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Research Note

Some Cultural Conditions for Maximum Bioextraction of Beet Pulp Pectin

ABSTRACT

The bioextraction of the beet pulp pectin by Kluyveromyces marxianus *was inhibited by ferrous sulphate penta hydrate and potassium dihydrogen phosphate, but stimulated by magnesium sulphate hepta hydrate salt. The pectin yields were also influenced by the addition of some enzymatic activators and some natural additives such as yeast extract.*

The characterization of both microbiologically and chemically extracted pectin samples indicated that the former had higher percentages of galacturonic acid, methoxyl groups and a higher degree of esterification and thus possesses superior qualities to chemically-extracted pectin.

INTRODUCTION

The decomposition of protopectin with the liberation of water-soluble pectic substance was originally attributed to protopectin-solubilizing enzymes (protopectinases) (Sakai & Yoshitake, 1984), these enzymes could be used in the production of pectin (Sakai & Okushima, 1980).

Commercial extraction of pectin is usually accomplished with mineral acids under conditions of pH, time, and temperature chosen to maximize yield while retaining quality. Materials, handling equipment and cost have also to be considered (Rouse & Crandall, 1978).

In a previous paper, the authors (Ghanem *et al.*, in press) found that *Kluyveromyces marxianus* was the most active yeast for the liberation of water-soluble pectin from the protopectin of Egyptian beet pulp (BP). The bioextraction of pectin was influenced by the pretreatment of BP, solid/liquid ratio, the age and size of inoculum and incubation period as well as the pH value.

In this paper we describe the results of an experiment to attain a better bioextraction of pectin from BP by *K. marxianus* by the use of some mineral salts, enzyme activators and some natural additives. The characteristics of the extracted pectin is also considered.

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MATERIALS AND METHODS

Beet pulp (BP)

The BP was kindly supplied by the Delta Sugar Company, Kafr El-Sheikh, Egypt. The dried BP was finely ground in a Wiley mill and passed through a 60 gauge mesh sieve to give a homogenous powder which was stored in a desiccator containing $CaCl_2$.

Maintenance and cultivation of the yeast

K. marxianus 70343 DSM (Deutsche Samplung Von Microorganismen) was maintained on agar slants containing 2% glucose, 0·2% pectin and 0·1% yeast extract at pH 5·0 (Sakai & Okushima, 1982). For the seed culture, standard inoculum (4 ml yeast suspension/100 ml medium) of 24 h old culture of the yeast was allowed to grow in a medium of 2% glucose, 0·4% peptone, and 0·2% yeast extract, pH 5·0 at 30°C for 24 h under shaking conditions. Incubation of the pectin was carried out with broth containing 5 g of BP and 80 ml of sterile distilled water dispensed in 250 ml Erlenmeyer flasks. The broth was inoculated with 8 ml seed culture and incubated at 30°C under shaking conditions (200 shakes/min, amplitude 7 cm) for 48 h.

Extraction and determination of pectic substances

At the end of fermentation, the supernatant was separated by centrifugation at 4000 rpm for 20 min and poured into 3 volumes of ethanol. The precipitated pectin was collected by centrifugation, washed with ethanol, dried under vacuum at 37°C and then accurately weighed (Sakai & Okushima, 1980). The pectin constituent of the BP was also extracted and estimated chemically as described by Abdel-Fattah and Edress (1972).

Characterization of extracted pectin

The microbially and chemically extracted BP pectins were characterized by the determination of free carboxyl groups by titration against standardized sodium hydroxide, methoxyl content (using the method of Myers & Baker, 1934), degree of esterification as percentage of methoxylated carboxyl groups to total carboxyl groups, relative viscosity of 5 ml of pectin solution (0·5%) at 30°C using an Ostwald viscometer and specific viscosity which was derived from the relative viscosity. The

galacturonic acid and sugar constituents were also determined as described by Hang and Larsen (1962) and Bitter and Muir (1962). Each treatment was carried out in triplicate and results obtained throughout this work were the arithmetic mean.

RESULTS AND DISCUSSION

Effect of some mineral salts

The results of the addition of 0·5 g/litre $MgSO_4.7H_2O$, KH_2PO_4, and 0·1 g/litre $FeSO_4.5H_2O$ (Fig. 1), one at a time or in mixtures, indicated that the addition of $FeSO_4.5H_2O$ and KH_2PO_4 in general had an inhibitory effect on pectin bioextraction. While the presence of Mg^{2+}, which is essential in maintaining the integrity of ribosomes and for the activity of many enzymes, enhance pectin bioextraction (10% increase).

Role of some enzymatic activators

NAD, NADP, ATP and ascorbic acid as growth and enzymatic activators were added at 5 mg/100 ml medium level. The results (Fig. 2) revealed that except NADP, the presence of such activators had a stimulatory effect on pectin bioextraction. The redox agents, NAD and ascorbic acid, raised the pectin extraction to 98·7 and 89·2%, respectively. While, NADP decreased this value to 62·4% The coenzymes NAD, NADP, have the same redox active part in their configurations and act in an identical manner, however, there is a difference in function at the subcellular level. ATP which is an important factor in kinase catalyzed reactions was found to improve pectin bioextraction (93·5%) by *K. marxianus.*

Effect of some natural additives

The bioextraction medium (5 g BP/80 ml distilled water) was modified by separate addition, one at a time, beef extract, peptone and yeast extract at different levels (1–4 g/litre). The results (Table 1) showed that pectin extraction was, in general, enhanced by these natural complex substances which may enhance the activity of the protopectin-solubilizing enzymes. The stimulatory effect of these supplements is largely dependent on its level. Thus yeast extract at 1 g/litre showed the highest yield of pectin bioextraction (99%).

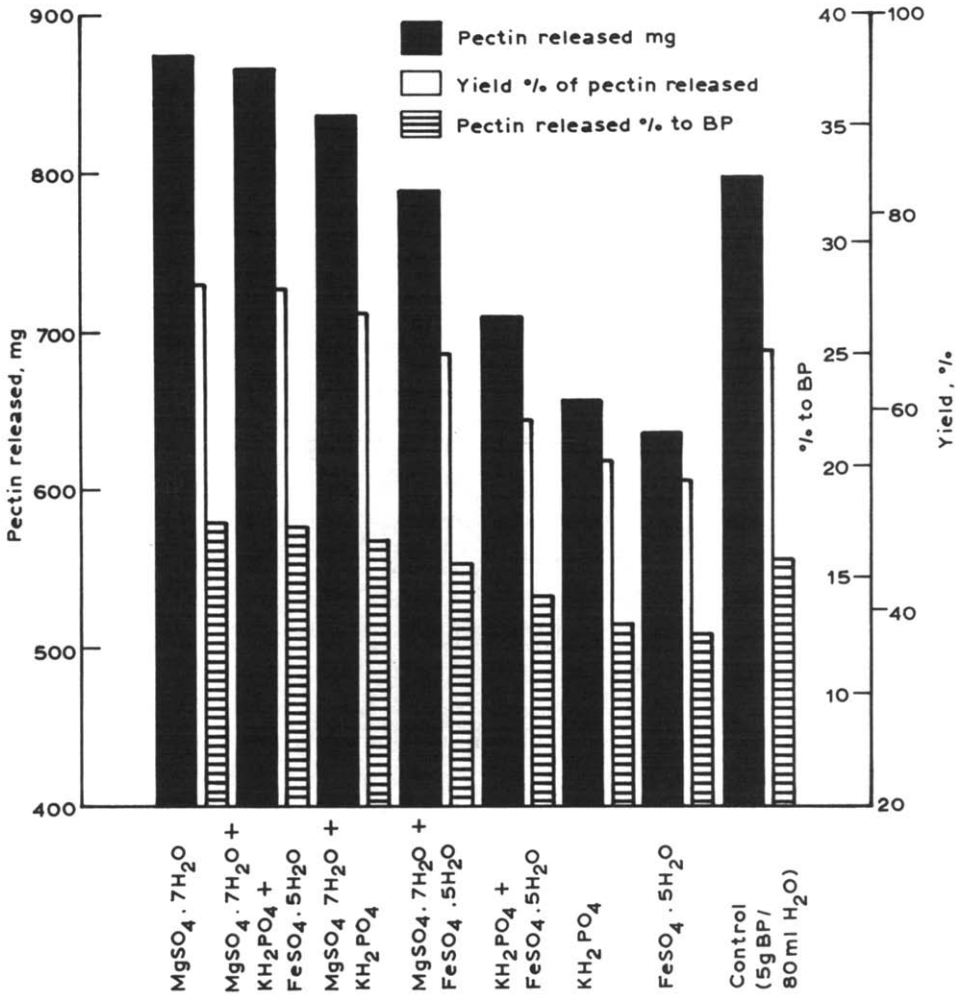

Fig. 1. Pectin bioextraction by *K. marxianus* in the presence of different mineral salts.

$$\% \text{ of BP} = \frac{\text{Bioextracted pectin}}{\text{BP weight}} \times 100$$

$$\text{Yield } \% = \frac{\text{Bioextracted pectin}}{\text{Whole pectin in BP}} \times 100$$

Some characteristics of the BP pectin extracted chemically and microbiologically

As both yield and quality are important factors in determining the suitability of the extraction method the microbiologically extracted

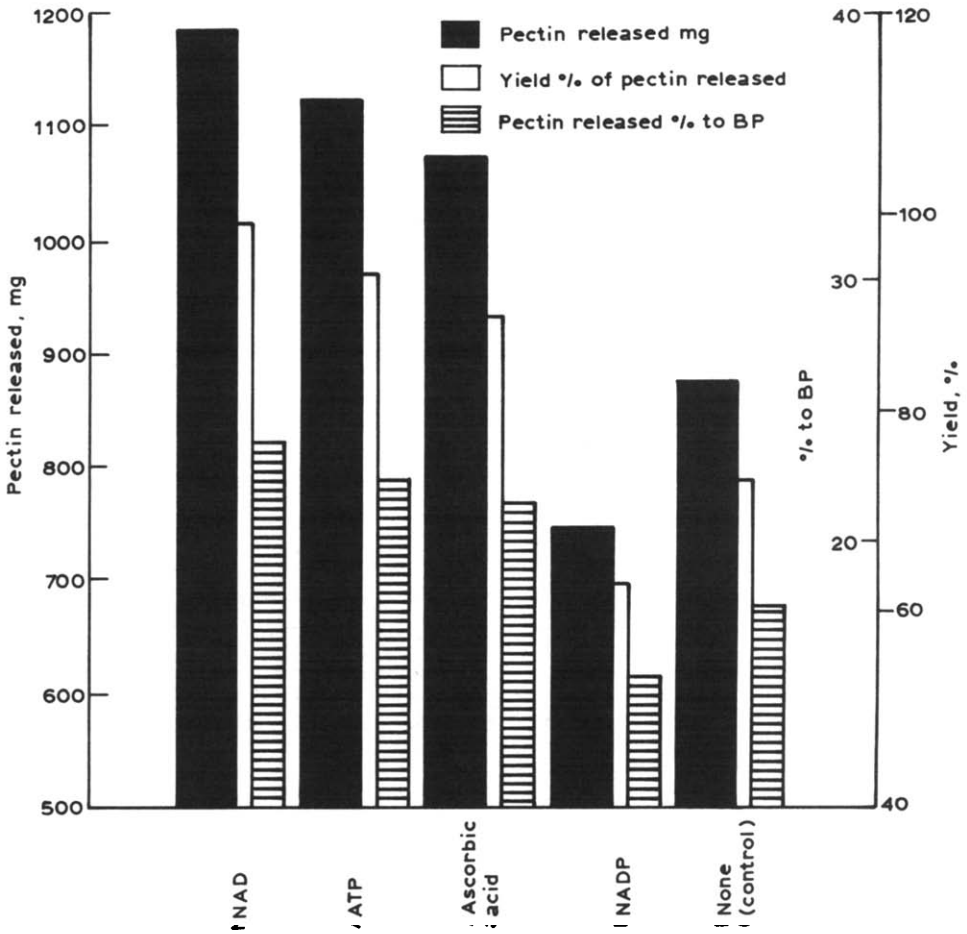

Fig. 2. Pectin bioextraction by *K. marxianus* in the presence of some activators.

pectin (MEP) was chemically characterized in comparison with a chemically extracted pectin (CEP) sample. The results (Table 2) revealed that both samples contain relatively low galacturonic acid residues (74·4% for CEP and 82% for MEP) compared to pectin prepared from onion skin (96–96·6%) (Abdel-Fattah & Edrees, 1972), but nearly similar to that of lemon and citrus (80·3–85%) (Sakai & Okushima, 1980). The lower quantity of galacturonic acid for the CEP might be due to partial decarboxylation of the residues during the chemical extraction process. In contrast, it was found that the citrus pectin extracted by fermentation had a lower galacturonic acid percentage compared to acid extracted pectin (Sakai & Okushima, 1980).

The CEP was richer in hemicelluloses, as shown by the higher arabinose and galactose contents. These polysaccharides are not

TABLE 1
Pectin Bioextraction by *K. marxianus* as Influenced by Different Levels of Natural Additives

Natural additives (g/litre)	*Pectin released*		
	mg	*% BP*[a]	*Yield %*[b]
None (control)[c]	876	17·5	73·0
Beef extract			
1	986	19·7	82·2
2	992	19·8	82·7
3	1067	21·3	88·9
4	1022	20·4	85·2
Peptone			
1	908	18·2	75·7
2	977	19·5	81·4
3	988	19·8	82·3
4	1006	20·1	83·8
Yeast extract			
1	1188	23·8	99·0
2	958	19·2	79·8
3	944	18·9	78·7
4	931	18·6	77·6

[a]$\%\ \text{to BP} = \dfrac{\text{Bioextracted pectin}}{\text{BP weight}} \times 100$

[b]$\text{yield}\ \% = \dfrac{\text{Bioextracted pectin}}{\text{Whole pectin in BP}} \times 100$

[c]Extracted without inoculation.

TABLE 2
Composition and Some Characteristics of the Chemically Extracted Pectin (CEP) and Microbially Extracted Pectin (MEP) Samples

Pectin samples	*Galacturonic acid (%)*	*Galactose (%)*	*Arabinose (%)*	*Methoxyl (%)*	*Degree of esterification (%)*	*Relative viscosity (%)*	*Specific viscosity (%)*
CEP	74·36	12·51	10·34	8·45	73·48	1·12	0·4
MEP	82·00	9·73	7·18	12·40	92·91	1·04	0·3

constituents of the pectin chains but co-precipitate with pectin and can be removed by purification processes (Kertesz, 1951).

The MEP is also characterized by its higher methoxyl content, and hence higher degree of esterification. In any case, the apparent high degree of esterification of both pectin samples may be due to the saponifiable acetyl groups in addition to the methoxyl groups. Beet pectin was reported to contain acetyl groups (Kertesz, 1951). It was also reported (Sakai *et al.*, 1982) that, when the methoxyl and galacturonic acid contents increase, the molecular weight increases giving a marked influence in gelling ability (Fogarty & Ward, 1974). The existence of carboxylic acids with relatively high degree of esterification in the MEP is partially responsible for the strong water binding capacity of the BP.

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