

VII

CONTENTS

DECLARATION	ii
APPROVAL	iii
ABSTRACT	iv
ACKNOWLEDGEMENTS	v
CONTENTS	vii-ix
LIST OF TABLES	x-xiii
LIST OF FIGURES	xiv-xv
LIST ANNEXES	xvi
GENERAL INTRODUCTION	1
CHAPTER 1	
<b>QUALITY CHANGES ASSOCIATED WITH THE IMMEDIATE &amp;     DELAYED ICING OF TRENCHED SARDINES <u>Amblygaster</u>     <u>sirm</u> &amp; THEIR RELATED MICROBIAL FLORA</b>	
1.1 INTRODUCTION	5
1.1.1 Nature & distribution of bacterial flora on fish	5
1.1.2 Bacterial spoilage of fish	7
1.1.3 Biochemical changes occurring during fish storage	7
1.2 MATERIALS & METHODS	11
1.2.1 Fish harvesting	11
1.2.2 Fish handling	11
1.2.3 Test methods used	11
1.2.3.1 Microbiological analysis of fish	11
1.2.3.2 Characterization of isolates	12
1.2.3.3 Chemical analysis of fish	12
1.2.3.4 Biochemical tests used in the characterization of isolates	15
1.2.4 Visual Observation	26
1.3 RESULTS	27
1.4 DISCUSSION	49
1.5 Annexes	53-57

## CHAPTER 2

STUDIES ON MICROBIAL CHARACTERISTICS & QUALITY  
 CHANGES OF VACUUM PACKED TRENCHED SARDINES  
Amblygaster sirm STORED UNDER REFRIGERATION  
 CONDITIONS

2.1	INTRODUCTION	60
2.1.1	Vacuum packaging in relation to quality, storage & distribution	60
2.1.2	The use of potassium sorbate dips	62
2.1.3	The use of irradiation treatment	64
2.2	MATERIALS & METHODS	69
2.2.1	Handling of fish	69
2.2.2	Pretreatment of dressed fish	69
2.2.2		
(a)	Potassium sorbate dip	69
(b)	Irradiation	69
(c)	Saturated salting	70
(d)	Control sample	70
2.2.3	Packaging of fish	70
2.2.4	Storage of vacuum packed fish	70
2.2.5	Microbiological analysis of vacuum packed fish	70
2.2.6	Characterization of isolates	71
2.2.7	Chemical analysis of refrigerated vacuum packs	71
2.2.8	Organoleptic evaluation	72
2.3	RESULTS	73
2.3.1	Storage aspects of untreated fish held at 4°C	73
2.3.2	Storage aspects of sorbate treated fish held at 4°C	79
2.3.3	Storage aspects of irradiated fish held at 4°C	85
2.3.4	Storage aspects of salt saturated fish held at 4°C	91
2.3.5	Storage aspects of untreated fish held at 15°C	97
2.3.6	Storage aspects of sorbate treated fish held at 15°C	103

## IX

2.3.7	Storage aspects of salt saturated fish held at 15°C	109
2.3.8	Storage aspects of irradiated fish held at 15°C	115
2.4	DISCUSSION	122
CHAPTER 3		
PROCESSING & STORAGE OF VACUUM PACKED, HEAT STERILIZED TRENCHED SARDINES <u>Amblygaster sirm</u>		
3.1	INTRODUCTION	129
3.1.1	The retort pouch & its applications	129
3.2	MATERIALS & METHODS	134
3.2.1	Fish handling for retorted vacuum pouches	134
3.2.2	Vacuum packaging	134
3.2.3	Fish processing - determination of shelf life of retorted vacuum pouches	134
3.2.4	Organoleptic evaluation	134
3.2.5	Chemical analysis	134
3.2.6	Fish processing - determination of $F_0$ (Thermal process time) BY A THERMAL PROCESS	135
3.2.7	Determination of $F_0$ (Thermal process time) BY GILLESPIE'S METHOD	136
3.3	RESULTS	137
3.4	DISCUSSION	149
3.5	Annexes	152-165
4.0	CONCLUSION	166
	BIBLIOGRAPHY	167

## LIST OF TABLES

## CHAPTER 1

TABLE 1	Observations of fish iced at site (0 hour), 5 hour & 10 hour after landing	30
TABLE 2	Proximate composition of <u>Amblygaster sirm</u>	31
TABLE 3	Oil content (%) of <u>Amblygaster sirm</u>	31
TABLE 4	Protein (%) content in <u>Amblygaster sirm</u>	32
TABLE 5	Effect of delayed icing on the total volatile nitrogen in <u>Amblygaster sirm</u>	33
TABLE 6	Effect of delayed icing on the pH of <u>Amblygaster sirm</u>	34
TABLE 7	Effect of delayed icing on the major grouping of microflora	35
TABLE 8	Effect of delayed icing on the gram positive microflora on <u>Amblygaster sirm</u>	36
TABLE 9	Effect of delayed icing on the gram negative microflora on <u>Amblygaster sirm</u>	37
TABLE 10	Further identification of some <u>Pseudomonas</u> spp. - colony & cultural appearance	38
TABLE 11	Biochemical identification of some <u>Pseudomonas</u> species	39
TABLE 12	Identity of some <u>Pseudomonas</u> species	40
TABLE 13	Further identification of <u>Micrococcus</u> spp. - colony & cultural appearance	41
TABLE 14	Biochemical identification of some <u>Micrococcus</u> species	42
TABLE 15	Identity of some <u>Micrococcus</u> species	43
TABLE 16	Further identification of <u>Bacillus</u> spp. - colony & cultural & appearance	44
TABLE 17	Biochemical identification of some <u>Bacillus</u> species	45
TABLE 18	Identity of some <u>Bacillus</u> species	46

## CHAPTER 2

TABLE 1	Observations of vacuum packed <u>Amblygaster sirm</u> stored at 4°C	74
TABLE 2	Total volatile nitrogen, trimethyleamine, & pH <u>Amblygaster sirm</u> stored at 4°C	75

XI

TABLE 3	Total bacterial counts of vacuum packed <u>Amblygaster sirm</u> stored at 4°C	76
TABLE 4	Dominant microflora isolated from vacuum packed <u>Amblygaster sirm</u> at initial & final stages of storage (4°C)	77
TABLE 5	Observations of 2% potassium sorbate dipped, vacuum packed <u>Amblygaster sirm</u> stored at 4°C	80
TABLE 6	Total volatile nitrogen, trimethylamine, & pH in 2% potassium sorbate dipped, vacuum packed <u>Amblygaster sirm</u> stored at 4°C	81
TABLE 7	Total bacterial counts in potassium sorbate (2%) dipped vacuum packed <u>Amblygaster sirm</u> stored at 4°C	82
TABLE 8	Dominant microflora isolated from 2% potassium sorbate dipped & vacuum packed <u>Amblygaster sirm</u> at the initial & final stages of storage (4°C)	83
TABLE 9	Observations of vacuum packed & irradiated <u>Amblygaster sirm</u> stored at 4°C	86
TABLE 10	Total volatile nitrogen, trimethylamine, & pH in vacuum packed, irradiated <u>Amblygaster sirm</u> stored at 4°C	87
TABLE 11	Total bacterial counts in vacuum packed <u>Amblygaster sirm</u> , irradiated & stored at 4°C	88
TABLE 12	Dominant microflora isolated from vacuum packed, irradiated <u>Amblygaster sirm</u> at the initial & final stages of storage (4°C)	89
TABLE 13	Observations of salt saturated (5%) & vacuum packed <u>Amblygaster sirm</u> stored at 4°C	92
TABLE 14	Total volatile nitrogen, trimethylamine, & pH in salt saturated (5%), vacuum packed <u>Amblygaster sirm</u> stored at 4°C	93
TABLE 15	Total bacterial counts in salt saturated (5%), & vacuum packed <u>Amblygaster sirm</u> stored at 4°C	94
TABLE 16	Dominant microflora isolated from salt saturated (5%), vacuum packed <u>Amblygaster sirm</u> at the initial & final stages of storage (4°C)	95
TABLE 17	Observations of vacuum packed <u>Amblygaster sirm</u> stored at 15°C	98

TABLE 18	Total volatile nitrogen, trimethylamine & pH in vacuum packed <u>Amblygaster sirm</u> stored at 15°C	99
TABLE 19	Total bacterial counts on vacuum packed <u>Amblygaster sirm</u> stored at 15°C	100
TABLE 20	Dominant microflora isolated from vacuum packed <u>Amblygaster sirm</u> at the initial & final stages of storage (15°C)	101
TABLE 21	Observations of 2% potassium sorbate dipped & vacuum packed <u>Amblygaster sirm</u> stored at 15°C	104
TABLE 22	Total volatile nitrogen, trimethylamine & pH in 2% potassium sorbate dipped & vacuum packed <u>Amblygaster sirm</u> stored at 15°C	105
TABLE 23	Total bacterial counts of 2% potassium sorbate dipped, vacuum packed <u>Amblygaster sirm</u> stored at 15°C	106
TABLE 24	Dominant microflora isolated from 2% potassium sorbate dipped & vacuum packed <u>Amblygaster sirm</u> at the initial & final stages of storage	107
TABLE 25	Observations of salt saturated (5%) & vacuum packed <u>Amblygaster sirm</u> stored at 15°C	110
TABLE 26	Total volatile nitrogen, trimethylamine & pH in salt saturated (5%), vacuum packed <u>Amblygaster sirm</u> stored at 15°C	111
TABLE 27	Total bacterial counts of salt saturated (5%) & vacuum packed <u>Amblygaster sirm</u> stored at 15°C	112
TABLE 28	Dominant microflora isolated from salt saturated (5%) & vacuum packed <u>Amblygaster sirm</u> at initial & final stages of storage	113
TABLE 29	Observations of vacuum packed & irradiated <u>Amblygaster sirm</u> stored at 15°C	116
TABLE 30	Total volatile nitrogen, trimethylamine & pH in vacuum packed, irradiated <u>Amblygaster sirm</u> stored at 15°C	117
TABLE 31	Total bacterial counts of vacuum packed, irradiated <u>Amblygaster sirm</u> stored at 15°C	118
TABLE 32	Dominant microflora isolated from vacuum packed & irradiated <u>Amblygaster sirm</u> at the initial & final stages of storage (15°C)	119

## CHAPTER 3

TABLE 1(a)	Proximate analysis of immediately iced <u>Amblygaster sirm</u> both before packaging (raw) & during vacuum packaged (heat sterilized) storage, (batch A)	139
	(b) Organoleptic & visual characteristics	139
TABLE 2(a)	Proximate analysis of immediately iced <u>Amblygaster sirm</u> both before packaging (raw) & during vacuum packed (heat sterilized) storage, (batch B)	140
	(b) Organoleptic & visual characteristics	140
TABLE 3(a)	Proximate analysis of 5 hour delayed iced <u>Amblygaster sirm</u> both before packaging (raw) & during vacuum packed (heat sterilized) storage, (batch A)	141
	(b) Organoleptic & visual characteristics	141
TABLE 4(a)	Proximate analysis of 5 hour delayed iced <u>Amblygaster sirm</u> both before packaging (raw) & during vacuum packed (heat sterilized) storage, (batch B)	142
	(b) Organoleptic & visual characteristics	142
TABLE 5(a)	Proximate analysis of 10 hour delayed iced <u>Amblygaster sirm</u> both before packaging (raw) & during vacuum packaged (heat sterilized) storage, (batch A)	143
	(b) Organoleptic & visual characteristics	143
TABLE 6(a)	Proximate analysis of 10 hour delayed iced <u>Amblygaster sirm</u> both before packaging (raw) & during vacuum packed (heat sterilized) storage, (batch B)	144
	(b) Organoleptic & visual characteristics	144
TABLE 7	$F_0$ values calculated by thermal process (vacuum packs / retorted)	145
TABLE 8	Values obtained by Gillespy's method	145
TABLE 9	Percentage drained mass of vacuum packed, heat sterilized <u>Amblygaster sirm</u>	146
TABLE 10	Proximate composition of vacuum packed heat sterilized <u>Amblygaster sirm</u>	147

## LIST OF FIGURES

<b>CHAPTER 1</b>		
FIGURE 1	Change in total bacterial count of <u>Amblygaster sirm</u> stored in ice at 0, 5 & 10 hours after landing	47
FIGURE 2	Total bacterial count of <u>Amblygaster sirm</u> from flesh & surface when stored at 30°C & 20°C at 0, 5 & 10 hours after landing	47
<b>CHAPTER 2</b>		
FIGURE 1	Change in total aerobic (ae) & anaerobic (an) counts at incubation temperatures of 30°C, 5°C (ae) & 35 °C (an) obtained from vacuum packed <u>Amblygaster sirm</u> stored at 4°C	78
FIGURE 2	Change in total aerobic (ae) & anaerobic (an) counts at incubation temperatures of 30°C, 5°C (ae) & 35°C (an) obtained from potassium sorbate (2%) treated vacuum packed <u>Amblygaster sirm</u> stored at 4°C	84
FIGURE 3	Change in total aerobic (ae) & anaerobic (an) counts at incubation temperatures of 30°C, 5°C (ae) & 35°C (ae) obtained from irradiated & vacuum packed <u>Amblygaster sirm</u> stored at 4°C	90
FIGURE 4	Change in total aerobic (ae) & anaerobic (an) counts at incubation temperatures of 30°C, 5°C (ae) & 35°C (an) obtained from salt saturated(5%), & vacuum packed <u>Amblygaster sirm</u> stored at 4°C	96
FIGURE 5	Change in total aerobic (ae) & anaerobic (an) counts at incubation temperatures of 30°C, 5°C (ae) & 35°C (an) obtained from vacuum packed, <u>Amblygaster sirm</u> stored at 15°C	102
FIGURE 6	Change in total aerobic (ae) & anaerobic (an) counts at incubation temperatures of 30°C, 5°C (ae) & 35°C (an) obtained from potassium sorbate (2%) treated & vacuum packed <u>Amblygaster sirm</u> stored at 15°C	108



- FIGURE 7 Change in total aerobic (ae) counts at incubation temperatures of 30°C, 5°C (ae) obtained from salt saturated (5%) & vacuum packed Amblygaster sirm stored at 15°C 114
- FIGURE 8 Change in total aerobic (ae) counts at incubation temperatures of 30°C, 5°C (ae) obtained from irradiated & vacuum packed Amblygaster sirm stored at 15°C 120

## LIST OF ANNEXES

## CHAPTER 1

ANNEX 1	Scheme prepared according to Shewan, Hobbs & Hodgkiss (1960), Cowan & Steel (1977), Lee & Pfeifer (1975) for the preliminary identification of microflora	53
ANNEX 2	Scheme prepared according to Hendrie & Shewan (1979) for the identification of some oxidative <u>Pseudomonas</u> species	54
ANNEX 3	Scheme prepared according to Hendrie & Shewan (1979) for the identification of some <u>Pseudomonas</u> & <u>Alteromonas</u> species which do not produce acid from O/F medium	55
ANNEX 4	Scheme prepared according to Baird-Parker (1974) & (1979), Genus <u>Micrococcus</u> <u>cohn</u> 1872, 151	56
ANNEX 5	Scheme prepared according to Gibson & Gorden (1974) Genus <u>Bacillus</u> <u>Cohn</u> 1872, 174	57

## CHAPTER 3

ANNEX 1	Calculation of $F_0$ - By a thermal process	152-157
ANNEX 2	Calculation of $F_0$ - By Gillespy's method	158-164