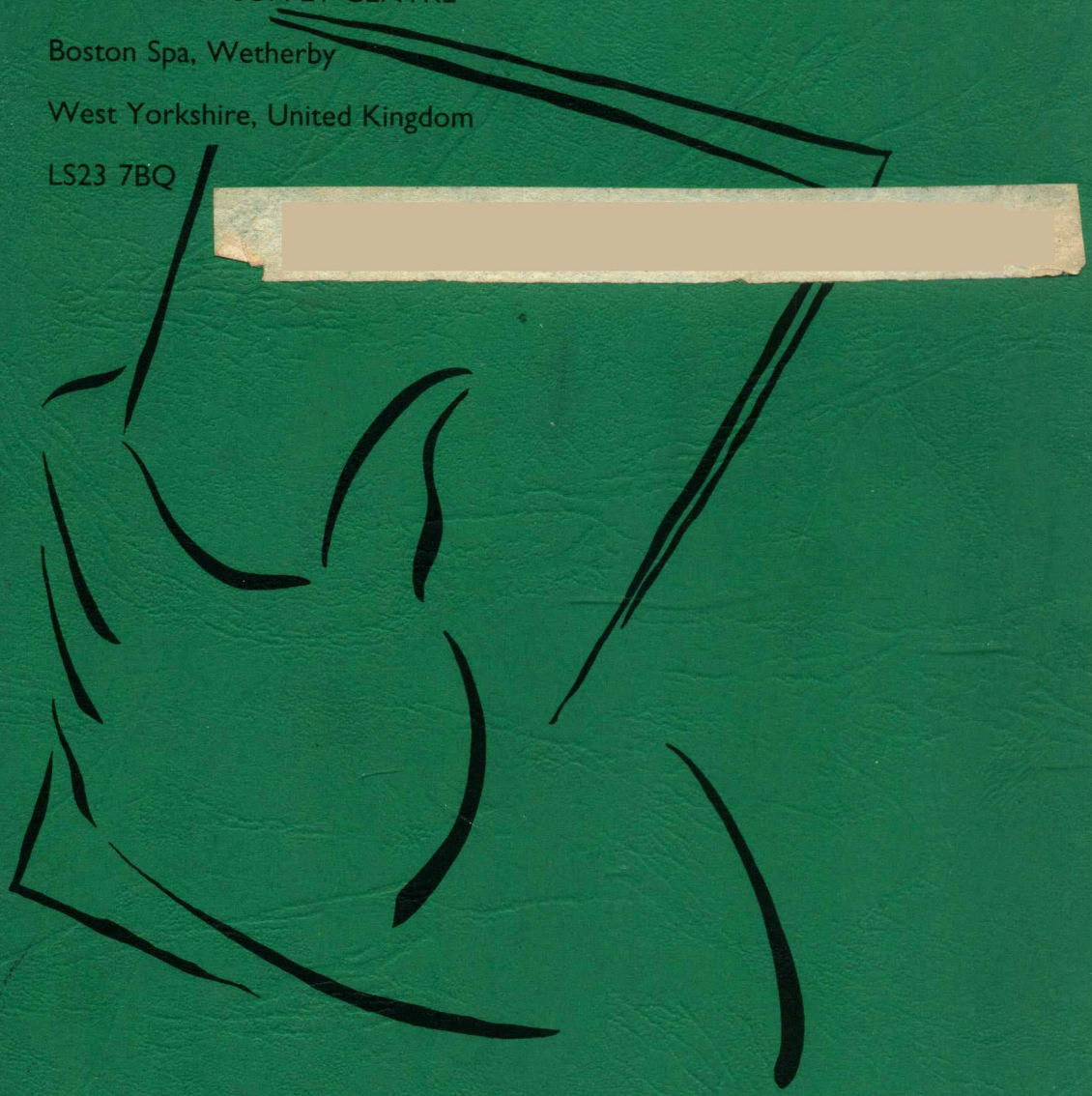

TITLE Oxygen Free-Radical-Scavenging Enzymes in Malt, Yeast and Soya Beans

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Oxygen Free-Radical-Scavenging Enzymes in Malt, Yeast and Soya Beans

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by

Simon Paul Clarkson B.Sc., G.R.S.C.

October 1990

SUMMARY OF THESIS SUBMITTED FOR Ph.D DEGREE

BY SIMON PAUL CLARKSON

ON

OXYGEN FREE-RADICAL-SCAVENGING ENZYMES IN MALT, YEAST AND SOYA BEANS

1. The effects of malting on barley superoxide dismutase, catalase, peroxidase and polyphenol oxidase were investigated. Polyphenol oxidase was lost. All other enzyme levels were found to increase.

2. On mashing, substantial activity of malt peroxidase was found to survive. Superoxide dismutase activity was lost after 15 minutes. No catalase activity was detected. The addition of superoxide, peroxide or hydroxyl radicals to mashes was found to have no effect on run-off, hot-water extract or haze of worts.

3. Mashing with a fungal catalase preparation (L-43) caused an increase in colour and haze and a decrease in the polyphenol content of worts. This phenomenon was dependent on mash temperature and thickness, but was absent in mashes using denatured (enzymically inactive) malt. Hydrogen peroxide had the same effect. These effects were caused by glucose oxidase in the L-43 and native peroxidases in the malt.

4. Purification of malt peroxidase by CM-Sepharose chromatography produced five active fractions with different pH optima and thermal stability. Some of the fractions contained more than one peroxidase isoenzyme and were kinetically dissimilar.

5. Brewing yeast strains BB1 (ale) and BB11 (lager) when subjected to sudden changes in oxygen tension showed no significant changes in growth rate, ethanol production, or the specific activities of alcohol dehydrogenase and pyruvate decarboxylase.

6. Transitions from anaerobiosis to aerobiosis caused yeast superoxide dismutase activity to increase, whereas the reverse caused it to fall. Catalase activity in the ale yeast followed that of superoxide dismutase, whereas in the lager yeast it remained unchanged by the transitions.

7. Anaerobic cultures of the two yeast strains lost viability on making them aerobic or by addition of superoxide radicals (0.25 mM). Aerobic cells were unaffected by this treatment.

8. The cupro-zinc superoxide dismutase of soya beans, a possible food-compatible preparation, was purified 300-fold. It had a molecular weight of 36,300 (two subunits of 18,000),and was very resistant to elevated temperatures (65°C and above). Isoelectric focussing produced three electromorphs, with isoelectric points of 4.73, 4.55 and 4.37.

9. Optimum stability of soya bean CuZn-superoxide dismutase preparations was at 4°C in 60% glycerol, 20mM potassium phosphate pH 7.8. These may have potential usefulness in the food industry.

"Ask, and it will be given to you; seek, and you will find; knock, and the door will be opened to you. For everyone who asks receives, and he who seeks finds, and to him who knocks it will be opened."

Matthew 7: 7 + 8

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ABBREVIATIONS

ABTS 2,2'-azinobis(3-ethylbenzthiazolinesulphonic acid)

ADH alcohol dehydrogenase

ASF ammonium sulphate fractionation

BB1 ale yeast strain

BB11 lager yeast strain

CS citrate synthase

CuZn-SOD cupro-zinc superoxide dismutase

DMSO dimethyl sulphoxide

DTNB 5,5'-dithiobis-(2-nitrobenzoic acid)

18-crown-6 1,4,7,10,13,16,-hexaoxacyclooctadecane

GOD glucose oxidase

HWE hot-water extract

L-43 catalase preparation L-43

Mn-SOD manganese superoxide dismutase

NBT nitro blue tetrazolium

$O_2^{\cdot -}$ superoxide radical

$OH^{\cdot}$ hydroxyl radical

PAGE polyacrylamide gel electrophoresis

PDC pyruvate decarboxylase

POD peroxidase

PPO polyphenol oxidase

SASPL saturated ammonium sulphate precipitation limit

SOD superoxide dismutase

TEMED N,N,N,'N'-tetramethylethylenediamine

INTRODUCTION

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