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Short contribution

Inhibition of the red yeast *Phaffia rhodozyma* by saponin

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Summary. Production of cell mass and astaxanthin by the yeast *Phaffia rhodozyma* was inhibited by saponin. The inhibition was partially reversed by oleic and linoleic acids and by ergosterol and cholesterol, thereby suggesting a possible interference of saponin with the yeast cell membrane.

Introduction

Saponins are surface-active compounds containing a glycosidic side chain attached to an aglycone moiety which may be a steroid or a triterpenoid They occur naturally in a wide variety of plants including alfalfa and other legumes. During a process which extracts leaf protein (for nutritional purposes) from the alfalfa plant, the saponins become distributedin various fractions which include alfalfa solubles or alfalfa residual juice (ARJ), the ultimate waste product (Livingston et al. 1979).

There are many reports on adverse effects of saponins on living systems (Birk 1969). Among the recorded effects are depression of growth in poultry and other animals, toxicity to fish and amphibia, haemolysis of red blood cells and inhibition of seed germination . Fungistatic activity of the compounds is also known, but only filamentous fungi such as *Sclerotium rolfsii* (Gestetner et al. 1971) and *Trichoderma viride* (Livingston et al. 1977) appear to have received much consideration; literature seems to be deficient on the effects of saponins on yeasts. In an earlier study (Okagbue and Lewis 1984), it was observed that use of ARJ as a substrate for propagation of the red yeast *Phaffia rhodozyma* was inhibitory to the formation of astaxanthin, the major and potentially useful carotenoid of the yeast. Since ARJ was known to contain saponin (Livingston et al. 1979), it was thought to be the potential inhibitor of astaxanthin formation. This hypothesis prompted the study of the effects of commercial grade saponin on the red yeast *Phaffia rhodozyma*.

Materials and methods

Organism and growth conditions: *Phaffia rhodozyma* (the type strain 67 - 210) was obtained from the culture collection of the Department of Food Science and Technology, University of California, Davis, USA, and was maintained on YM (Difco) agar slants at 20°C.

A medium containing different concentrations of saponin was prepared by adding appropriate volumes of sterile 2.5% (w/v) aqueous solution of saponin (Sigma Chemical Co., St. Louis, Mo, USA) to 40-ml of a basal medium (contained in 500-ml baffled flasks) to give final concentrations ranging from 0% (control flasks) to 0.5%. If necessary, the final volume of medium in each flask was made up to 50-ml with sterile distilled water. The basal medium contained (as final concentrations in 50ml) 0.02% $MgSO_4.7H_2O$, 0.5% of each of KH_2PO_4, yeast extract and peptone and 1.5 glucose in 0.02M phthalate buffer, pH 5.6; glucose and the buffered basal medium were autoclaved separately at 120°C for 15 min. There-

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after, 2ml of an aqueous suspension of 20h-old *P. rhodozyma* (containing approximately 1.5mg/ml cell mass on a dry weight basis) was inoculated into each flask which was then shaken at 200 rpm (Orbital shaker, New Bruns-wick Scientific, Edison, N.J., USA) at 20°C for 48h. Drops of 10% (v/v) solution of sterile antifoam (FG-10, Dow corning) were added intermittently to control foaming.

Analyses: Washed cell pellets from the flasks were assayed for yields of cell mass and astaxanthin. Cell mass was determined as cell dry weight per ml of culture. Astaxanthin concentration (expressed as µg/ml) was determined as described previously (Okagbue and Lewis 1984).

Results and Discussion

Figure 1 shows the effects of various concentrations of saponin on *Phaffia rhodozyma*. As expected, the compound inhibited astaxanthin formation; total inhibition was caused by 0.075% concentration of the inhibitor. A less drastic effect was observed on cell mass production. At 0.025% concentration the saponin appeared to have a stimulatory effect on cell yield. Higher concentrations inhibited growth of the yeast but total inhibition was not observed even at 0.5% level of the saponin. The observed inhibition on cell mass production was unexpected because ARJ known to contain saponin (Livingston et al. 1979), had no corresponding effect on *Phaffia rhodozyma* (Okagbue and Lewis 1984); apparently, the commercial grade saponin had a more toxic effect on the yeast than ARJ. It is likely that the inhibitory effect (on cell mass production) of the saponin component of ARJ was neutralised by other components of the complex medium. It has been pointed out (Birk 1969) that toxicity of certain saponins to red blood cells and to certain digestive enzymes can be inhibited by proteins and other natural companions of the saponins.

Toxicity of saponin has been attributed to its adverse effect on permeability of biological membrances (Bangham and Horne 1962). It is probable that the inhibitory effects observed on *Phaffia rhodozyma* in this study could be due (at least partially) to some disturbance in the integrity of the lipid-rich plasma membrane of the yeast. To examine this possibility, we tested the ability of some agents (known to enrich plasma membrane lipids or to enhance carotenoid biosynthesis in fungi) to reverse the inhibitory effects of saponin on the yeast. Table 1 shows that only the unsaturated fatty acids (oleic and linoleic acids) and the sterols (ergosterol and cholesterol) were totally or partially effective It appears

Table 1. Effects of some supplements on inhibitory effects of saponin on *Phaffia rhodozyma*.

Supplement	Cell mass[a] mg/ml	Astaxanthin[a] µg/ml	µg/g
none (control)	2.0	0.12	60.00
biotin(0.22µg/100ml)	2.1	0.07	33.33
Ca-pantothenate (240µg/100ml)	1.5	0.07	46.70
inositol (1mg/100ml)	2.1	0.12	57.14
B-ionone (0.2%, v/v)	scanty growth	ND	NA
$CaCl_2.2H_2O$ (2mg/100ml)	3.3	0.21	63.6
$MgSO_4.7H_2O$ (2mg/100ml)	2.1	0.13	61.9
Oleic acid (2%)	4.0	ND	NA
linoleic acid (2%)	10.6	ND	NA
Cholesterol (10µg/ml)	6.7	1.69	238.81
Ergosterol (10µg/ml)	7.2	1.12	155.56

ND = Not determined (cells white)

NA = Not applicable

[a]All data are averages from duplicate flasks, two determinations per flask.

In this experiment, *Phaffia rhodozyma* was grown in a basal medium containing 0.075% saponin and various supplements. The supplements were added from appropriate stock solution; recorded concentrations were the final in 50ml of medium. Ergosterol and cholesterol were dispersed in tween 80 (Andreason and Stier 1953); stock suspensions contained 0.05g in 10ml. An amount of 1ml of 95% ethanol was used to aid dispersion. Other conditions for this experiment were as described in "Materials and methods".

that the saponin adversely affected permeability of the yeast cell membrane by interferring with synthesis of the unsaturated fatty acids (which also predominate in *Phaffia rhodozyma* (Johnson et al. 1980) and the sterols; the two groups contribute to stability and biological activity of yeast lipids and membranes (Rattray et al 1975). The apparent disturbance in the integrity of the plasma membrane probably contributed to inhibition of astaxanthin formation since carotenoids in some fungi are associated with lipid globules and membranes (Mitzka-Schnabel and Rau 1980).

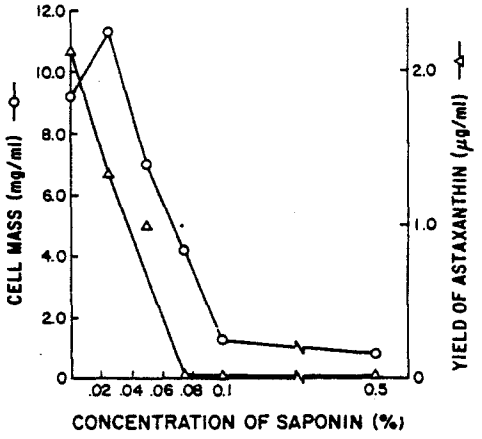

Fig 1: The effect of saponin at different concentrations on *Phaffia rhodozyma*.

References

Andreasen, A A, Stier, T J B (1953) Anaerobic nutrition of *Saccharomyces cerevisiae*. 1. Ergosterol requirement for growth in a defined medium. J Cell and Comp Physiology 41:23 - 36

Bangham, A D, Horne, R W (1962) Action of saponin on biological cell membranes. Nature 196 : 952-953

Birk, Y (1969) Saponins. In: *Toxic Constituents of Plant Foodstuffs*, Liener, E I (ed). Academic Press: New York

Gestetner, B, Assa, Y, Henis, Y, Birk, Y, Bondi, A (1971) Lucerne saponins. IV. Relationship between their chemical constitution, and haemolytic and antifungal activities. J Sci Food and Agric 22:168 - 172

Johnson, E A, Villa, T G, Lewis, M J (1980) *Phaffia rhodozyma* as an astaxanthin source in salmonid diets. Aquaculture 20:123 - 134

Livingston, A L, Knuckles, B E, Edwards R H, de Fremery, D, Miller, R E, Kohler, G O (1979) Distribution of saponin in alfalfa protein recovery systems. J Agric Food Chem 27:362 - 365

Livingston, A L, Whitehead, L C, Kohler, G O (1977) Microbiological assay for saponin in alfalfa products. J Assoc Official Analyt Chem 60:957 - 960

Mitzka-Schnabel, U, Rau, W (1980) The subcellular distribution of carotenoids in *Neurospora crasa*. Phytochem. 19:1409 - 1413

Okagbue, R N, Lewis, M J (1984) Use of alfalfa residual juice as a substrate for propagation of the red yeast *Phaffia rhodozyma*. Eur J Appl Microbiology and Biotechnol. In Press

Rattray, J B M, Schibeci, A, Kidby, D K (1975) Lipids of yeasts. Bacteriol Rev 39: 197 - 231

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