Bioextraction of Grapefruit Pectin by *Kluyveromyces Marxianus*

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Abstract

Microbial and chemical extraction of pectin from some agro-industrial wastes (grapefruit, orange fruit, mango fruit, lemon fruit and onion scales) proved that grapefruit waste (GFW) contains more amounts of pectin than the other wastes. GFW contains about 37.5% pectin, 17.2% soluble sugars, and 14.3% holocellulose of its dry weight. The highest bioextraction of GFW pectin by Kluyveromyces marxianus (35.6%), extracellular protein(5.5%) and protopectinase activity(12.02μ/ml), as well as, yeast gowth (342x10^{8}cfu/ml) were obtained under the following optimized fermentation conditions: absence of yeast extract, 0.4% peptone, 1.0% glucose and 8 % grapefruit waste (added at zero time of incubation), under shaked conditions (200 shakes/min) for 18h at 30°C, pH 5 and seed culture of 24h (old) at 4 % level.

Key words: Grapefruit, Bioextraction, Pectin, *Kluyveromyces marxianus*

Introduction

Great amounts of agroindustrial wastes rich in polysaccharides, such as pectic substances, are produced worldwide. Some of these wastes are used for the production of pectin[1]. Pectin is a polysaccharide having properties such as gelatin and emulsion stabilization, which make it useful in the manufacture of food, cosmetics and medicine; it is a normal constituent of food and may therefore safely be ingested[2-4].

The water-insoluble parent pectic substance occurring in plants is known as protopectin. Upon limited hydrolysis it yields pectins of various molecular weights[5]. Currently, pectin is extracted at industrial scale by physicochemical means that comprise many disadvantages, but lately new biotechnological alternatives have been developed[6]. Many microorganisms are known to produce protopectin solubilizing enzymes including yeast[7,8], molds[9,10] and bacteria[11,12].

Grapefruit waste (GFW) is produced as a by-product of the juice factories and currently it is either used for animal feeding or is disposed of as an industrial waste. It contains considerable amounts of pectic substances. Therefore, this study aims to optimize some cultural conditions of *Kluyveromyces marxianus* to obtain maximum pectin yields.

Material and Methods

Agroindustrial wastes: Five different substrates containing considerable amount of pectin substances were screened for their suitability in pectin bioextraction. The wastes were grapefruit (*Citrus paradisi*), orange fruit (*Citrus sinensis*), mango fruit (*Mangifera indica* L.), lemon fruit (*Citrus liman*), and onion scales (*Allium cepa* L.). They were obtained from the local market, dried in an oven at 60°C for constant weight. The dried wastes were finely ground in a Wiley mill and passed through a 60 gauge mesh to give a homogenous powder, which was stored in a desiccator containing $CaCl_2$.

Analyses of grapefruit waste (GFW): The moisture content was estimated by drying a sample of GFW at 105°C for 24 h in a drying oven. The ash content was estimated by the sulphated method[13]. The pectin substances were extracted using acidified boiling water (pH 2.0) for 3 h, was precipitated by ethanol[2] and was also extracted by ammonium oxalate and estimated chemically as described by Abdel-Fattah and Edrees[14]. The dried, powdered GFW sample was defatted by methanol: chloroform (1: 1, v / v) and delignified by sodium chlorite and glacial acetic acid; the residual material was washed with water and ethanol, dried and weighed to give the holocellulose content. Hemicellulose was removed by treating the obtained holocellulose with 10 % NaOH solution (1: 20, w / v) at 80°C in a water bath for 3h. The residue was washed with water, ethanol and ether; and then dried at 105°C to constant weight. The amount of hemi-cellulose was derived by subtracting the amount of alkali-treated residue from that of holocellulose, and the pure cellulose was estimated by subtracting the amount of ash from the alkali-treated residue[15]. Lignin was determined spectrophotometrically at 280 nm after acetylation[16]. Crude protein was estimated as total micro Kjeldahl nitrogen multiplied by 6.25. Total lipids were extracted by methanol: chloroform (1: 1, v / v) for 4h[17] and the total soluble sugars were estimated colourmetrically by phenol and sulphuric acid[18].

Maintenance and cultivation of the yeast: *K.marxianus* 70343DSM (Deutsch Sampling Von Microorganismens) was maintained on agar slants containing 2 % glucose, 0.2 % pectin and 0.1 % yeast extract at pH 5.0 [19]. For the seed culture, standard inoculum (4 ml yeast suspension / 100 ml

recorded[10]. The experimental yeast was characterized by producing pectin in the cultures in a relatively short time (24h). So, in preliminary experiments, non-sterilized substrate (GFW) was added and the risk of contamination was assessed by microscopic examination that revealed absence of contaminants. This property represents an advantage for future technological applications of the studied system by reducing the costs of sterilization. Similar observations were recorded by other workers [2,7]. Statistical differences at $P<0.01$ were considered to be significant.

The proximate chemical composition of GFW, as the chosen experimental substrate, shows that it contains (% to GFW dry weight): moisture content, 0.6; pectin, 37.5 ; soluble sugars, 17.2; holocellulose, 14.3; crude protein, 5.1; lipid, 1.7; lignin, 0.9 and total ash, 0.7. The low content of GFW of holocellulose as compared with other agro-industrial wastes may stimulate protopectinases for pectin bioextraction.

Effect of yeast extract: The supplementation of the culture medium with yeast extract showed an adverse effect on pectin production and low values of protopectinase activity were detected with increasing the levels of yeast extract (Fig.2). Maximum pectin bioextraction, as well as protopectinase activity were observed when yeast extract was omitted from the medium. These results are in partial agreement with those reported by some workers[25] that maximum pectin extraction by *K. marixanus* from beet pulp was obtained when a relatively low yeast extract concentration was added to the fermentation medium. The analysis of variance indicated that the variations with the different yeast extract level in extracellular protein, pectin bioextraction, growth and protopectinase activity were significant ($P<0.01$).

Influence of time of substrate (GFW) addition: The results (Fig.3) revealed that the highest protopectinase activity was reached when the substrate was added at the beginning of the experiment (zero time), while the lowest enzyme activity was obtained when the addition of substrate was delayed to 16h after incubation. Also, both extracellular protein and growth of *K. marixanus* showed the same figure (their amounts decreased) as substrate addition to the culture delayed. The analysis of variance indicated that the variations with the different time of substrate addition in extracellular protein, pectin bioextraction, growth and protopectinase activity were significant ($P<0.01$).

Effect of incubation period: The results (Fig.4) indicated that after 18h of incubation the amount of pectin extracted by the yeast reached its maximum (32.32 %). Extension of fermentation period over 18h showed a decrease in the yield of bioextracted pectin. This may be due to the consumption of solubilized pectin as a carbon source. It was observed that there is a parallel relationship between the quantity of the extracted pectin and the protopectinase activity in the fermentation medium, since maximum activity was recorded after 18h. It was reported [2] that an incubation period not less than 5h was needed for the beginning of pectin appearance in the culture broth containing citrus peels of *Trichosporon penicillatum*. Similarly, it was indicated that pectin began to exist after 8h of *K. marixanus* incubation in beet pulp containing medium [7]. However, mould culture of *Aspergillus japonicus* needed 122h to attain best pectinase activity under the optimum culture conditions [9]. The analysis of variance indicated that the variations with the different incubation period in extracellular protein, pectin bioextraction, growth and protopectinase activity were significant ($P<0.01$).

Effect of peptone level: The stimulatory effect of this natural supplement on pectin production by *K. marxianus* was largely dependent on its level (Fig.5). The lowest pectin output, protopectinase activity and growth were observed when peptone was omitted from the medium. While, 0.4% peptone levels was responsible for maximal pectin bioextraction and protopectinase activity. However, higher peptone levels have adverse effect on pectin bioextraction and protopectinase activity. It is appropriate to point out that peptone as a natural complex substance may provide specific compounds and elements essential for the production of an adequate level of the enzymes and co-factors catalyzing the protopectinase activity. In comparison, the pectin released by *K. marxianus* in cultures containing beet pulp as a substrate was accelerated with increasing peptone level up to 4 g/l [26]. The analysis of variance indicated that the variations with the different peptone level in extracellular protein, pectin bioextraction, growth and protopectinase activity were significant ($P<0.01$).

Effect of glucose level: Pectin bioextraction, growth and protopectin solubilizing enzymes were accelerated when the glucose level was increased from 0.0 to 1.0 % in the culture medium (Fig.6). While higher glucose concentrations were accompanied by a detectable drop in yeast activities of pectin accumulation and protopectinase activity. The presence of glucose, as a more accessible energy / carbon source, in the medium may enhance the growth of the yeast in earlier stages of fermentation. This probably provides a high number of yeast cells able to utilize GFW more efficiently and yielding higher quantities of pectin and protopectinase. It was reported that enzyme synthesis of pectinases is correlated with the quality and concentration of the carbon and nitrogen sources[9]. The analysis of variance indicated that the variations with the different glucose level in extracellular protein, pectin bioextraction, growth and protopectinase activity were significant ($P<0.01$).

Substrate level (solid/liquid): The quantity of pectin liberated was found to be dependent on GFW concentration in the medium (Fig.7). The total amount of extracted pectin, the yeast growth and the protopectinase activity increased by increasing GFW level in the medium from 2 to 8 %. Thus, pectin increased by about 1.12 fold and protopectinase

showed 1.3 fold increase under these conditions. Any further increase in the amount of the substrate in the medium showed adverse effects on pectin extraction and enzyme activity. High substrate concentration (up to 5 %) was reported to be favorable for microbial pectin extraction from citrus peels[2]. On the other hand, a reduction of pectin yield on using a beet pulp level higher than 6 % was reported[7]. However, it was indicated that the highest pectinase activity of *Aspergillus japonicus* was obtained from culture medium containing 0.5 % pectin and 0.5 % glucose and higher concentrations have a repression effect[9]. In pectin production the pectin level in broth is important, because the isolation procedure proceeds more readily with pectin solutions of higher concentrations. GFW: water ratio of 1: 12.5 (8%) was found to be favorable. Meanwhile, other workers[2] reported that a citrus peel: water ratio of 1:2 was more promising and others reported that a beet pulp: water ratio of 1:16 was more convenient[7]. These indicate that the ability of GFW to absorb water is more than citrus and less than beet pulp. The analysis of variance indicated that the variations with the different grapefruit waste level in extracellular protein, pectin bioextraction, growth and protopectinase activity were significant ($P<0.01$).

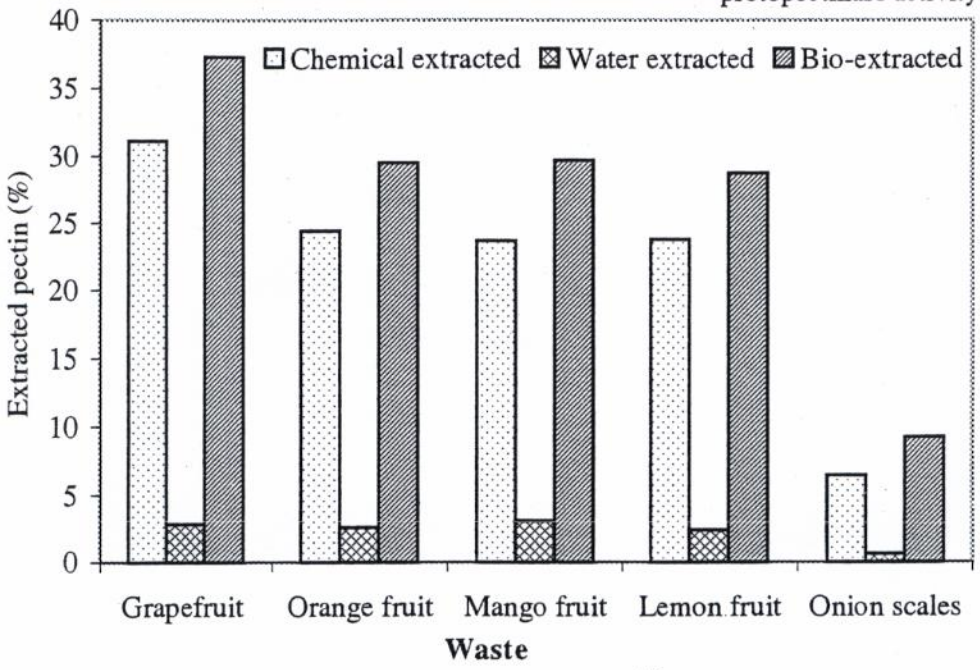

Fig.1: Microbial and chemical extraction of pectin from different substrates.

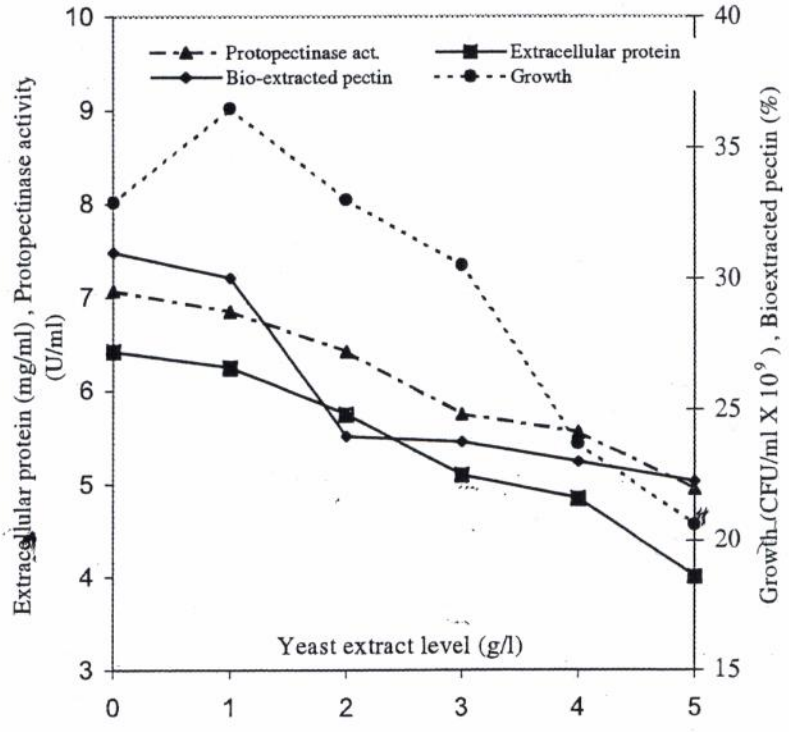

Fig. 2: Pectin bioextraction by *K.marxianus* as influenced with yeast extract level

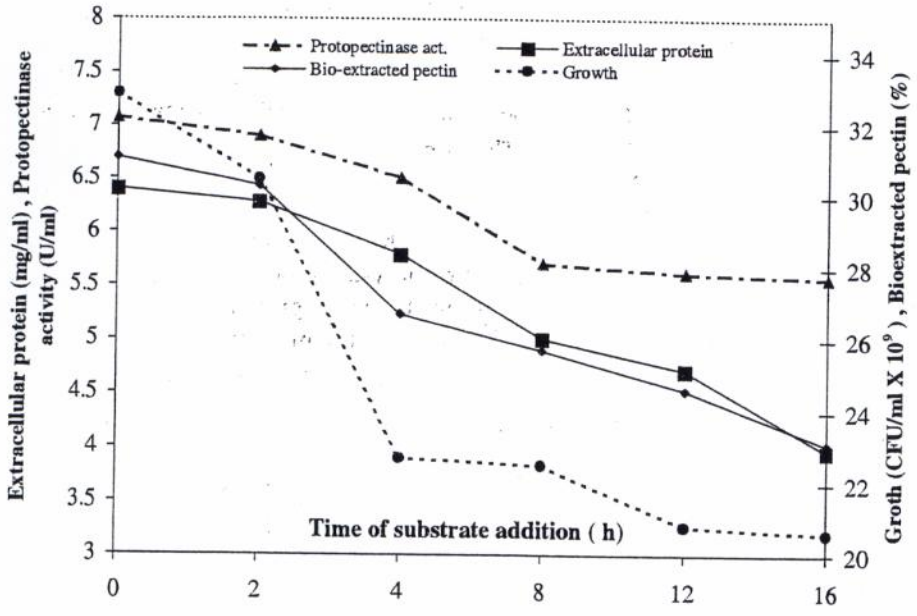

Fig. 3: Pectin bioextraction by *K. marxianus* as influenced with the time of substrate addition

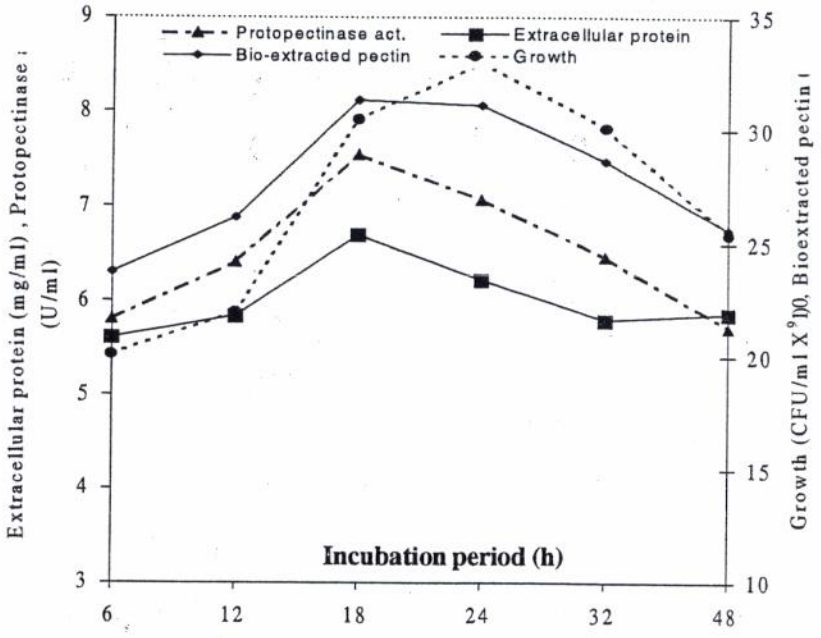

Fig. 4: Pectin bioextraction by *K.marxianus* at different incubation periods.

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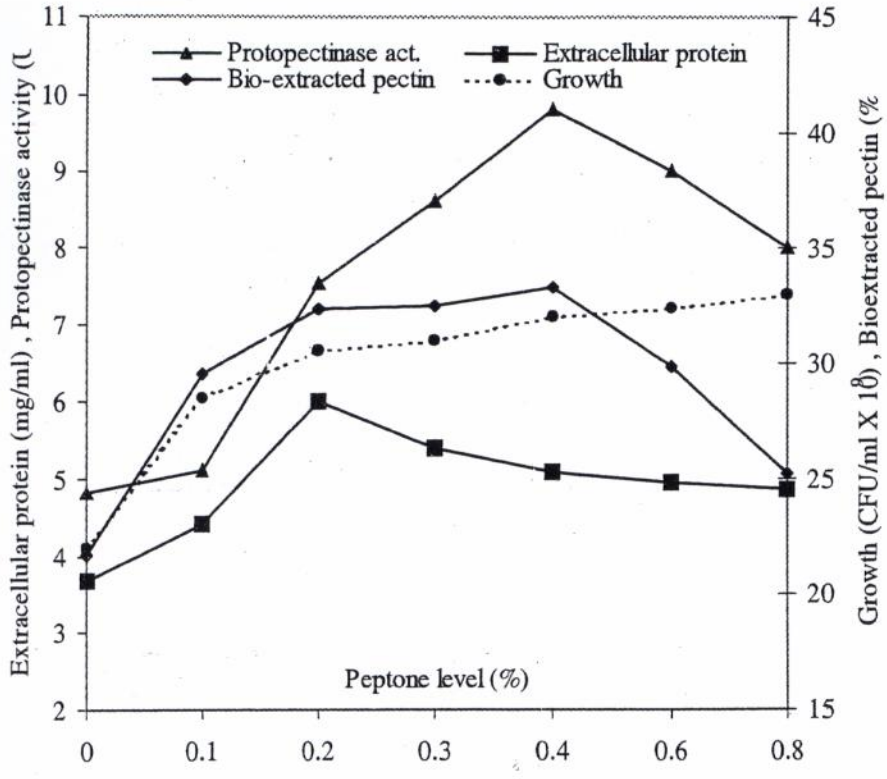

Fig. 5: Pectin bioextraction by *K.marxianus* at different peptone levels.

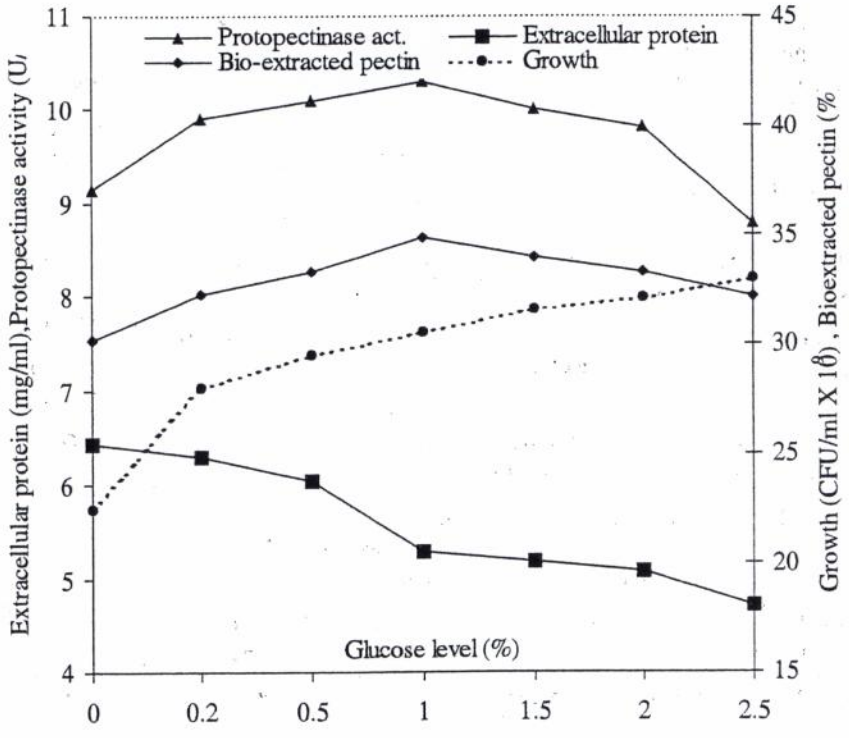

Fig. 6: Pectin bioextraction by *K.marxianus* at different glucose levels.

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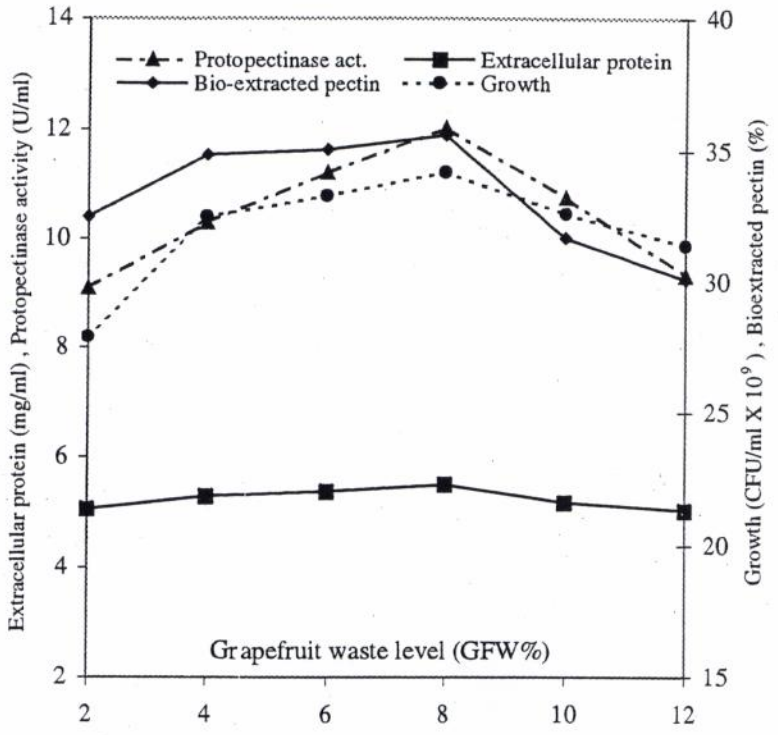

Fig. 7: Pectin bioextraction by *K.marxianus* as influenced with GFW level (solid/liquid)

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