

ABSTRACT

Abeer El-Sayed Abd El-Fattah, Microbial Production of Emulsifiers for the Utilization in Dairy Products. Unpublished Doctor of Philosophy Thesis, Ain Shams University, Faculty of Agriculture, Department of Food Science, 2006.

The main objective of this study was to produce bioemulsifier from yeast that could potentially be used in food and dairy industries and many other applications.

The heat extraction procedure allowed the isolation of emulsifying agent from all of the eleven yeast strains tested. *Saccharomyces cerevisiae* EMCC 69 strain produced the highest bioemulsifier yield with superior emulsification activity. Some yeast strains such as *Candida utilis* EMCC 120 produced higher extracellular bioemulsifier yield and activity, than that recorded for Glycerol monooleate as a control emulsifying agent. For this reason, and due to long history of safe human consumption, *Saccharomyces cerevisiae* EMCC 69 *Candida utilis* EMCC 120 were chosen for further studies.

The highest production of intracellular bioemulsifier in modified Czapek, s yeast broth by *S. cerevisiae* EMCC 69 strain was achieved with initial pH 7, inoculum level of 5%, agitation rate of 200 rpm, fermentation at 30oC for 72 h.. The highest production of extracellular bioemulsifier in modified Czapek, s yeast broth by *C. utilis* EMCC 120 strain was achieved with initial pH 7, inoculum level of 3%, agitation rate of 400 rpm, fermentation at 30oC for 60 h. Scale-up fermentation enhanced the yield of exteracellular bioemulsifier of *Candida utilis* EMCC 120 and intracellular bioemulsifier of *Saccharomyces cerevisiae* EMCC 69. This means high yield of

extracellular and intercellular bioemulsifiers with low production costs.

The pH of aqueous phase had little effect on the amount of the butter oil phase emulsified by *Candida* emulsifier between pH 2 and 11. Stability of emulsions with *S. cerevisiae* EMCC 69 emulsifier decreased with increasing pH values over 6. In the presence of 1 to 5% (w/v) sodium chloride, there was no loss of emulsion stability occurred. Stable emulsions were formed in the presence of up to 15% (w/v) sucrose. Emulsion for both of *S. cerevisiae* and *C. utilis* emulsifier stability was 79% after three cycles of freezing at -18°C for 16 h and thawing at 32°C for 8h. Emulsions with *S. cerevisiae* EMCC 69 and *C. utilis* EMCC 120 were not disrupted by pasteurization at 63°C for 30 min. Stability of emulsions did not change during storage at 4°C for 30 days. Much loss in emulsion stability during storage at room temperature 25°C for 15 days was observed. Emulsifiers extracted from *S. cerevisiae* EMCC 69 and *C. utilis* EMCC 120 emulsified all oils tested.

High quality ice cream and whipped cream with preferable texture and consistence, were produced with adding 0.2% or 0.3% bioemulsifiers, respectively.

Key words: Yeast, emulsifier, emulsification activity, emulsification stability, ice cream, whipped cream.

LIST OF CONTENTS

		Page
I	Introduction	1
II	Review of Literature	3
1.	Appropriate conditions for microbial emulsifier production	3
2.	Production of microbial emulsifiers and surfactants	6
3.	Use of microbial emulsifier in food processing	49
III	Materials and Methods	58
1.	Materials	58
1.12.	Chemicals	60
1.13.	Microbiological media	60
2.	Experimental procedure	60
2.1.	Screening yeast strains for bioemulsifier production	60
2.2.	Bioemulsifier production	60
2.3.	Emulsifier extraction	61
2.4.	UF- purification of bioemulsifier	61
2.5.	Emulsification stability	62
2.6.	Fermentation process	62
2.6.1.	Growth curve	62

2.6.2.	Bioreactor (one liter) as a batch culture (one stage)	63
2.6.3.	Effect of fermentation period	63
2.6.4.	Effect of nitrogen sources	63
2.6.5.	Bioreactor (14-litres) as a batch culture (one-stage)	63
2.7.	Utilization of yeast bioemulsifier in dairy products	64
2.7.1.	Ice cream	64
2.7.2.	Whipped cream.	65
3.	Analytical methods	66
3.1.	Fermentation process	66
3.1.1.	Yeast viable cell count	66
3.1.1.	Yeast viable cell count	66
3.1.2.	Biomass dry weight	66
3.1.3.	Emulsification activity	66
3.2.	Growth and biological activity parameters	67
3.2.1.	Growth parameters	67
3.2.2.	Sugar utilization efficiency	67
3.2.3.	Bioemulsifier yield (%)	68
3.3.	Bioemulsifier composition	68
3.3.1.	Protein content	68

3.3.2.	Total carbohydrate content	68
3.4.	Dairy products	68
3.4.1.	Microbiological analyses	68
3.4.2.	Chemical Analyses	68
3.4.2.1.	Dry matter content	68
3.4.2.2.	Fat content	69
3.4.2.3.	Protein content	69
3.4.2.4.	Measurement of the pH Value	69
3.4.2.5.	Titratable acidity	69
3.4.2.6.	Ash content	69
3.4.3.	Physical analyses	69
3.4.3.1.	Viscosity	69
3.4.3.2.	Freezing point	69
3.4.3.3.	Specific gravity	70
3.4.3.4.	Weight per gallon	70
3.4.3.5.	Overrun	70
3.4.3.6.	Melting resistance	70
3.4.4.	Organoleptic evaluation	70
IV	Results and discussion	71
Part I	Yeast strain selection	71

Section A	Production of interacellular bioemulsifier	71
Section B	Production of extracellular bioemulsifier	74
Part II	Achieving Ample Fermentation Protocol.	78
Section (A)	Growth parameters and prouductivity	78
1.	Growth parameters	78
2.	Prouductivity	88
Section (B)	Optimization of fermentation condition for the production of interacellular bioemulsifier by <i>S. cerevisiae</i> EMCC 69 and extracellular bioemulsifier by <i>Candida utilis</i> EMCC 120 grown in one liter bioreactor.	93
1.	Effect of pH value	93
2.	Effect of fermentation temperature	98
3.	Effect of inoculum level	103
4.	Effect of agitation rate	108
5.	Effect of fermentation period	113
6.	Effect of whey protein concentrate as nitrogen source on production of intracellular biemulsifier form <i>S. cereviciae</i> EMCC 69 .	119

7.	Effect of whey protein concentrate as nitrogen source on Production of extracellular biemulsifier form <i>Candida utilis</i> EMCC 120.	123
Section (C)	Scale-up fermentation process	127
Part III	Characteristics of Yeast Emulsifiers and their Emulsion Properties	131
1.	Emulsifiers characteristization	131
2.	Properties of the emulsions.	133
3.	The effect of Pasteurization (63 °C for 30 min), Freezing and thawing, and Storage at room temperature (25+1 °C) and at 4 °C on emulsification stability of bioemulsifier.	137
Part IV.	Application of bioemulsifier in Production of some dairy Product.	140
Section A.	The possibility of using bioemulsifi- -er in ice cream manufacture.	140
1.	The use of intracellular and extracellular biomulsifiers in Ice cream manufacture	140
1.	Mixes properties	141
1.1	Dry matter content..	141
1.2	Fat content.	141
1.3	Protein content.	142

1.4	Ash content.	142
1.5	Titratable acidity and pH.	142
2.	Physical properties of ice cream mix	145
2.1	Specific Gravity and weight per gallon.	145
2.2	Freezing point.	145
2.3	Viscosity.	146
3.	Physical properties of resultant ice cream	150
3.1.	Specific Gravity and weight per gallon.	150
3.2.	Overrun %.	150
3.3.	Melting resistance.	151
4.	Organoleptic profile of ice cream	155
5.	Bacteriological quality of ice cream	159
Section B.	The possibility of using biomulsifiers in the manufacture of whipped cream	163
1.	Physical properties of whipped cream with using extracellular bioemulsifiers	164
1.1	pH.	164
1.2	Viscosity.	164
1.3	Foam expansion (%).	165
1.4	Foam stability.	166
2.	Organoleptic profile of whipped cream with using extracellular bioemulsifiers	170

2.1.	Organoleptic profile of whipped cream	170
V.	Summary and Conclusion	174
-	References	181
VI.	Arabic Summary	1

LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
ATCC	American Type Culture Collection
°C	Degree centigrade
CMC	Sodium carboxy methylcellulose
cfu	colony forming unit
cfu/ml	colony forming unit per milliliter
DCW	Dry cell weight
DM	Dry matter
DSM	Deutsch Sammlung von Mikroorganismen
EMCC	Egyptian Microbiology Culture Collection
EXBE	Extracellular bioemulsifier
<i>eta</i>	Egyptian Office for Trading and Agencies
et al	and others (et alii)
g/g	gram per gram
g/l	Gram per liter
GMO	Glycerol monoleate
h	Hour
IBE	Interacellular bioemulsifier
kDa	Kilo dalton
min	minute
MIRCEN	Microbiological Resources Center
ml	Milliliter
N CYC	National Collection of Yeast Cultures
NRRL	Northern Regional Research Laboratory
OD ₆₀₀	Optical density at 600 nm
r.p.m.	Revolution per minute

SAS	Statistical Analysis System
sec	Second
SNF	Solids not fat
sp. gr.	Specific gravity
TA	Titrateable acidity
TBC	Total bacterial count
temp.	temperature
TN	Total nitrogen
UF	ultrafiltration
%	percentage
wt	weight
W / gal	Weight per gallon