with lactic or citric acid but with no GDL added were found to be lower than that of the control cheese. This could be caused by the absence of starter organisms and their enzymes in chemically acidified cheese. Lactic acid bacteria contain a wide range of proteinases and peptidases which are essential contributors to protein degradation in

TABLE 3

Nitrogen Fractions of Ras Cheese made from Milk Acidified with Lactic Acid as Affected by the Incorporation into the Curd of some Additives (% of Total N)

Nitrogen fraction	*Ripening period (months)*	*Control cheese (with starter)*	*Without GDL*[a]			*With GDL*[a]		
			A	*B*	*C*	*A*	*B*	*C*
Soluble N	Fresh	9·6	6·4	10·0	10·0	7·0	10·2	10·3
	2	16·8	10·0	21·0	22·0	12·4	22·2	22·8
	4	24·5	15·2	30·4	31·0	17·0	32·2	32·8
Non-protein N	Fresh	1·76	1·00	1·80	1·96	1·10	1·60	1·88
	2	4·69	2·80	6·49	6·69	2·92	6·66	6·72
	4	6·24	3·92	8·42	8·59	4·00	9·00	9·02
Amino acid N	Fresh	0·50	0·20	0·60	0·62	0·22	0·82	0·88
	2	1·00	0·40	1·20	1·30	0·38	1·24	1·30
	4	1·94	0·96	2·10	2·14	0·98	2·40	2·44

[a] A, B and C, as for Table 1.

TABLE 4
Nitrogen Fractions of Ras Cheese made from Milk Acidified with Citric Acid as Affected by the Incorporation into the Curd of some Additives (% of Total N)

Nitrogen fraction	*Ripening period (months)*	*Control cheese (with starter)*	*Without GDL*[a]			*With GDL*[a]		
			A	*B*	*C*	*A*	*B*	*C*
Soluble N	Fresh	9·6	7·0	10·0	10·0	7·1	10·2	10·3
	2	16·8	11·6	22·0	23·0	12·0	23·6	23·8
	4	24·5	16·3	32·5	32·1	17·2	32·9	33·0
Non-protein N	Fresh	1·76	1·20	1·96	1·98	1·20	1·98	2·00
	2	4·69	3·00	6·45	6·73	3·20	6·96	6·98
	4	6·24	4·06	9·00	9·10	4·18	9·20	9·26
Amino acid N	Fresh	0·50	0·20	0·79	0·82	0·22	0·90	0·90
	2	1·00	0·42	1·44	1·50	0·42	1·60	1·66
	4	1·94	0·94	2·00	2·00	0·98	2·40	2·44

[a] A, B and C, as for Table 1.

cheese made by normal methods (Desmazeaud & Zevaco, 1979). Incorporation into the cheese curd of GDL showed some stimulating effect on the formation of SN and NPN, probably owing to the increased acidity in cheese with added GDL. O'Keeffe *et al.* (1975) observed rapid proteolysis during the manufacture and early ripening of Cheddar cheese made by the direct acidification technique of Mabbitt *et al.* (1955). The authors explained this result on the basis that the rapid decline in pH, followed by the addition of GDL to milk, solubilized excessive amounts of colloidal calcium phosphate, rendering the micellar caseins susceptible to proteolysis. Holmes *et al.* (1977) have shown that the proportion of added rennet retained in Cheddar curd increases rapidly as the pH at setting decreases and this is likely to be an important contributory factor to excessive early proteolysis. The acidification schedule of Mabbitt *et al.* (1955) was modified by O'Keeffe *et al.* (1975, 1978) to solve the problem of rapid decline of pH of milk and curd. Milk was acidified to pH 6·5 with concentrated lactic acid, and after cutting, the curd/whey mixture was titrated with lactic acid to simulate the pH decline in the control cheese. After complete drainage, GDL was added to the curd. The level of proteolysis in curd and cheese was found to be similar or even less than that of the control starter cheese. In the present trials milk was acidified to pH 5·8 with concentrated lactic or citric acid and GDL was stirred well with the curd before hooping to stimulate gradual production of acid in the cheese through the hydrolysis of GDL.

It can also be seen from Tables 3 and 4 that the addition of selected mixtures containing Fromase 100 and Piccantase B or Fromase 100 and Capalase K showed similar stimulating effects on the formation of SN, NPN and AN in the chemically acidified Ras cheese. The effect of the added protease/lipase mixtures was greater in cheese with added GDL. The level of soluble nitrogen compounds in 2-month-old Ras cheese made from chemically acidified curd with added protease/lipase mixtures was found to be similar or even more than that of control cheese ripened for 4 months. The effectiveness of certain protease/lipase mixtures in accelerating the formation of soluble nitrogen compounds in Ras cheese made by the conventional methods was reported by Abdel Salam *et al.* (1979).

Free volatile fatty acids

As shown in Tables 5 and 6 the volatile fatty acids (C_2, C_3, C_4 and C_5, and higher) content of Ras cheese made from milk acidified with lactic acid or

TABLE 5

Free Fatty Acids (FFA) of Ras Cheese made from Milk Acidified with Lactic Acid as Affected by the Incorporation into the Curd of some Additives

Ripening period (months)	*FFA (ml 0·01 N NaOH/100 g cheese)*	*Control cheese (with starter)*	*Without GDL*[a]			*With GDL*[a]		
			A	*B*	*C*	*A*	*B*	*C*
Fresh	C_2	2·0	1·2	1·6	1·6	1·0	1·6	1·8
	C_3	0·2	0·2	0·2	0·2	0·2	0·2	0·2
	C_4	1·2	0·6	1·6	1·2	0·8	2·8	2·6
	C_5 and higher	12·0	9·0	13·4	13·0	10·0	14·0	13·0
	Total	15·4	11·0	16·8	16·0	12·0	18·6	17·6
2	C_2	3·2	1·6	2·8	2·6	1·6	2·8	2·6
	C_3	0·4	0·2	0·2	0·2	0·2	0·4	0·4
	C_4	2·4	1·2	3·8	3·6	1·4	4·8	4·2
	C_5 and higher	25·2	13·8	25·8	25·0	14·6	27·6	26·6
	Total	31·2	16·8	32·6	31·4	17·8	35·6	33·8
4	C_2	4·2	2·0	3·2	3·0	1·8	3·4	3·2
	C_3	0·5	0·2	0·4	0·4	0·2	0·6	0·6
	C_4	3·6	2·0	6·6	6·4	2·4	6·4	6·0
	C_5 and higher	30·6	17·0	29·8	28·5	17·8	32·2	31·0
	Total	38·9	21·2	40·0	38·3	22·2	42·6	40·8

[a] A, B and C, as for Table 1.

TABLE 6

Free Fatty Acids (FFA) of Ras Cheese made from Milk Acidified with Citric Acid as Affected by the Incorporation into the Curd of some Additives

Ripening period (months)	*FFA (ml 0·01 N NaOH/100 g cheese)*	*Control cheese (with starter)*	*Without GDL*[a]			*With GDL*[a]		
			A	*B*	*C*	*A*	*B*	*C*
Fresh	C_2	2·0	1·2	1·6	1·6	1·0	1·6	1·8
	C_3	0·2	0·2	0·2	0·2	0·2	0·2	0·2
	C_4	1·2	0·6	1·6	1·2	0·8	1·8	2·1
	C_5 and higher	12·0	4·8	16·8	15·6	4·8	15·0	14·6
	Total	15·4	6·8	20·2	18·6	6·8	18·6	18·7
2	C_2	3·2	1·6	2·8	2·6	1·6	2·8	2·6
	C_3	0·4	0·2	0·2	0·2	0·2	0·4	0·4
	C_4	2·4	1·2	3·8	3·6	1·4	3·8	3·6
	C_5 and higher	25·2	10·8	33·0	27·5	12·0	34·0	30·0
	Total	31·2	13·8	39·8	33·9	15·2	41·0	36·6
4	C_2	5·0	2·0	3·2	3·0	1·8	3·4	3·2
	C_3	0·5	0·2	0·4	0·4	0·2	0·5	0·6
	C_4	3·6	2·0	6·6	6·6	2·4	6·4	6·0
	C_5 and higher	30·6	15·6	44·0	42·0	18·0	46·5	44·2
	Total	39·7	19·8	54·2	52·0	22·4	56·8	54·0

[a] A, B and C, as for Table 1.

citric acid with and without the addition of GDL to cheese curd was lower than that of the control cheese throughout the ripening period. This might be due to the low level of simple nitrogen compounds, particularly the free amino acids, in Ras cheese made by direct acidification.

Incorporation into the curd of certain protease/lipase mixtures enhanced the formation of volatile fatty acids. Mixtures containing Piccantase B were more effective in this respect, in cheese with and without added GDL. This could be due to the stimulating effect of the added lipase on fat hydrolysis. Ohren and Tuckey (1969) showed that milk fat was the major source of free fatty acids in cheese, and amino acids were considered an insignificant source in this respect.

Organoleptic properties

Tables 7 and 8 show that Ras cheese made by the different acidification differed considerably from that made from milk ripened with added

TABLE 7

Organoleptic Properties of Ras Cheese made from Milk Acidified with Lactic Acid as Affected by the Incorporation into the Curd of some Additives

Ripening period (months)	*Cheese property*[a]	*Control cheese (with starter)*	*Without GDL*[b]			*With GDL*[b]		
			A	*B*	*C*	*A*	*B*	*C*
1	Appearance	8	7	8	8	7	8	8
	Body and texture	34	20	33	33	20	35	34
	Flavour	38	22	35	35	22	36	36
2	Appearance	8	7	8	8	7	8	8
	Body and texture	36	22	34	35	24	35	35
	Flavour	42	24	40	40	26	43	43
4	Appearance	9	7	8	8	7	9	9
	Body and texture	38	26	36	36	28	37	38
	Flavour	46	28	44	43	30	45	45

[a] Maximum organoleptic scores: appearance, 10; body and texture, 40; flavour, 50.
[b] A, B and C, as for Table 1.

TABLE 8

Organoleptic Properties of Ras Cheese made from Milk Acidified with Citric Acid as Affected by the Incorporation into the Curd of some Additives

Ripening period (months)	*Cheese property*[a]	*Control cheese (with starter)*	*Without GDL*[b]			*With GDL*[b]		
			A	*B*	*C*	*A*	*B*	*C*
1	Appearance	8	7	8	8	7	8	8
	Body and texture	34	20	32	32	21	34	34
	Flavour	38	23	34	34	23	36	36
2	Appearance	8	7	8	8	7	8	8
	Body and texture	36	22	34	34	22	36	36
	Flavour	42	25	41	41	25	42	43
4	Appearance	9	7	8	8	7	9	9
	Body and texture	38	25	36	36	25	38	38
	Flavour	46	28	44	44	28	46	46

[a] Maximum organoleptic scores: appearance, 10; body and texture, 40; flavour, 50.
[b] A, B and C, as for Table 1.

starter in that it had poor body and crumbly texture during the early stage of ripening. The body and texture were not greatly improved as ripening advanced. In addition, a weak flavour intensity was detected in most samples of chemically acidified Ras cheese. These observations could be due to the low levels of soluble nitrogen compounds and volatile acids in Ras cheese made by direct acidification (Tables 2, 4–6).

Incorporation into lactic acid curd or citric acid curd with and without GDL of a mixture containing Fromase 100 and Piccantase B or Fromase 100 and Capalase K resulted in a cheese with better body and more pronounced flavour intensity than that of chemically acidified Ras cheese without enzymes, comparable to control cheese made by the conventional method. Addition of Fromase 100 and Piccantase B was more effective in this respect, particularly when they were added to the chemically acidified curd with added GDL. The best organoleptic score was given to Ras cheese made from milk acidified with lactic acid or citric acid coupled with the incorporation into the curd of GDL and a Fromase 100/Piccantase B mixture.

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