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# AUTOLYSIS OF THE RED YEAST *PHAFFIA RHODOZYMA:* A POTENTIAL TOOL TO FACILITATE EXTRACTION OF ASTAXANTHIN.

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SUMMARY

Distilled water and 0.02 molar citrate buffer pH 7.0, are suitable autolysing systems for the red yeast *P. rhodozyma*. Autolysis renders astaxanthin extractable from the yeast. Of six strains of the yeast tested, 67-484 was most susceptible to autolysis.

## INTRODUCTION

*Phaffia rhodozyma* is a carotenoid-producing yeast which has potential commercial value as a dietary source of astaxanthin for poultry and pen-reared salmonids (Johnson et al, 1977; 1980). Disruption of cell walls in the yeast biomass prior to its inclusion in animal diets is essential for solvent extraction and intestinal absorption of the pigment. Methods of cell wall disruption which have been applied or discussed include mechanical breakage (Johnson et al, 1977), chemical (acid or alkaline) hydrolysis, and digestion by yeast-wall lytic enzymes of bacterial origin (Johnson et al, 1978, 1980; Okagbue and Lewis 1983). Unfortunately, the methods either denature the carotenoids or they are cumbersome and difficult to apply on a large scale. The purpose of the present study was to examine autolysis as a possible tool for processing *P. rhodozyma* to ensure preservation and extractability of astaxanthin. Different strains of the yeast have been compared for their susceptibility to autolysis.

## MATERIALS AND METHODS

Organisms and growth conditions: Six strains of *P. rhodozyma* listed in Table 2 were used. They were maintained on YM (Difco) agar slants. Each strain was grown (with shaking at 200 rpm at 20°C for 48 hr) in 500ml buffled side-arm flask (Bellco Glass Inc., Vineland, N.J.) in a total of 120ml of medium containing (as final concentration) 0.7% yeast nitrogen base (Difco), 1.5% glucose and 0.1M phosphate buffer pH 7.0. Foaming was controlled by intermittent addition of 10% v/v solution of sterile antifoam (FG-10, Dow Corning).

Cell mass harvested by centrifugation for use in autolysis experiments was washed once with sterile distilled water. Various autolysing systems were then used to suspend the biomass at a concentration of 6mg/ml in large test tubes. Each suspension was incubated at 37°C without agitation; higher temperatures caused rapid deterioration of the yeast pigmentation. After 26 or 72hr incubation, cell pellet centrifuged from each tube was visually scored for retention of the characteristic salmon-pink colour of *P. rhodozyma* and assayed for extractability of astaxanthin.

Quantitative Analyses:

pH: All measurements were performed using a pH meter (Orion research, model 601A digital ionalyzer).

Extraction and estimation of astaxanthin: Astaxanthin content of autolysed yeast biomass was determined as 'free' and as 'total' astaxanthin. The two parameters were determined and percent extractability of astaxanthin calculated as described previously (Okagbue and Lewis, 1983).

## RESULTS AND DISCUSSION

Table 1 shows that astaxanthin was extractable, to various extents, from *P. rhodozyma* autolysed in various systems at 37°C. Only distilled water alone or citrate buffer (0.02M) with or without dithiothreitol (DTT) could be considered satisfactory autolysing systems. Though 2% (v/v) toluene did not appear to adversely affect the autolytic effect of distilled water, the organic solvent would be unattractive for commercial use because it dissolved some of the carotenoids of *P. rhodozyma* and thus contributed to an apparent decrease in pink colour of the yeast pellet. 15% NaCl had an inhibitory effect on autolysis; this was unexpected since Kihlberg (1972) stated that up to 25% solution of salt is added to yeast to accelerate autolysis in yeast extract manufacture. DTT enhanced the autolytic effect of citrate buffer, probably in a manner analogous to its (DTT) potentiating effect on extractability of protein from yeast (Shetty and Kinsella, 1978). Unfortunately, the pungent odour of the thiol would probably preclude its use in a commercial system for processing *P. rhodozyma* for use in animal feeds. In general, this study has shown that autolysis of the red yeast in distilled water or in 0.02M citrate buffer pH 7.0 could be usefully employed to preserve its pink colour and render astaxanthin extractable (and presumably nutritionally available for absorption). Of all the strains of the yeast tested (Table 2), 67-484 was the most susceptible to autolysis. It would appear to be very useful for commercial application since all its pigments became extractable after only 26 hr incubation in distilled water.

## REFERENCES


Johnson, E.A., Conklin, D.E., and Lewis, M.J. (1977). J. Fish Res. Board Can, 34; 2417 - 2421.

Johnson, E.A., Villa, T.G., Lewis, M.J., and Phaff, H.J. (1978). Appl. Environ. Microbiol. 35; 1155 - 1159.

Johnson, E.A., Lewis, M.J., and Grau, C.R. (1980). Poultry Sci. 59; 1777 - 1782.

Kihlberg, R. (1972). Ann. Rev. Microbiol. 26; 427 - 466.

Okagbue, R.N. and Lewis, M.J. (1983). Biotechnol. Lett. 5; 731 - 735.

Shetty, K.J., and Kinsella, J.E. (1978). Biotech. and Bioeng. 20; 755 - 766.

Table 1

Effect of autolysing medium on pigmentation and extractability of astaxanthin from *P. rhodozyma*

| Autolysing medium | Final pH | Yield of total astaxanthin(µg/ml) in cell suspension | Yield of extractable astaxanthin (µg/ml) | % Extractability | Colour of cell pellet |
|---|---|---|---|---|---|
| Distilled water | 7.20 | 1.74 | 1.36 | 78.2 | ++ |
| Distilled water + toluene | 6.53 | 1.46* | 1.02 | 69.9 | - |
| Distilled water + 15% NaCl | 5.70 | 1.74 | 0.00 | 0 | - |
| Medium of yeast growth(as is) pH 6.43 | 6.53 | 1.20 | 0.32 | 26.7 | - |
| Medium of yeast growth pH adjusted to 7.20 | 7.12 | 1.20 | 0.14 | 11.7 | - |
| 0.1M Tris buffer, pH8.0 | 8.11 | 1.60 | 0.47 | 29.4 | + |
| 0.1M Tris buffer pH8.0 + .01M dithiothreitol | 8.12 | 1.78 | 0.40 | 22.5 | + |
| 0.02M citrate buffer pH7.0 | 8.40 | 1.69 | 1.22 | 72.2 | ++ |
| 0.02M citrate buffer pH7.0 + 0.01M dithiothreitol | 6.42 | 1.60 | 1.36 | 85.0 | ++ |

++ very good retention of pink colour

\+ good

\- Appreciable loss of colour

* Some pigment dissolved in the autolysing medium.

Table 2

Susceptibility of strains of *P. rhodozyma* to autolysis*

| Strain | Autolysing medium: 0.02M citrate buffer pH 7.0 + 0.01M DTT | | | Distilled water | | |
|---|---|---|---|---|---|---|
| 67 - 202 | (1.41) | 6.76 | (5) | (1.46) | 13.01 | (5) |
| 67 - 203 | (1.60) | 5.94 | (6) | (1.97) | 45.69 | (4) |
| 67 - 210 | (1.36) | 41.90 | (3) | (1.17) | 64.10 | (3) |
| 67 - 385 | (1.13) | 75.20 | (2) | (1.32) | 78.03 | (2) |
| 67 - 484 | (1.39) | 87.80 | (1) | (1.41) | 100.00 | (1) |
| 68 - 653C | (0.80) | 17.50 | (4) | (0.80) | 11.88 | (6) |



*Duration of autolysis was 26 hr at 37°C

All cultures tested were grown for 48 hr at 20°C

Under each autolysing medium, unbracked data refer to % extractability of astaxanthin.

Bracketed data on the left indicate total concentrations of astaxanthin (µg/ml) in the suspension used for calculating % extractability, while those on the right indicate the order in which the strains responded to the treatment.