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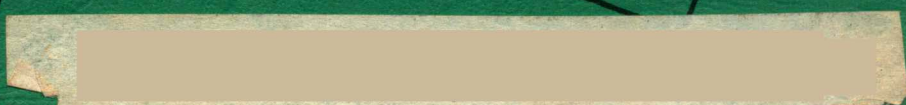
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Oxygen Free-Radical-Scavenging Enzymes in

Malt, Yeast and Soya Beans

AUTHOR

Simon Paul Clarkson

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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.

ACKNOWLEDGEMENTS

First, many thanks to my wife Lesley, for her considerable patience in transcribing my dictations, for the typing of the text, and for putting up with me during the production of this thesis.

My thanks to my supervisors Dr Peter Large and Dr Charles Bamford, for their advice and suggestions during the course of this study. Thanks also to Dr Chris

"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."

Thanks also to Anne Robinson for her copywork in the laboratory — for the drawing of the figures, and to Dr Alastair Anderson for his assistance with the translation work.

Matthew 7: 7 + 8

To my family and friends, for their encouragement and friendship. Thanks and God bless you all.

Finally, thank you to the AFRC and to Borealis for the funding of this work.

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Thanks also to Anne Robertson for her company in the laboratory and for the drawing of the figures, and to Dr Alistair Anderson for his assistance with the fermentation work.

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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

1.1. Oxygen and its toxicity

Oxygen (O_2) is the most abundant element in combined form (53.8%) in the earth's crust and the most abundant elemental di-oxygen accounts for some 21% of dry air. This makes the earth's atmosphere unique in the solar system. Although it is indispensable for growth of many organisms including man and yeast, oxygen in liquid or concentrations in excess of those normally present in air is toxic to aerobic organisms. Even at normal concentrations, damaging effects manifest themselves slowly.

The major deleterious effects do not seem to be due to the oxygen molecule in the ground state, but rather the activated forms of oxygen formed by combination of additional electron in the π orbitals. Univalent reduction of dioxygen leads to the successive formation of superoxide anion ($O_2^{\cdot -}$), peroxide (H_2O_2), the hydroperoxide (HO_2^{\cdot}) and finally water (Figure 1).

INTRODUCTION



Figure 1. Univalent reduction of dioxygen

Fluxes of $O_2^{\cdot -}$ generated enzymically or photochemically have been shown to induce various defects. Hydroperoxidation of organic membranes and cell walls (Fridovich 1978, Fridovich 1985, Halliwell and Gutteridge 1989). It is also known to cause degradation of DNA, lipid and polysaccharides, inactivate enzymes and lysosomes (Mickelson et al 1977). The high reactivity of $O_2^{\cdot -}$ is due to: 1) its negative charge, which enables it to act as a strong base or nucleophile and 2) its unpaired electron which causes it to behave as a free radical (hydrogen abstraction, addition reactions and radical coupling). It can also act either as a one-electron reductant or as an oxidant (Primer 1981).

CONTENTS

	Page
LIST OF FIGURES, FRAMES AND TABLES	viii
SUMMARY	xii
ABBREVIATIONS	xiv
INTRODUCTION	
1.1. Oxygen and its toxicity	1
1.2. Oxygen, malting and mashing	3
1.3. Oxygen and fermentation	6
1.4. Soya beans, a food grade source of superoxide dismutase	8
1.5. Structure of this thesis	8
METHODS	
2. OXYGEN-SCAVENGING ENZYMES IN BARLEY AND MALT	11
2.1. Barleys, malts and malting conditions	11
2.2. Grain extraction	11
2.3. Levels of oxygen-scavenging enzymes during the malting process	11
2.4. SOD and catalase levels in various malts	12
2.5. Comparison of oxygen-scavenging enzymes in barley and lager and ale malt	12
2.6. Enzyme assays	12
2.6.1. Superoxide dismutase	12
2.6.2. Catalase	12
2.6.3. Peroxidase	13
2.6.4. Polyphenol oxidase	13
2.7. Protein estimation	13
3. OXYGEN-SCAVENGING ENZYMES AND THEIR EFFECTS DURING MASHING	14
3.1. The effects of mashing on various oxygen-scavenging enzymes in malt	14
3.1.1. Mash procedure for laboratory mashing in a BRF mashing bath	14
3.1.2. Effect of mashing on malt SOD	15
3.1.2.1. Activity of malt SOD during the course of mashing	15
3.1.2.2. PAGE of worts obtained in time course mashes	15

3.1.2.3.	Dialysis of worts from the time course mashes	16
3.1.3.	The effect of mashing on malt catalase	16
3.1.4.	The effect of mashing on malt peroxidase	16
3.2.	The effects of oxygen radicals on mashing parameters	16
3.2.1.	Radical generation	16
3.2.2.	Mash parameters measured	17
3.3.	The effects of catalase preparation L-43 on mash parameters	17
3.4.	Mashing with additions of catalase L-43	18
3.4.1.	Haze production	18
3.4.2.	Wort colour	18
3.4.3.	Determination of total polyphenols	18
3.4.4.	The effect of L-43 concentration in mash	19
3.4.5.	The effect of the time of addition of L-43 to a mash	19
3.4.6.	The effect of adding L-43 to wort	19
3.4.7.	The effect of mashing temperatures on L-43- induced changes in wort	19
3.4.8.	The effect of mash thickness on L-43- induced changes in wort	20
3.4.9.	The effect of adding L-43 and hydrogen peroxide to mash	20
3.4.10.	The effect of adding tannic acid to a mash containing L-43	20
3.4.11.	The effect of adding L-43 and hydrogen peroxide to mash of denatured malt	20
3.4.12.	Saturated ammonium sulphate precipitation limits of worts from L-43-treated and boiled L-43-treated mash	21
3.4.13.	Enzyme analysis of L-43	21
3.4.14.	The effects of mashing with individual components of L-43	22
3.5.	The effect of glucose oxidase and peroxidase on fresh wort	22
3.6.	The effect of glucose oxidase and purified malt peroxidase isoenzymes on fresh wort	23
4.	MALT PEROXIDASE: PURIFICATION AND PROPERTIES	24
4.1.	Assay of malt peroxidase	24
4.2.	Studies on crude extracts of malt peroxidase	24
4.2.1.	Preparation of crude extract	24
4.2.2.	Ammonium sulphate fractionation of crude extract	24
4.2.3.	PAGE of crude extract	25
4.2.4.	Detection of peroxidase activity on polyacrylamide gels	25

4.2.5.	The effect of pH on crude malt peroxidase activity	25
4.2.6.	Heat stability of crude malt peroxidase	25
4.3.	Partial purification of malt peroxidase	25
4.4.	Studies on partially purified malt peroxidase isoenzymes	26
4.4.1.	PAGE analysis	26
4.4.2.	Effect of pH on peroxidase isoenzyme activities	27
4.4.3.	Temperature stability of malt peroxidase isoenzymes	27
4.5.	Kinetic studies of crude extract and isoenzymes of malt peroxidase	27
5.	EFFECT OF CHANGES IN OXYGEN CONCENTRATION ON ENZYME ACTIVITIES IN BREWING YEAST	28
5.1.	Yeasts	28
5.2.	Media	28
5.2.1.	Media preparations	28
5.2.1.1.	Solution A	28
5.2.1.2.	Solution B	29
5.2.1.3.	<i>Saccharomyces</i> salts	29
5.3.	Fermenter set-up	29
5.4.	Inoculum culture	30
5.5.	Fermentation conditions	30
5.5.1.	Aerobic growth	31
5.5.2.	Aerobic to anaerobic transition during stationary phase	31
5.5.3.	Aerobic to anaerobic transition during early exponential phase	31
5.5.4.	Anaerobic to aerobic transition during stationary phase	31
5.5.5.	Anaerobic to aerobic transition during early exponential phase	32
5.5.6.	The effect of an air pulse on an anaerobic stationary phase yeast culture	32
5.5.7.	The effect of an air pulse on an anaerobic stationary phase yeast culture in the presence of cycloheximide	32
5.5.8.	The effect on cell viability of adding superoxide radicals to a culture of BB1	32
5.5.8.1.	Aerobic culture of BB1	33
5.5.8.2.	Anaerobic culture of BB1	33
5.6.	Harvesting of cells	33
5.7.	Preparation of cell-free extracts	34
5.8.	Yeast viability	34
5.8.1.	Preparation of dye for the viability stain	34
5.9.	Analysis of medium	34
5.9.1.	Total carbohydrate	34

5.9.2.	Glucose	35
5.9.3.	Ethanol	35
5.9.4.	pH Measurement	35
5.10.	Cell-free extract analysis - enzyme assays	35
5.10.1.	SOD	35
5.10.2.	Catalase	35
5.10.3.	Citrate synthase	36
5.10.4.	Pyruvate decarboxylase	36
5.10.5.	Alcohol dehydrogenase	36

6.	PURIFICATION AND CHARACTERISATION OF SOYA BEAN SUPEROXIDE DISMUTASE	37
6.1.	Preparation of crude soya bean extracts	37
6.2.	Polyacrylamide gel electrophoresis	37
6.3.	Soya SOD purification	38
6.3.1.	Ammonium sulphate precipitation	38
6.3.2.	QAE-Sepharose chromatography	38
6.3.3.	Sephacryl S-300 gel permeation chromatography	39
6.3.4.	Hydroxyapatite chromatography	39
6.3.5.	Polyacrylamide gel electrophoretic analysis	39
6.4.	Properties of purified soya SOD	40
6.4.1.	Determination of molecular mass	40
6.4.1.1.	Relative molecular mass	40
6.4.1.2.	Subunit molecular mass	40
6.4.2.	Isoelectric point determination	41
6.4.3.	Effect of cyanide on soya bean SOD	41
6.4.4.	Inhibition of SOD by hydrogen peroxide	41
6.4.5.	Effect of pH on soya SOD	42
6.4.6.	Effect of temperature and pH on the stability of soya bean SOD	42

RESULTS

7.	OXYGEN-SCAVENGING ENZYMES IN BARLEY AND MALT	44
7.1.	Oxygen-scavenging enzyme levels during the malting process	44
7.2.	SOD and catalase levels in various malts	44
7.3.	Comparison of oxygen-scavenging enzymes in barley, lager and ale malts	47
8.	OXYGEN-SCAVENGING ENZYMES AND THEIR EFFECTS DURING MASHING	49
8.1.	The effect of mashing on oxygen-scavenging enzymes in malt	49
8.1.1.	The effect of mashing on malt SOD	49
8.1.2.	The effect of mashing on malt catalase	49
8.1.3.	The effect of mashing on malt peroxidase	49
8.2.	The effects of oxygen radicals on mash parameters	53