MAGNESIUM FORTIFICATION IN CAKES BY USING DOUBLE EMULSIONS WITH LEGUME FLOURS

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ABSTRACT

MAGNESIUM FORTIFICATION IN CAKES BY USING DOUBLE EMULSIONS WITH LEGUME FLOURS

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Magnesium is a trace element that plays an important role in contraction and relaxation of muscles, enzymatic cellular reactions, production and transport of energy and protein synthesis. Magnesium fortification of food is necessary to prevent magnesium deficiency. However, magnesium addition in foods can induce chemical degradations, protein aggregation and results in unacceptable taste. In order to overcome these drawbacks, in this thesis, it was aimed to encapsulate magnesium by using water-oil-water double emulsions and to analyze its baking stability in cake. Double emulsions were prepared by using different emulsifiers and by using high speed homogenization and ultrasonication. As a natural raw material, lentil and pea flours were used in the outer phase of double emulsions and obtained emulsions were characterized. Emulsions prepared by different legume flour concentrations (15%, 20%, 25%, 30%) and homogenization methods (high speed homogenization and ultrasonic homogenization) were analyzed in terms of particle size, rheology, stability, optic view, nucleic magnetic resonance and efficiency and optimum parameters were determined. Double emulsions with lower particle size, higher

viscosity and higher stability were obtained at higher legume flour concentrations. Increasing legume flour concentration form 15% to 30%, resulted in the 15% increase in the stability of double emulsions. Ultrasonic homogenization also contributed to produce double emulsions with higher stability (> 99%). Optimum conditions for preparing double emulsion was at least with 20% legume flour concentration and with ultrasonic homogenization.

Emulsions with high stability and efficiency were integrated into cakes and the heat stability of emulsions in the baked product were analyzed. The aim was to enrich foods with magnesium without taste and quality degradation. Therefore, cakes containing magnesium in the form of double emulsion, in the form of primary emulsion, and without any protection were compared by means of hardness, specific volume, weight loss and crust color and the similar quality parameters were obtained. Moreover, sensory results showed that, cakes containing magnesium encapsulated in double emulsion had similar taste with cakes containing no magnesium. Cakes with double emulsion had lower encapsulation efficiencies than heated double emulsions. However, double emulsions in cakes had about 80% and 89% higher baking stability than control and 13% and 24% higher baking stability than the primary emulsion, for lentil and pea flour, respectively. Digestion of the obtained emulsions and cakes containing emulsions were analyzed in gastric and intestinal juices and it was seen that magnesium can be released and digested in human body. Considering all these results, encapsulation of magnesium by double emulsion with legume flours and its integration into food was fulfilled successfully.

Keywords: Magnesium, Double Emulsion, Encapsulation, Lentil, Pea

ÖZ

BAKLİYAT UNU İÇEREN ÇİFT KATMANLI EMÜLSİYON KULLANILARAK KEKE MAGNEZYUM TAKVİYESİ YAPILMASI

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Magnezyum kasların kasılıp gevşemesi, enzimatik hücre reaksiyonlarının gerçekleşmesi, enerji üretimi ve taşınması ve protein sentezi gibi birçok yaşamsal faaliyette önemli rol oynayan ancak az rastlanan bir elementtir. Magnezyum eksikliğinin giderilmesi için gıdalara magnezyum takviyesi yapılması gereklidir. Ancak gıdalara eklenen magnezyum kimyasal bozulma ve protein kümelenmesine, sonucunda da istenmeyen tat değişikliğine sebep olmaktadır. Bu sorunlara çözüm olmak adına, bu çalışmada magnezyumun su-yağ-su ikili emülsiyon ile kapsüllenmesi ve magnezyumun kekin pişirilmesi sırasında ısıya karşı stabilitesinin belirlenmesi amaçlanmıştır. İkili emülsiyonlar, farklı emülgatörler ve yüksek hızlı homojenizasyon ve ultrasonikasyon yöntemleri kullanılarak hazırlanmışlardır. İkili emülsiyonların dış fazında emülgatör olarak doğal bir hammadde olan mercimek ve bezelye unları kullanılmış ve elde edilen emülsiyonların özellikleri analiz edilmiştir. Farklı baklagil unu konsantrasyonlarına (15%, 20%, 25%, 30%) sahip ve farklı homojenizasyon yöntemleri (yüksek hızlı homojenizasyon ve ultrasonik homojenizasyon) ile hazırlanan emülsiyonların parçacık boyutu, reolojik özellikleri, stabiliteleri, optik görünümleri ve verimleri incelenerek en uygun parametreler

belirlenmiştir. Yüksek un konsantrasyonlarında daha küçük parçacık boyutuna, daha yüksek viskoziteye ve daha yüksek stabiliteye sahip ikili emülsiyonlar elde edilmiştir. Baklagil unu konsantrasyonunu %15'den %30'a yükseltmek, ikili emülsiyonların stabilitesinde %15 artış sağlamıştır. Ultrasonik homojenizasyon da yüksek stabiliteye sahip emülsiyon oluşturmaya katkı sağlamıştır (>99%). Baklagil unları ile ikili emülsiyon oluşturulmasında en az %20 un konsantrasyonu ve ultrasonik homojenizasyon kullanılması en uygun koşullar olarak belirlenmiştir.

Yüksek verime ve stabiliteye sahip emülsiyonlar seçilip kek içerisine eklenerek ısıl işlem görmüş gıda içerisindeki stabiliteleri incelenmiştir. Bu çalışmada magnezyumca zenginleştirilen ürünün tat ve kalitesinin bozulmaması amaçlanmıştır. Bu sebeple, ikili emülsiyon şeklinde magnezyum eklenen kekler, kaplanmamış magnezyum ve birincil emülsiyon ile kaplanmış magnezyum kullanılarak üretilen kekler ile karşılaştırılmış ve sertlik, özgül hacim, ağırlık kaybı ve kabuk rengi baz alınarak benzer kalite değerlerine sahip oldukları görülmüştür. Duyusal analiz sonuçları da ikili emülsiyon ile kaplanmış halde magnezyum içeren keklerin magnezyum ilavesi yapılmamış kekler ile benzer beğenilirliğe sahip olduğunu göstermiştir. Kek içerisine eklenen ikili emülsiyonların kapsülleme verimi ısıl işleme maruz kalan ikili emülsiyonlardan daha düşük bulunmuştur. Buna rağmen, kek içerisine eklenen ikili emülsiyonlardan mercimek unu ile hazırlananlar kontolden %80, birincil emülsiyondan %13, bezelye unu ile hazırlananlar ise kontrolden %89, birincil emülsiyondan %24 daha fazla pişirme stabilitesi göstermiştir. Elde edilen emülsiyonların ve emülsiyon içeren keklerin mide ve bağırsak sıvılarındaki sindirimleri analiz edilmiş ve magnezyumun insan vücudunda açığa çıkarak sindirilebildiği görülmüştür. Bu sonuçlar göz önüne alınarak magnezyumun baklagil unları ile kapsüllenmesi ve gıda içerisine entegre edilmesi başarıyla gerçekleştirilmiştir.

Anahtar Kelimeler: Magnezyum, İkili Emülsiyon, Kapsülleme, Mercimek, Bezelye

To my beloved family,

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TABLE OF CONTENTS

ABSTRACT v

ÖZ vii

ACKNOWLEDGMENTS x

TABLE OF CONTENTS xii

LIST OF TABLES xvi

LIST OF FIGURES xxii

CHAPTERS

1 INTRODUCTION 1

1.1 Magnesium 1

1.2 Double Emulsion 3

1.2.1 Double Emulsions in Food Systems 7

1.2.2 Preparation Methods 9

1.2.3 Homogenization Methods 11

1.3 Surfactants 15

1.3.1 Lipophilic Surfactant 16

1.3.2 Hydrophilic Surfactant 17

1.4 Legumes 21

1.4.1 Lentil 23

1.4.2 Pea 26

1.5 Encapsulation Efficiency of Double Emulsions 28

1.5.1 Atomic Absorption Spectrophotometry 28

1.5.2 Nuclear Magnetic Resonance 30
1.6 Objective of the Study 31
2 MATERIALS & METHODS 33
2.1 Materials 33
2.2 Preparation of Double Emulsions 33
2.2.1 Preparation of W/O Primary Emulsion 34
2.2.2 Preparation of W/O/W Double Emulsion 35
2.3 Analyses of Double Emulsion 36
2.3.1 Particle Size Analysis 36
2.3.2 Rheology 36
2.3.3 Instant Stability 37
2.3.4 Long Term Stability 37
2.3.5 Encapsulation Efficiency 38
2.3.6 Nuclear Magnetic Resonance Analysis 41
2.3.7 Optical Imaging 42
2.4 Incorporation of Double Emulsions into Cakes 42
2.4.1 Preparation of Cakes 42
2.4.2 Characterization of Cakes 43
2.5 Baking Stability 45
2.6 Simulated In-Vitro Digestion of Magnesium in the Cakes 46
2.6.1 Simulated Gastric Digestion 46
2.6.2 Simulated Intestinal Digestion 47
2.7 Statistical Analysis 47
2.8 Experimental Design 48

3 RESULTS & DISCUSSION 49
3.1 Effect of Osmotic Balance on Stability 49
3.2 Analysis of Magnesium Amount in Legume Flours 50
3.3 NMR T_2 Relaxometry to Analyze the Effect of Magnesium on the Solubility of Legume Proteins 51
3.4 Characterization of Magnesium-Loaded Double Emulsions 52
3.4.1 Particle Size and Distribution 52
3.4.2 Rheology 61
3.4.3 Instant Stability 69
3.4.4 Long Term Stability 73
3.4.5 Optical Imaging 75
3.4.6 Encapsulation Efficiency 80
3.4.7 Nuclear Magnetic Resonance Relaxometry 86
3.5 Characterization of Magnesium-Loaded Cakes 91
3.5.1 Hardness 91
3.5.2 Specific Volume 92
3.5.3 Weight Loss 93
3.5.4 Color 95
3.5.5 Sensory Analysis 96
3.6 Baking Stability and In-Vitro Digestion 97
3.6.1 Baking Stability 97
3.6.2 In-Vitro Digestion 101
4 CONCLUSION 105
REFERENCES 107

APPENDICES 133

A. Statistical Analaysis 133

CURRICULUM VITAE 185

LIST OF TABLES

TABLES

Table 1.1 Recommended Dietary Allowances (RDA) for Magnesium (Mg) 2
Table 1.2 Formula and Bioavailability Properties of Magnesium Salts..... 3
Table 1.3 The common general characteristics and differences between of proteins and polysaccharides as functional biopolymers in food systems 17
Table 1.4 Amino acid content of lentil and pea..... 23
Table 1.5 Proximate composition of whole dry lentil seeds 24
Table 1.6 Proximate composition of field pea seeds..... 26
Table 2.1 Experimental Design of the Study..... 48
Table 3.1 Particle size of double emulsions prepared with different lentil and pea flour concentrations and homogenization methods (HSH: high-speed homogenization, UH: ultrasonic homogenization)..... 57
Table 3.2 Consistency index and flow behavior index of double emulsions prepared with different lentil and pea flour concentrations and homogenization methods (HSH: high-speed homogenization, UH: ultrasonic homogenization)..... 65
Table 3.3 Instant stability, particle size and consistency index of double emulsions prepared with different lentil and pea flour concentrations and homogenization methods (HSH: high-speed homogenization, UH: ultrasonic homogenization) 70
Table 3.4 Encapsulation efficiency of double emulsions prepared with different lentil and pea flour concentrations and homogenization methods (HSH: high-speed homogenization, UH: ultrasonic homogenization)..... 84
Table 3.5 Relaxation time and fraction of integrated peak area of double emulsions prepared with lentil and pea flour at different concentrations..... 87
Table 3.6 Relaxation time and fraction of integrated peak are of primary emulsion 88
Table 3.7 Relaxation time and fraction of integrated peak are of double emulsions prepared with lentil and pea flour at different concentrations with addition of 2%, 10 mM $MnCl_2$ addition..... 89

Table 3.8 Hardness (N) of cakes containing double emulsions prepared with lentil and pea flour 92
Table 3.9 Specific volume (ml/g) of cakes containing double emulsions prepared with lentil and pea flour 93
Table 3.10 Weight loss (%) of cakes containing double emulsions prepared with lentil and pea flour 94
Table 3.11 Color difference in crust of cakes containing double emulsions prepared with lentil and pea flour 95
Table 3.12 Sensory analysis of cakes 97
Table 3.13 Baking stability of cakes 98
Table 3.14 Release of magnesium in gastric juice when directly added, encapsulated within primary emulsion and double emulsion containing 20% legume flour 102
Table 3.15 Release of magnesium in intestinal juice when directly added, encapsulated within primary emulsion and double emulsion containing 20% legume flour 102
Table 5.1 Two way Anova and Tukey's Comparison Test for stability of double emulsions containing 20% lentil flour with and without NaCl addition into the outer aqueous phase 133
Table 5.2 Three way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil and pea flour concentrations 134
Table 5.3 Two way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil flour concentrations 137
Table 5.4 Two way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% pea flour concentrations 138
Table 5.5 Three way ANOVA and Tukey's Comparison Test for consistency index of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil and pea flour concentrations 139

Table 5.6 Two way ANOVA and Tukey's Comparison Test for consistency index of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil flour concentrations 143
Table 5.7 Two way ANOVA and Tukey's Comparison Test for consistency index of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% pea flour concentrations 144
Table 5.8 Three way ANOVA and Tukey's Comparison Test for flow behavior index of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil and pea flour concentrations 145
Table 5.9 Two way ANOVA and Tukey's Comparison Test for flow behavior index of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% pea flour concentrations 149
Table 5.10 Two way ANOVA and Tukey's Comparison Test for flow behavior index of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% pea flour concentrations 150
Table 5.11 Three way ANOVA and Tukey's Comparison Test for instant stability of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil and pea flour concentrations 151
Table 5.12 Two way ANOVA and Tukey's Comparison Test for instant stability of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil flour concentrations 154
Table 5.13 Two way ANOVA and Tukey's Comparison Test for instant stability of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% pea flour concentrations 156
Table 5.14 Three way ANOVA and Tukey's Comparison Test for encapsulation efficiency of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil and pea flour concentrations 157
Table 5.15 Two way ANOVA and Tukey's Comparison Test for encapsulation efficiency of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil flour concentrations 160

Table 5.16 Two way ANOVA and Tukey's Comparison Test for encapsulation efficiency of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% pea flour concentrations 162
Table 5.17 Two way ANOVA and Tukey's Comparison Test for hardness of cakes prepared with double emulsions containing 20%, 25% and 30% lentil and pea flour concentrations 163
Table 5.18 One way ANOVA and Tukey's Comparison Test for hardness of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% lentil flour concentrations 164
Table 5.19 One way ANOVA and Tukey's Comparison Test for hardness of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% pea flour concentrations 165
Table 5.20 Two way ANOVA and Tukey's Comparison Test for specific volume of cakes prepared with double emulsions containing 20%, 25% and 30% lentil and pea flour concentrations 165
Table 5.21 One way ANOVA and Tukey's Comparison Test for specific volume of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% lentil flour concentrations 166
Table 5.22 One way ANOVA and Tukey's Comparison Test for specific volume of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% pea flour concentrations 167
Table 5.23 Two way ANOVA and Tukey's Comparison Test for weight loss of cakes prepared with double emulsions containing 20%, 25% and 30% lentil and pea flour concentrations 168
Table 5.24 One way ANOVA and Tukey's Comparison Test for weight loss of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% lentil flour concentrations 169
Table 5.25 One way ANOVA and Tukey's Comparison Test for weight loss of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% pea flour concentrations 170

Table 5.26 Two way ANOVA and Tukey's Comparison Test for color of cakes prepared with double emulsions containing 20%, 25% and 30% lentil and pea flour concentrations 171
Table 5.27 One way ANOVA and Tukey's Comparison Test for color of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% lentil flour concentrations 172
Table 5.28 One way ANOVA and Tukey's Comparison Test for color of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% pea flour concentrations 173
Table 5.29 One way Anova and Tukey's Comparison Test for taste (from sensory analysis) of cakes without magnesium, with direct magnesium addition and with magnesium encapsulated in double emulsions containing 20% lentil and pea flour 173
Table 5.30 One way Anova and Tukey's Comparison Test for hardness (from sensory analysis) of cakes without magnesium, with direct magnesium addition and with magnesium encapsulated in double emulsions containing 20% lentil and pea flour 174
Table 5.31 One way Anova and Tukey's Comparison Test for color (from sensory analysis) of cakes without magnesium, with direct magnesium addition and with magnesium encapsulated in double emulsions containing 20% lentil and pea flour 175
Table 5.32 Two way ANOVA and Tukey's Comparison Test for baking stability of cakes prepared with double emulsions containing 20%, 25% and 30% pea flour concentrations 176
Table 5.33 One way ANOVA and Tukey's Comparison Test for baking stability of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% lentil flour concentrations 177
Table 5.34 One way ANOVA and Tukey's Comparison Test for baking stability of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% pea flour concentrations 177

Table 5.35 One way ANOVA and Tukey's Comparison Test for baking stability of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% lentil and pea flour concentrations 178
Table 5.36 Two way ANOVA and Tukey's Comparison Test for heat stability of double emulsions containing 20%, 25% and 30% lentil and pea flour..... 179
Table 5.37 Two way ANOVA and Tukey's Comparison Test for heat stability of double emulsions containing 20%, 25% and 30% lentil flour concentrations and the cakes containing them 180
Table 5.38 Two way ANOVA and Tukey's Comparison Test for heat stability of double emulsions containing 20%, 25% and 30% lentil flour concentrations and the cakes containing them 181
Table 5.39 One way ANOVA and Tukey's Comparison Test for release of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, lentil and pea flour concentrations in simulated gastric juice.... 182
Table 5.40 One way ANOVA and Tukey's Comparison Test for release of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, lentil and pea flour concentrations in simulated intestinal juice182

LIST OF FIGURES

FIGURES

Figure 1.1. Types of Emulsions 4
Figure 1.2 Types of Double Emulsions 6
Figure 1.3. One Step Emulsification 10
Figure 1.4. Two Step Emulsification 11
Figure 3.1. Stability (%) of double emulsions without NaCl; (■) and with NaCl; (■) in the outer aqueous phase 50
Figure 3.2. T_2 values of solutions prepared with lentil and pea flour with and without magnesium chloride 52
Figure 3.3. Particle size distribution of double emulsions prepared with high speed homogenization containing lentil flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30% 54
Figure 3.4. Particle size distribution of double emulsions prepared with ultrasonic homogenization containing lentil flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30% 54
Figure 3.5. Particle size distribution graph of double emulsions prepared with high speed homogenization containing pea flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30% 55
Figure 3.6. Particle size distribution graph of double emulsions prepared with ultrasonic homogenization containing pea flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30% 55
Figure 3.7. Sauter mean diameters of double emulsions prepared with different lentil flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■) 59
Figure 3.8. Sauter mean diameters of double emulsions prepared with different pea flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■) 61

Figure 3.9. Apparent viscosity of double emulsions prepared with high speed homogenization containing lentil flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30% 62
Figure 3.10. Apparent viscosity of double emulsions prepared with ultrasonic homogenization containing lentil flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30% 63
Figure 3.11. Apparent viscosity of double emulsions prepared with high speed homogenization containing pea flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30% 63
Figure 3.12. Apparent viscosity of double emulsions prepared with ultrasonic homogenization containing pea flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30% 64
Figure 3.13. Appearent viscosity of double emulsions prepared with different lentil flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■) 67
Figure 3.14. Appearent viscosity of double emulsions prepared with different pea flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■) 68
Figure 3.15. Instant stability of double emulsions prepared with different lentil flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■) 71
Figure 3.16. Instant stability of double emulsions prepared with different pea flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■) 72
Figure 3.17. Long term stability of double emulsions containing 15% lentil flour at different environments; (●):4 ºC, (▲):20 ºC 74
Figure 3.18. Optical images of double emulsion with 15% lentil flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image) 76

Figure 3.19. Optical images of double emulsion with 20% lentil flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image) 76
Figure 3.20. Optical images of double emulsion with 25% lentil flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image) 77
Figure 3.21. Optical images of double emulsion with 30% lentil flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image) 77
Figure 3.22. Optical images of double emulsion with 15% pea flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image) 78
Figure 3.23. Optical images of double emulsion with 20% pea flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image) 78
Figure 3.24. Optical images of double emulsion with 25% pea flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image) 79
Figure 3.25. Optical images of double emulsion with 30% pea flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image) 79
Figure 3.26. Encapsulation efficiency of double emulsions prepared with different lentil flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■)..... 81
Figure 3.27. Encapsulation efficiency of double emulsions prepared with different pea flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■)..... 82
Figure 3.28. T_2 results of outer water phases prepared with lentil (■) and pea (■) flour at 20% concentration with addition of manganese at different concentrations 90

Figure 3.29. Heat stability of double emulsions prepared with lentil flour (■) and cakes containing double emulsions prepared with lentil flour (■)..... 99
Figure 3.30. Heat stability of double emulsions prepared with pea flour (■) and cakes containing double emulsions prepared with pea flour (■) 100

CHAPTER 1

INTRODUCTION

1.1 Magnesium

Magnesium is an essential mineral which has a critical role for biochemical functioning of various metabolic pathways. It is the second most abundant intracellular cation and the fourth most abundant cation in the body. Since magnesium plays role as a cofactor in more than 300 enzymatic reactions, it impacts nearly all of the systems of the body (Schwalfenberg & Genuis, 2017). It is a fundamental ion in the body due to its vital cellular and molecular functions in carbohydrate, fat, protein and electrolyte metabolism (Chakraborti et al., 2002). Most importantly, it has ability to compete with calcium for binding sites on proteins and membranes and it takes place in the formation of chelates with important intracellular anionic-ligands, especially ATP. Moreover, magnesium is essential for the synthesis of nucleic acids and proteins, for intermediary metabolism and for specific actions in different organs such as the neuromuscular and cardiovascular systems (Swaminathan, 2000).

Magnesium is widely distributed in plant and animal foods and in beverages. Green leafy vegetables, such as spinach, legumes, nuts, seeds, and whole grains, are the major sources for magnesium. However, the dietary intake of magnesium is decreasing due to changing habits of nutrition. Consumption of processed and cooked foods and demineralized soft water lead to a decrease in the daily intake of magnesium for most people. The recommended daily intake of magnesium determined by FDA is about 400 mg for adults (Table 1.1), but only a few people can fulfill this value. Although the exact number is not clear, magnesium deficiency is estimated to be around 11% in the world (Wong et al., 1983). The results of National Health and Nutrition Examination Survey revealed that almost half of the

individuals have inadequate intakes of magnesium. The percentage of inadequacy is greater for the children between 14-18 and adults over 71 years (Nutrient & From, 2009). Similarly, the studies showed that 48% of the population could not satisfy the recommended magnesium intake from food in the United States (Rosanoff et al., 2012).

Table 1.1 Recommended Dietary Allowances (RDA) for Magnesium (Mg)

Age	Male	Female	Pregnancy	Lactation
0-6 months	30	30		
7-12 months	75	75		
1-3 years	80	30		
4-8 years	130	130		
9-13 years	240	240		
14-18 years	410	360	400	360
19-30 years	400	310	350	310
31-50 years	420	310	360	320
51+ years	420	320		

Magnesium deficiency causes serious health problems, like type 2 diabetes, cardiovascular diseases, ischemic heart diseases, high blood pressure, asthma, migraines and depression (Ismail et al., 2018). Studies showed that the risk of many chronic diseases can only be reduced by consuming 300 mg more than the recommended daily intake of magnesium (300-420 mg / day). Despite this, most people cannot even fully meet the recommended daily allowance (DiNicolantonio et al., 2018).

It is possible to prevent magnesium deficiency and the disturbances related to it by magnesium fortification of foods. A study claimed that magnesium fortification of water played role in inhibiting atherogenesis in male mice (Sherer et al., 1999). Magnesium could be integrated into food products in the form of various magnesium salts such as magnesium chloride (21CFR184.1426), magnesium carbonate

(21CFR184.1425), magnesium hydroxide (21CFR184.1428), magnesium oxide (21CFR184.1431) or magnesium citrate (21CFR184.1449) as they are confirmed to be generally recognized as safe (GRAS) and can be used with no limitation as a nutrient supplement (Table 1.2) . However, integration of magnesium into foods can cause chemical degradations and protein aggregation due to its highly active nature. Taste of foods can be affected negatively from the chemical reactions. An effective way to avoid from these drawbacks is to encapsulate magnesium before entrapping into a food product.

Table 1.2 Formula and Bioavailability Properties of Magnesium Salts

Magnesium salts	**Formula**	**Bioavailability Properties**
Magnesium chloride	$MgCl_2$	high bioavailability
Magnesium hydroxide	$Mg(OH)_2$	poorly absorbed; used as an antacid and a cathartic
Magnesium oxide	MgO	bioavailability is poor but better than magnesium carbonate
Magnesium lactate	$C_6H_{10}MgO_6$	have higher and similar bioavailability to magnesium chloride
Magnesium citrate	$C_6H_6MgO_7$	significantly better absorbed than oxide since it is more soluble
Magnesium carbonate	$MgCO_3$	bioavailability is poor

1.2 Double Emulsion

An emulsion is a mixture of two or more liquids that are immiscible in each other. Emulsions consists of a dispersed phase and a continuous phase. The phase that is present in the form of droplets is called as dispersed phase, whereas the phase that surrounds the dispersed phase is called as continuous phase. Emulsions are mainly grouped as the content of their dispersed and continuous phases. Emulsions having

dispersed oil droplets in the continuous water phase are called as oil-in-water (O/W) emulsions, whereas emulsions with water droplets dispersed in the continuous aqueous phase are called as water-in-oil (W/O) emulsions (Figure 1.1). Oil-in-water (O/W) emulsions are most common type which include mayonnaise, homogenized whole milk, egg yolk and salad dressings. Margarine and butter are the most frequently used examples of water-in-oil (W/O) emulsions.

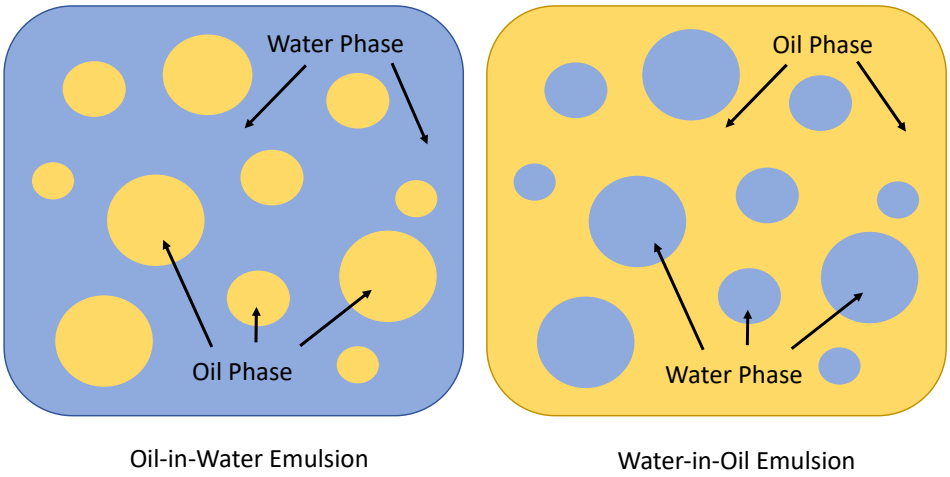

Figure 1.1. Types of Emulsions

Double emulsions are more complex systems as compared to single emulsions. In double emulsions, the dispersed droplets in the continuous phase also contain another smaller dispersed droplets. That is, the outer continuous phase and the inner dispersed phase are separated by a third phase containing liquid which is immiscible with other two liquid phases. These nested structures can also be defined as emulsions of emulsions (Iqbal et al., 2015).

Double emulsions have shown promise in many fields, most importantly in pharmaceutical, cosmetics, agriculture and food industry. They are studied on various applications in different industries. Mainly, they are used for sustained and prolonged release and encapsulation of water soluble active materials in drugs, cosmetics and foods. Thanks to double emulsion, active compounds could be entrapped in the inner dispersed phase and they are protected from external factors, such as light or oxygen. Moreover, slow and controlled release of active compound

from inner phase to outer phase in double emulsions makes it important in the field of encapsulation. Microencapsulation of active compounds (Blanco-Príeto et al., 1997), target delivery of drugs (Farago et al., 2008), peptides (Giroux et al., 2016), hormones (Cournarie et al., 2004), reformation of vaccines (Hilbert et al., 1999), oral administration of vaccines (Hearn et al., 1996), encapsulation of bioactive compounds (Shaddel et al., 2018), production of functional food (Kheynoor et al., 2018) and low calorie food manufacturing (Paximada et al., 2021) are the examples for the applications of double emulsions.

Despite their many potential applications, there are a few commercial products in the market. The main reason of that is the intrinsic thermodynamic and kinetic instability problem due to higher interfacial area. Also, the difficulty in controlling the release rate and relatively larger droplet size pose an obstacle on the commercial production. As the main limitation in double emulsions is the instability, it is important to understand and characterize the reasons behind to minimize them. Instability can be defined as the deterioration in the double emulsion structure. The main physicochemical mechanisms causing instability in double emulsions are phase separation, coalescence, flocculation and Ostwald ripening (Lamba et al., 2015). Phase separation is the separation of aqueous and oil phases due to the difference in their densities under the effect of gravitational force. Coalescence is the irreversible association of two or more droplets to form a larger droplet, whereas flocculation is the aggregation of droplets which result in formation of a bigger composite containing small droplets with a ruptured interfacial film. On the other hand, Ostwald ripening is the phenomena in which smaller droplets dissolve in the continuous phase and then redeposit upon larger ones due to difference in their chemical potentials. It is also referred as isothermal distillation (Taylor, 1998). Despite all these instability problems, it is possible to produce stable double emulsions by adjusting the preparation conditions. For double emulsions it is quite important to pay attention to various parameters such as preparation method, homogenization method, time and intensity of the shear applied, operation temperature, ratio of the phases, type and concentration of the surfactant, osmatic balance between the two aqueous phases, etc. (Lamba et al., 2015).

If we are to classify double emulsions according to their types, there are two main types of double emulsion; water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) (Figure 1.2). The most common type is W/O/W emulsions. W/O/W double emulsions are composed of an aqueous continuous phase and an oily dispersed phase in which aqueous smaller droplets exist. Similarly, oil-in-water-in-oil O/W/O double emulsions contain dispersed aqueous droplets, involving oily droplets, in the continuous oily phase. Emulsifiers are needed for both double emulsion types. For the W/O/W type emulsions, firstly lipophilic emulsifier is used to form inner single emulsion of water-in-oil. Then, in the emulsification of double emulsion, hydrophilic emulsifier is used. Also, for the O/W/O type emulsions, hydrophilic emulsifier take place in the formation of inner interface of the oil-in-water single emulsion and the lipophilic emulsifier is employed for the outer water-in-oil emulsion.

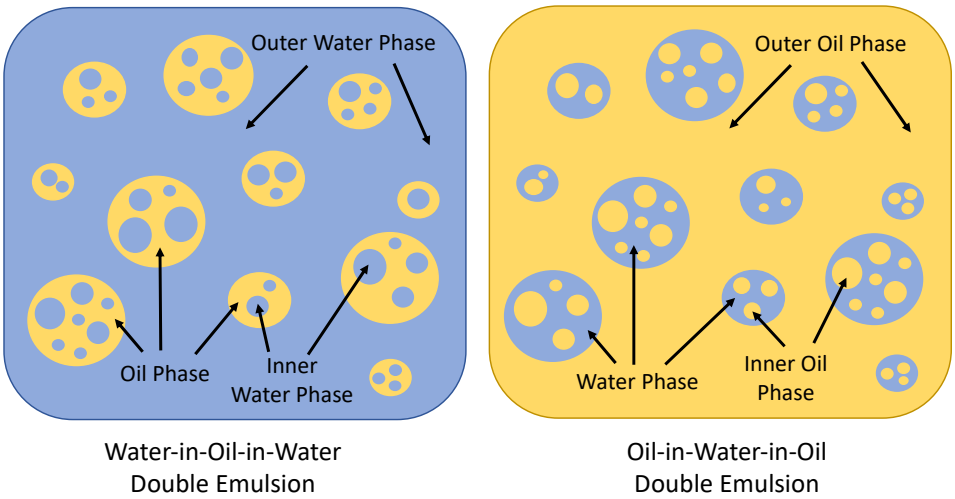

Figure 1.2 Types of Double Emulsions

W/O/W double emulsions are mainly used to encapsulate water soluble active compounds to protect them from external conditions and to provide controlled release of these active compounds. On the other hand, O/W/O double emulsions are suitable for the oil soluble active compounds and they are also used to produce low calorie foods.

1.2.1 Double Emulsions in Food Systems

There are many studies on usage of double emulsions in the food systems. Incorporation of double emulsion into foods provides to produce functional and low-fat foods. Double emulsions have a potential to encapsulate and protect both water soluble and oil soluble components, like vitamins, minerals, antioxidants, flavoring agents or aroma ingredients, and release them during digestion. Also, replacement of oil-in-water emulsion in foods by water-in-oil-in-water emulsion results in low calorie foods with similar texture (Muschiolik & Dickinson, 2017).

In the study conducted by Lobato-Calleros and colleagues, they found that it is possible to produce low fat cheese from milk containing double emulsion. Although structural arrangements differ from the full fat one, produced low fat cheese had similar textural characteristics (Lobato-Calleros et al., 2008). Moreover, in another study, it is achieved to reduce oil content of mayonnaise by 36.6 % with usage of double emulsion (Yildirim et al., 2016). Low fat ice cream with acceptable quality was also obtained by incorporation of double emulsion containing guar gum-gum tragacanth blend (Tekin et al., 2017).

Encapsulating active compounds to produce functional foods are also within researchers' area of interest. In food industry, double emulsions are mainly used to encapsulate valuable materials like vitamins (Díaz-Ruiz et al., 2021), minerals (Dima & Dima, 2020), anthocyanins (Shaddel et al., 2018), polyphenols (Tian et al., 2021) and probiotics (Huerta-Vera et al., 2017). Benichou et al. (2007), encapsulated vitamin B_1 in the inner aqueous phase of double emulsion and produce a stable emulsion with the combination of whey protein isolate and polysaccharide complexes. They achieved thick and efficient barrier against release of vitamin $B_{1.}$ Ilyasoglu Buyukkestelli & El (2019), produced double emulsions containing iron in the inner aqueous phase with different primary phase to outer water phase ratios and characterize them in terms of droplet size, encapsulation efficiency, rheology, stability and bioaccessibility. They observed that, while double emulsions with 40:60 ratio had lower particle size, higher viscosity and higher encapsulation efficiency

and higher stability, emulsions with 20:80 ratio showed highest iron bioaccessibility. Li et al. (2012), encapsulated both a water soluble vitamin (vitamin B_2) and a lipid soluble vitamin (vitamin E) together in the same double emulsion stabilized by whey protein isolate with combination of low methoxyl pectin and carrageenan. They stated that the usage of protein-polysaccharide complex retarded the release of vitamins as compared to pure whey protein and polysaccharide. Similarly, in the study of Artiga-Artigas et al. (2019), a hydrophilic (chlorophyllin) and a hydrophobic (lemon grass essential oil) active compound was encapsulated in the double emulsion together without changing emulsion characteristics. Eisinaitė et al. (2021), incorporated black chokeberry pomace extract into the double emulsion and analyzed its effect on the physical and oxidative stability of emulsions. They observed that, higher amounts of the extract resulted in smaller particle size, higher viscosity and higher oxidative stability due to considerable amount of polyphenolics content. Ying et al. (2021) encapsulated soy peptides in the double emulsions prepared with different lipophilic emulisifers (PGPR, Span 60, lecithin) and a hydrophilic emulsifier (octenyl succinic anhydride starch and maltodextrin mixture). They determined the optimum conditions for the encapsulation of soy peptides in terms of inner phase to oil phase ratio, peptide concentration and homogenization condition of the secondary step. Xu et al. (2018) analyzed the in vitro digestion behavior of the double emulsion containing anthocyanin and stabilized by PGPR and soy protein isolate. They observed that, double emulsions protected anthocyanins against mouth and gastric digestion and the release of anthocyanins took place in the intestinal digestion. Marquez & Wagner (2010) produced double emulsions with calcium and soybean milk as an alternative to whipped dairy cream. They produced emulsions with higher consistency when they increased calcium chloride content. The produced double emulsion had high calcium and low fat content with accepted texture.

In the study conducted by Bonnet et al. (2009), magnesium was chosen as the active compound encapsulated in the double emulsion. They used PGPR and sodium caseinate as emulsifiers and produced stable emulsions with small droplet size. They found that magnesium release was mainly due to diffusion and permeation

mechanisms rather than coalescence. They also analyze the effect of pancreatic lipase and proved that enzyme hydrolase the lipids and release magnesium at the intestinal site. Moreover, in another study, Bonnet et al. (2010) investigated the effect of location and concentration of sodium caseinate on magnesium release from double emulsions. They observed higher release rate at higher concentrations of sodium caseinate at the outer phase, while lower release rates with the addition of sodium caseinate into inner phase at the same concentration with outer phase.

1.2.2 Preparation Methods

There are two main methods to prepare double emulsion; one-step emulsification and two-step emulsification. One-step emulsification is based on mixing continuous and dispersed phases and two different type emulsifiers at once and then forming double emulsion (Figure 1.3). It is important to choose emulsifiers with suitable hydrophilic-lipophilic balance. To form double emulsion, mechanical agitation or phase inversion methods are applied. In mechanical agitation, double emulsions are formed at the conversion stage after mixing the oil phase containing lipophilic emulsifier and water phase containing hydrophilic emulsifier. Differently, phase inversion method involves inversion of phases due to temperature or concentration change. At this method, first water-in-oil single emulsion is formed and then either water addition or the given heat leads to formation of double emulsion (Saffarionpour, 2021). By using one-step emulsification method it is difficult to control the process and reproduce emulsion with similar properties, so it is not widely used (Garti & Bisperink, 1998).

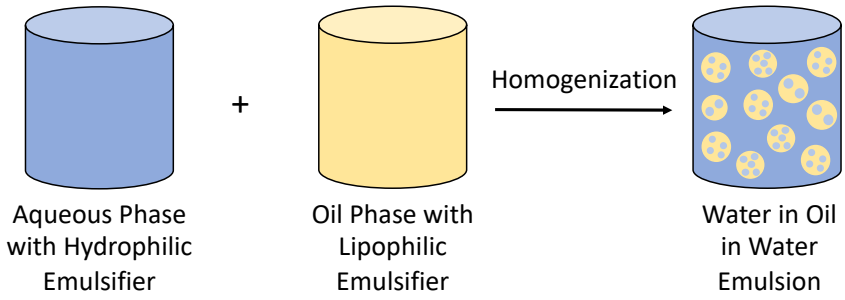

Figure 1.3. One Step Emulsification

Two-step emulsification is generally preferred due to its controllability. In the two-step emulsification method, firstly primary emulsion is formed by mixing aqueous and oily phase and relatively high amount of hydrophobic emulsifier. They are homogenized at high shear in order to obtain small particle sized droplets. Then, in the second step, primary emulsion, outer aqueous phase and hydrophilic emulsifier are homogenized gently not to disrupt primary emulsion (Garti & Aserin, 1996) (Figure 1.4).

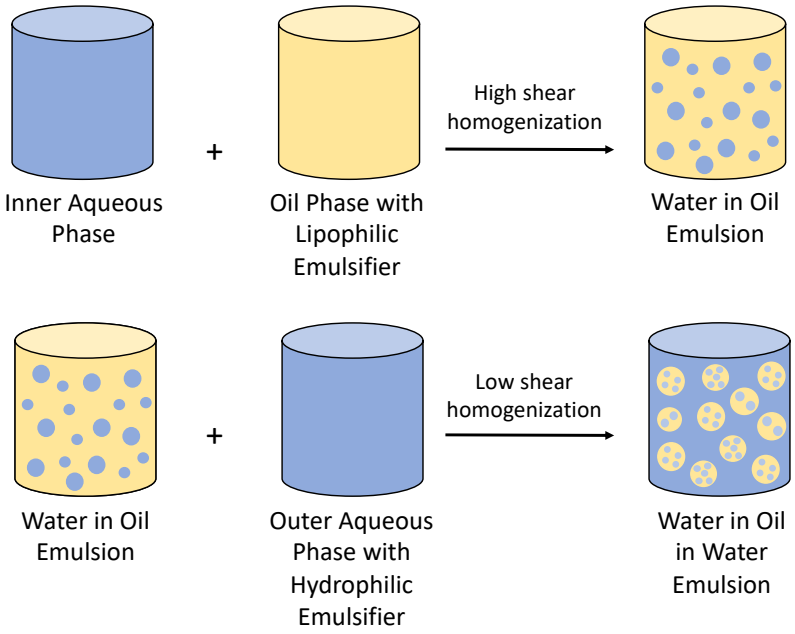

Figure 1.4. Two Step Emulsification

1.2.3 Homogenization Methods

Homogenization is a unit operation using a class of processing equipment referred to as homogenizers that are geared towards reducing the size of droplets in liquid-liquid dispersions (Weiss, 2008). Homogenization can be categorized as primary and secondary homogenization. Formation of an emulsion in the presence of two separate liquids and suitable emulsifier is referred to as primary homogenization. On the other hand, secondary homogenization can be defined as the process in which particle size reduction occur in the existing emulsion. (Akoh & Min, 2002). In many food processes, it is efficient to produce emulsion using two homogenization steps. First, immiscible oil and water phases are converted to emulsion with the help of emulsifier and then droplet size is reduced. Homogenizer type, so energy density and shear applied have an important impact on the product character so homogenization step should be evaluated carefully.

1.2.3.1 High Speed Homogenization

High speed homogenizer is a rotor-stator system which can produce emulsion by mixing, stirring, blending, dissolving and de-agglomerating. High speed homogenizer have capacity to supply energy to the rotary head to turn it at a speed of 10-50 m/s and also it can reach to high shear rates up to 20 000 min^{-1} (Zhang et al., 2012). In this homogenizer, fluid enters in the center of the system and fluid is accelerated and decelerated during process in order to create high tangential forces. This accelerated turning of rotating head result in horizontal, vertical, longitudinal and radial velocity components (McClements, 2004). The high shear produced by cavitation and turbulence in high speed homogenizer also helps to decrease particle size of the droplets in addition to forming emulsion by interfering the water-oil interphase.

High speed homogenization is the most frequently used process for emulsion preparation in food industry. The degree of the shear produced by the high speed homogenizer has a great effect on the stability of double emulsion. Although high shear rate is required for the formation of primary emulsion to overcome the Laplace pressure of the droplets, lower shear rate is applied for the secondary step in double emulsion preparation to avoid damaging the emulsion structure (Lamba et al., 2015). The intensity of the shear used during emulsion preparation directly affects the particle size of the double emulsion which in turn determines the emulsion stability and encapsulation efficiency. The studies conducted by Bi et al. (2014), also proved this phenomenon. They found that increasing rotational speed of the high speed homogenizer significantly decreased the particle size of the droplets and polydispersity index. Moreover, Bamba et al. (2018), greatly discussed the effect of speed and time of high speed homogenizer for both first and second step in the double emulsion formation. They observed that the longer homogenization time applied in the primary emulsion formation step resulted in obtaining emulsions with smaller droplet diameter and higher encapsulation efficiency. On the other hand, in the second step of the double emulsion preparation, a critical homogenization speed was determined. Particle size of droplets decreased with increasing homogenization

speed up to a limit since enough mechanical energy was supplied to break down the oil droplets and to adsorb emulsifier at the oil-water interface. However, excessive homogenization speed caused higher droplet velocity and collision rate which increased the possibility of coalescence. As a result, it is important to handle the speed and intensity of the homogenization carefully to obtain stable and efficient double emulsions.

1.2.3.2 Ultrasonic Homogenization

Ultrasonic homogenization is a mechanical process in which sound energy is applied to agitate and homogenize the particles of the sample. Ultrasound waves, with frequencies above 20 kHz, are generated by piezo-electric or magneto-restrictive transducers. Pressure gradients, created by ultrasound waves, cause deformation of droplets. Cavitation caused by drop of local pressure of the solvent generates turbulent flow and high shear (Weiss, 2008). Cavitation is the main effect of ultrasonic homogenization and it can be defined as the sudden formation of vapor bubbles in the liquid due to reduced pressure. At lower pressures, cavitation bubbles expand and collapse leading high local turbulence and shear rate. Also intense shockwaves are created in the liquid and they cause reduced particle size (Tang et al., 2013).

There are many studies on ultrasonic homogenization showing its effect in emulsion preparation and comparing it with other possible homogenization methods. In a study which compare ultrasonic homogenization with mechanical agitation, it is found that emulsions prepared by ultrasonication have smaller droplet sizes, they are less polydispersed and more stable. Moreover, ultrasound reduce the energy consumption and the desired surfactant amount (Canselier et al., 1999). Another study comparing ultrasonication with microfluidization, also support its ability to produce emulsions with small droplet size as microfluidizer.

Studies in the literature on obtaining double emulsion using ultrasonic homogenization are promising but limited. Ultrasonic homogenization was found as

the most suitable method for the production of biodegradable protein loaded poly(lactic-co-glycolic acid) microparticles microparticles among rotor-stator homogenizer and high pressure homogenizer, since smaller particle size and higher efficiency and stability were achieved with ultrasonication (Wischke & Borchert, 2006). Similarly, ultrasonication produced relatively more monodisperse droplets with greater stability as compared to high pressure homogenizer for the W/O/W double emulsions prepared with skim milk in both aqueous phases (Leong et al., 2018). Moreover, stable and efficient double emulsions could be obtained by using ultrasonic homogenization in the second emulsification step when milk proteins were used in the aqueous phase (Silva, et al., 2020). However, it is important to adjust sonication time carefully, because, although smaller droplet sizes could be obtained with incrasing sonication time of the second step until the equilibrium, after that point, droplet size remain same but the leakage of inner material continues to increase (Khadem & Sheibat-Othman, 2019).

The main drawbacks of ultrasonication are stated as producing emulsion droplets within a wide range and its dependence on primary emulsion (Jafari et al., 2006). In a study which covered the effect of homogenization methods on stability of double emulsion, it was found that ultrasonic application decreased the Sauter mean diameter of particles significantly for samples containing xanthan gum. However, inverse effect was observed for the other samples which are already in the micron size. The particle size increased due to over-processing phenomenon (Yildirim et al., 2017). Over-processing is a phenomenon in which high intensity of the homogenization conditions cause recoalescence of the droplets due to droplet disruption and increased contact area. Over-processing is observed mainly when

* the smaller droplet formation is faster than the surfactant absorption on the droplets so the oil-water interface is not covered completely.

* the surfactant concentration is insufficient to cover the increased surface area resulted from decreased droplet diameter.

* the temperature increase caused by acoustic cavitation during homogenization alter the emulsifying properties of the surfactant used (Vélez-Erazo et al., 2018).

1.3 Surfactants

Surfactants, namely surface active agents, are compounds that are capable of reducing surface tension between a surface and a liquid or between two or more immiscible liquids. Generally, surfactants are long chain carbon molecules having amphiphilic properties. They have both polar and non-polar functional groups at each end. In an emulsion, hydrophilic head attract water molecules whereas lipophilic tail repels water and have affinity to oil molecules. Thanks to this amphiphilic structure, surfactants combine two phases in emulsions and stabilize them by hindering the coalescence and aggregation. Since double emulsions contain two interfaces, it is necessary to use at least two different surfactants. The one that is used in the formation of primary emulsion should be lipophilic, while hydrophilic surfactant should be used for the secondary step of the preparation of double emulsion. Selection of surfactant mainly depends on continuous and dispersed phases of the emulsion and it plays a critical role in the formation and stabilization of the double emulsion.

There is an empirical expression which stands for the relation of hydrophilic and lipophilic parts called HLB (Hydrophilic Lipophilic Balance) value. In the HLB System, each surfactant is assigned a numerical value between 0 and 20 and this value express the size and strength of polar parts to non-polar parts of the molecule numerically. Surfactants showing lipophilic characteristics are assigned by low HLB number and surfactants with hydrophilic characteristics are assigned by high HLB number (Gadhave, 2014). That is, for the water-in-oil emulsion preparation, surfactants with small HLB value should be used while surfactants with high HLB value should be preferred for oil-in-water emulsion. It is easy and time saving to use HLB value while deciding on the proper surfactant for a specific system.

1.3.1 Lipophilic Surfactant

Lipophilic surfactants are the surface active agents that are more soluble in oil (having low HLB value) and used in the formation of water-in-oil emulsions. Mainly used lipophilic surfactants in the food production are polyglycerol polyricinoleate (PGPR), lecithin, monoglyceride and Span 80. PGPR is the predominant surfactant used for water-in-oil emulsion and double emulsion preparations in the food industry (Dickinson, 2011). It is preferred due to its high stability and strong lipophilic character. It is synthetic surfactant and produced by esterification of castor oil fatty acid. Although it is generally recognized as safe (GRAS) according to Food and Drug Administration (Food and Drug Administration, 2006), it is recommended to be consumed within certain limits. The acceptable daily intake for PGPR is defined as 2.64 mg/kg body weight/day (Wilson et al., 1998). Therefore, researchers investigate to decrease the amount of PGPR used by partially replacing it with other surfactants. In the study conducted by Su et al. (2006), W/O/W emulsions were prepared with PGPR alone and in combination of sodium caseinate and they achieved to reduce used PGPR concentration when sodium caseinate is also added into the inner aqueous phase without affecting the stability and yield of the emulsions. Moreover, Okuro et al. (2019), performed the partial replacement of PGPR with lecithin up to 50% of the surfactant amount and produced emulsions with high kinetic stability.

Lecithin is another lipophilic emulsifier commonly used in food industry. It is a natural emulsifier which is produced from soybean and egg. It is a low molecular weight surfactant composed of various phospholipid species like phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol (Zhu & Damodaran, 2013). Lecithin is mostly preferred due to its natural and low molecular weight structure since it can be used without any health consideration and it can greatly enhance the stability of emulsions. Although lecithin is considered as a hydrophobic molecule, natural lecithin is a zwitterionic surfactant which has hydrophilic parts almost as much as the lipophilic ones and their HLB value is around 8. Therefore, it is not suitable to be used to stabilize water-in-oil emulsions alone. Lecithin is generally used together with surfactants having more lipophilic

properties, that is having lower HLB value. In the study of Altuntas et al. (2017), it was observed that lecithin produced oil-in-water emulsions when used alone so it was used together with PGPR to form water-in-oil emulsion. Among the different surfactant ratios, the most stable emulsions were obtained with the usage of PGPR and lecithin at equal amounts.

1.3.2 Hydrophilic Surfactant

Hydrophilic surfactants are hydrophilic molecules with high HLB value (>10) which stabilize the oil-in-water emulsions. In the double emulsion preparation, they are used in the second step of emulsification for stabilization of the outer water phase and primary phase. Macromolecules like proteins and polysaccharides can also be used as a hydrophilic surfactant thanks to their emulsification and stabilization properties. Table 1.3 (Dickinson, 2003) summarizes the similarities and differences between proteins and polysaccharides as functional food biopolymers.

Table 1.3 The common general characteristics and differences between of proteins and polysaccharides as functional biopolymers in food systems

Similarities
Natural polymers
Widespread in food colloids
Used in pharmaceuticals, cosmetics, personal products
Environmentally friendly polymers
Complicated structure
Complex aggregation behavior
Gelling / stabilizing agents

Table 1.3 (Continued)

Differences	
Proteins	*Polysaccharides*
Wide-ranging structures	Similar structures
Reactive	Unreactive
Monodisperse	Polydisperse
Many segment types	Few segment types
Linear chain	Linear or branched
Flexible chain	Stiff chain
Medium molecular weight	High molecular weight
Small molecular volume	Large molecular volume
Amphiphilic	Hydrophilic
Surface-active	Not surface-active
Polyelectrolyte	Non-ionic or charged
Emulsifying / foaming	Thickening / waterholding
Temperature sensitive*	Temperature insensitive
Strong surfactant binding	Weak surfactant binding

*That is the structure and properties of most proteins can change drastically when heated above a characteristic 'denaturation temperature'.

1.3.2.1 Proteins

Proteins are widely used as surfactant due to their ability to adsorb at the water-oil interface and form stabilizing layer around oil droplets. During emulsion formation, they are adsorbed at the surface of oil droplets in the form of a densely packed layer. They decrease interfacial tension and prevent coalescence by forming a physical barrier at the interphase. When pH of the emulsion is away from their isoelectric point, they decrease the oil droplet aggregation by electrostatic repulsion also. Moreover, they increase viscosity of continuous phase which leads to slowing down

droplet movement and increase in emulsion stability (Karaca et al., 2011). There are many studies on the emulsifying properties of animal-based proteins like whey protein isolate (Hunt & Dalgleish, 1994), soy protein isolate (Keerati-u-rai & Corredig, 2009), sodium caseinate (Amine et al., 2014) and bovine serum albumin (Haque & Kinsella, 1988). Animal based proteins are also used in double emulsions (Bamba et al., 2018; Benichou et al., 2004; Bonnet et al., 2010; Yildirim et al., 2016).

Legume proteins, on the other hand, are relatively new in the emulsion studies. There is an increasing trend to replace animal based products with plant based ones with the awareness of increasing population and climate change. Along with its sustainable nature and nutritional value, high emulsifying abilities of legume proteins are excellent substitutes for animal based and allergenic surfactants. Researches showed that legume proteins have comparable emulsifying properties with milk whey protein, β-lactoglobulin and form stable, negatively charged emulsions which demonstrated the potential use of legume proteins as alternative to animal-based proteins (Benjamin et al., 2014). The results of another study also indicated that surface active properties of wheat and pea proteins are higher than even gelatin at the same concentration (Ducel et al., 2004).

In contrast to great interest in using legume proteins as emulsifiers in primary emulsions, their use in double emulsions are very limited. Although pea took place in double emulsions in the form of pea protein-pectin conjugate as a hydrophilic emulsifier (Tamnak et al., 2016), so far, legume proteins have not been used to stabilize secondary phase of double emulsions alone.

1.3.2.2 Polysaccharides

Carbohydrates, mainly polysaccharides are also commonly used as hydrophilic surfactant. Polysaccharides are molecules which have the ability to form hydrogen bonds and establish networks and binding centers with proteins, lipids and other molecules to generate a stable multicomponent system (Tang & Huang, 2022). Natural polysaccharides are mainly hydrophilic and suitable to form oil-in-water

emulsion. Gum Arabic (Reiner et al., 2010), alginate (Yang et al., 2012), carrageenan (Melanie et al., 2020), chitosan (Zinoviadou et al., 2011), corn fiber gum (Cirre et al., 2014), basil seed gum (Osano et al., 2014), xanthan gum, guar gum, carboxymethyl cellulose (Hayati et al., 2016), beet pectin (Bai et al., 2017) and modified starch (Yusoff & Murray, 2011) are the examples of polysaccharides used to form a food grade emulsion. Most of the polysaccharides show high stability against pH and temperature. They increase the viscosity of the aqueous phase which results in increase in stability of emulsion. While the emulsifying activity of polysaccharides are attributed to their proteinaceous parts or their interactions with proteins, emulsion stability is ensured by the carbohydrate parts which form a hydrated layer preventing coalescence and flocculation (Nakauma et al., 2008). Hence, they both act as surfactants and stabilizers in the emulsions. However, the emulsifying ability of natural polysaccharides can be insufficient to form stable emulsion in some cases. Therefore, polysaccharides are generally used together with proteins or other polysaccharides. The study conducted by Hayati et al. (2016), proved the synergistic effect of polysaccharide mixtures on the basis of improved droplet characteristics and flow behavior.

1.3.2.3 Protein-Polysaccharide Complexes

Studies show that combination of polysaccharides with proteins have greater effect on emulsion droplet stability (Murray et al., 2011). Polysaccharides mainly used to increase protein stabilization since they can be readily attached to them via Maillard reaction and provide good solvent compatibility (Wooster & Augustin, 2007). Researches show that protein modification through polysaccharide conjugation results in enhanced functional properties such as solubility, emulsification and encapsulation stability, thermal stability and gelation properties (Drapala et al., 2016). Li et al. (2012) used whey protein isolate with combination of low methoxyl pectin and carrageenan for the double emulsions which encapsulate vitamin B_2 and vitamin E together. They found that both combinations improved the controlled release, that is, retard the release of vitamin in simulated gastric juice and undergo

enteric liberation of vitamins in simulated intestinal conditions. Drapala et al. (2016) found out that the conjugation of whey protein hydrolysate with maltodextrin created an excellent thermal stability for the emulsions primarily due to enhanced steric interactions. Moreover, Wooster & Augustin (2007) analyzed the chemistry behind protein-carbohydrate complexes by conjugating β-lactoglobulin with maltose and maltodextrin. They reached to the result that the high stability of conjugates comes from the combined effects of weak electrostatic repulsion from protein surface and the steric repulsion from attached carbohydrate layer.

Flours are natural substances obtained from raw grains, roots, beans, nuts or seeds. They are composed of proteins, starch, lipids, carbohydrates and enzymes. They intrinsically contain high amounts of protein and starch. The protein contents of legume flours are even higher than the cereal flours. Therefore, using legume flours, containing high amount of legume protein and starch, could be a good option for the hydrophilic surfactant. Although there are considerable amount of study analyzing the emulsifying properties of flours (Hung & Zayas, 1992; Mcwatters & Cherry, 1977; Siddiq et al., 2010), studies on emulsions stabilized by flours are quite limited. Romero et al. (2008) studied the rheology and droplet size distributions of oil-in-water emulsions produced with crayfish flour. Okezie & Bello (1988) compared winged bean flour with winged bean isolate and soy protein isolate and obtained that winged bean flour showed the highest emulsifying capacity. In addition, a recent study revealed the fact that oil in water emulsions prepared with pea flour and pea protein concentrate had similar interfacial and physical properties, so purification was stated as unnecessary (Sridharan et al., 2020).

1.4 Legumes

Legumes, such as common bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), soybean (*Glycine max L. Merr*.), cowpea (*Vigna unguiculate*) and peanut (*Arachis hypogaea*) constitute a considerable part of world's crop production and play an important role in diets of both vegetarian and nonvegetarian people due to their high nutrition value (Singh et

al., 2013). They come from after cereals in agricultural importance considering harvested area and total production. They play an important role as a rotation crop contributing to system sustainability by fixing atmospheric nitrogen through symbiotic association with bacteria which in turn result in improved soil health (Erskine et al., 2011).

Legumes constitute approximately one third of all dietary protein nitrogen and one-third of processed vegetable oil in human diet. They contain about 20%-40% protein. Different from the cereal proteins deficient in lysine, legume proteins are generally lack of sulfur-containing amino acids. They contain essential minerals and form health promoting secondary components that enhance the immune system. Moreover, they have a hypoglycemic effect due to their feature of reducing blood cholesterol. (Gepts et al., 2005).

Legume proteins mainly consist of globulins (about 70%) and albumins (less than 20%) as storage protein. Globulins are classified as legumins (11S) and vicilins (7S) according to their sedimentation coefficients (Benjamin et al., 2014). While vicilin is a trimetric protein, legumin proteins exist in the hexameric ring structure with α- and β-chains linked by disulfide bridges. Globulins dissociate into its monomers with the change in environmental conditions like pH and ionic strength. The ratio of globulins and albumins and ratio of legumins and vicilins affect the physicochemical and emulsifying properties of legumes (Karaca et al., 2011).

Among legumes, lentil and pea were chosen due to their high protein content and emulsifying abilities. They both include essential amino acids at sufficient levels except methionine. They also contain other amino acids at adequate levels (Table 1.4). Moreover, they contain magnesium at comparable amounts with other legumes. Lentil and pea contains about 4.5 mg/100 g and 4.2mg/100g magnesium mineral (Iqbal et al., 2006).

Table 1.4 Amino acid content of lentil and pea

Amino acids (% of protein)	Lentil	Pea
Arginine	7.8b ± 0.03	7.2d ± 0.04
Histidine	2.2c ± 0.05	2.4b ± 0.05
Isoleucine	4.1b ± 0.05	4.5a ± 0.06
Leucine	7.8b ± 0.05	7.4b ± 0.05
Lysine	7.0b ± 0.03	8.1a ± 0.07
Methionine	0.8c ± 0.02	1.1b ± 0.03
Phenylalanine	5.0b ± 0.12	5.2b ± 0.04
Threonine	3.5a ± 0.04	3.8a ± 0.05
Tryptophan	0.7a ± 0.03	0.8a ± 0.02
Valine	5.0a ± 0.05	5.0a ± 0.09
Total	43.9	45.5
Alanine	4.2b ± 0.04	5.2a ± 0.04
Aspartic acid	11.8a ± 0.08	11.0b ± 0.06
Cystine	0.9b ± 0.04	1.8a ± 0.03
Glutamic acid	21.5a ± 0.07	17.5b ± 0.06
Glyine	3.6b ± 0.05	4.5a ± 0.01
Proline	3.5b ± 0.03	3.8a ± 0.03
Serine	5.2a ± 0.05	5.1a ± 0.54
Tyrosine	32a ± 0.06	3.7a ± 0.03
Total	53.9	52.9

1.4.1 Lentil

Lentil is the fourth most widely grown legume in the world ranks after bean, pea and chickpea (Coyne et al., 2020). It has their origin in the Neolithic times, being one of the first crops domesticated in the Near East. Lentil has relatively high resistance to drought and cultivated throughout the world. Production of lentil is primarily supplied by Canada, Turkey, India and America (Singh et al., 2013).

Lentil has an excellent macro and micronutrient profile with high protein, starch and mineral content (Thavarajah et al., 2011). Besides differences from different lentil varieties and environmental conditions, lentil contain about 27.2% protein, 43.7% starch and 13.9% fiber (Wang & Daun, 2006) (Table 1.5). Moreover, lentil involves considerable amounts of minerals (Ca, Cu, P, K, Fe, Zn, Mn, Mg) and vitamins

(Niacin, Vitamin A, Ascorbic Acid and Inositol) (Erskine et al., 2011). The essential amino acid amount and pattern in the protein determine its nutritive value. Nutritive value increases when one or more essential amino acids exist in sufficient amounts. Lentil proteins contain histidine, isoleucine, lysine, phenylalanine, tyrosine, threonine and valine at amounts higher than FAO/WHO requirement patterns. On the other hand, limiting amino acids were determined as tryptophan and the sulphur-containing amino acids (methionine and cystine) (Wang & Daun, 2006). Lentil does not involve gluten protein so its consumption is also suitable for people having celiac disease (Aydogdu et al., 2018).

Table 1.5 Proximate composition of whole dry lentil seeds

Component (%)	**Range**	**Mean**
Protein	24.3-30.2	27.2
Carbohydrates	41.5-46.5	43.7
Fat	1.0-1.3	1.1
Total fibre	12.7-16.0	13.9
Ash	2.3-3.5	2.9

Lentil is also a good source of naturally occurring prebiotic carbohydrates which constitute high portion of dietary fiber. Lentil contains raffinose-family oligosaccharides, sugar alcohols, fructooligosaccharides, and resistant starch. The raffinose family oligosaccharides provide pathogen reduction and increased calcium absorption, resistant starch enhance insulin sensitivity of metabolic syndrome patients, fructooliogosaccharides show great prebiotic action and sugar alcohol displace pathogens from gastrointestinal tract (Johnson et al., 2013).

Additionally, lentil proteins have good emulsifying properties and form oil-in-water emulsions acting as a surfactant. There are some studies investigating the emulsifying properties of lentil and optimizing the conditions for a better emulsification in the literature. In their study, Joshi et al. (2012), compared the interfacial and emulsifying properties of lentil protein isolate with animal-based

proteins. The stability of emulsions stabilized by lentil protein isolate was found as slightly lower than the ones with whey protein isolate and sodium caseinate. However, higher emulsion stability index was obtained by increasing the protein concentration. Also, both emulsion activity index and emulsion stability index were improved with the reduction in disulfide bonds. The results of the study conducted by Karaca et al. (2011), in which the emulsifying properties of legume proteins were analyzed, revealed that lentil protein isolate had the highest surface charge and solubility and emulsion capacity among legume proteins. It also displayed high emulsion activity and stability higher than other legume protein isolates and comparable to soy protein isolate.

Moreover, Ladjal-Ettoumi et al. (2016), found out that emulsifying properties of lentil protein are strongly dependent on the acidity. Minimum emulsifying abilities were observed at the isoelectric point of lentil protein (pH 4.5) and stable emulsions were obtained as pH moved away from the isoelectric point, since protein solubility plays important role in emulsifying activities. Similarly, Karaca, et al. (2011) determined the optimum pH as 3.0 or 8.0 since higher protein solubility, smaller droplet size, higher net droplet charge, lower creaming were achieved at the pH values analyzed outermost the isoelectric point. A study worked on the functional properties of lentil flour reached to the result that boiling the lentil flour as a thermal processing increased its water holding capacity, fat binding capacity and gelling capacity (Ma et al., 2011).

Lentil flour containing high amount of lentil protein could be a good option as a hydrophilic surfactant to eliminate purification step which cause high energy consumption and mass loss. The usage of lentil flour instead of lentil protein brings about the involvement of starch and other plant derived molecules and these can also contribute to the overall effect (Ma et al., 2011).

1.4.2 Pea

Among legumes, pea is the second widely grown legume following bean (Coyne et al., 2020). It is one of the oldest domesticated crops in the world and its initial domestication lies in the Middle East (Singh et al., 2013). The major pea cultivators are Canada, Russia, America and India (Dahl et al., 2012).

Peas are recognized as low-cost, readily available source of protein, starch, vitamins and minerals, which makes them a valuable food commodity (Dahl et al., 2012). Peas contain about 26.5% protein, 41.2% starch, 2.35% fat and 16.4% non-starch polysaccharides (Nikolopoulou et al., 2007) (Table 1.6). Pea protein can be classified into four major groups; albumin, globulin, prolamin, and glutelin. Globulin constitutes about 60% of total proteins and brings sensitivity to extreme pH and ionic strength (Lu et al., 2020). Pea protein contains high amounts of glutamine, aspartic acid, arginine and lysine, while methionine, tryptophan and cysteine found at small amounts. The in vitro digestibility of pea protein is higher than other legume proteins. The high protein content helps to improve cardiovascular diseases and assists in controlling body weight. Pea protein is a good source of bioactive proteins which hydrolase to peptides like angiotensin I-converting enzyme inhibitor activity, which has antioxidant activity (Dahl et al., 2012).

Table 1.6 Proximate composition of field pea seeds

Component (%)	**Range**	**Mean**
Protein	24.3-32.5	26.5
Starch	33.4-47.5	41.2
Fat	0.76-3.95	2.35
Non-starch polysaccharides	14.4-19.5	16.4
Ash	3.05-4.06	3.5

Pea is a low glycemic index food due to the properties of starch and fiber it contains. It also reduces blood cholesterol thanks to its fiber content. Moreover, thanks to its high dietary fiber content (raffinose-family and other galactose-containing oligosaccharides) and ability to promote growth of Bifidobacterium more than bean and lentil, pea has a potential to be used as prebiotic (Queiroz-Monici et al., 2005). In addition, pea is a source of various minerals such as zinc, magnesium, calcium, iron and potassium (Alonso et al., 2001).

Besides its sustainable nature and nutritional value, pea proteins have high emulsifying abilities which resulted in the increased interest on the pea protein as an alternative to soy protein and animas-based proteins. There are many studies proving that pea proteins perform good emulsions comparable to casein and albumin (Rangel et al., 2003), whey protein (Kornet et al., 2021), sodium caseinate (Amine et al., 2014) and soy protein (Aluko et al., 2009). According to the study conducted by Aluko et al. (2009), pea protein isolate had better emulsifying properties than soy protein isolate at pH 7.0, so could be a suitable replacement to soybean stabilized emulsions. In the study of Hinderink et al. (2021), emulsion stability of pea protein fractions were analyzed and compared with whey protein isolate. Although emulsions prepared with full pea protein isolate were unstable due to droplet coalescence and flocculation, insoluble fraction of the pea proteins formed stable emulsions under both non-oxidative and pro-oxidant conditions. Amine et al. (2014), reached to the result that, higher interfacial elasticity and higher emulsion stability were obtained at pH 10, since unfolding of protein led to lower interfacial tension and smaller droplets. Additionally, Gharsallaoui et al. (2010) stated that the pea globulins present as a hexamer at neutral pH and dissociate at low and high pH conditions. Especially, acidic pH values affect more and cause complete dissociation to monomers with good interfacial properties.

Moreover, the interfacial properties of pea protein concentrate and pea flour were compared to analyze the use of pea flour without the necessity of purification step. The results were quite promising that both had similar interfacial properties and so, the emulsions prepared with pea protein concentrate and pea flour showed similar

droplet size and rheological properties. This indicated that pea proteins are the main interfacially active components of pea flour and starch did not affect the interfacial behavior (Sridharan et al., 2020).

1.5 Encapsulation Efficiency of Double Emulsions

Encapsulation efficiency of double emulsion is the measure for the percentage of inner phase that is successfully entrapped into the double emulsion system. Although it is one of the most important parameters for the double emulsion quality, it is hard to be measured since the analysis methods are long and complicated. In order to analyze encapsulation efficiency, the amount of inner water phase transferred into the outer water phase is measured mainly. There are many methods for measuring encapsulation efficiency of double emulsions. The most common methods are conductivity (Yildirim et al., 2017), atomic absorption spectroscopy (M. Bonnet et al., 2009) , spectrophotometry (Hino et al., 2001) and fluorimetry (Hai and Magdassi, 2004) measurements. All these methods need a separation procedure of outer water phase and the primary emulsion in order to analyze the outer water phase and decide the amount of inner phase in it. However, nuclear magnetic resonance spectroscopy method, enable measurement of encapsulation efficiency without any separation of phases or addition of a marker compound.

1.5.1 Atomic Absorption Spectrophotometry

Atomic absorption spectroscopy is a technique, which studies absorption of electromagnetic radiations in relationship to molecular structure. It is a technique for measuring the concentration of various elements in the sample through their absorption of light. It is a relatively simple and reliable technique, which uses absorption of optical radiation by free atoms for determining the contents of different elements. Atomic absorption spectroscopy is based on the principle that when a beam of electromagnetic radiation is passed through a substance, the radiation may either be absorbed or transmitted depending upon the wavelength of the radiation. The

absorption of radiation would bring about an increase in the energy of the molecule. The energy gained by the molecule is directly proportional to the wavelength of radiation. The increase in the energy of the molecule due to absorption leads to the electronic excitations where electrons jump to higher energy levels. The analyte concentrations are determined from the amount of absorption. The relationship between the amount of light absorbed and the concentration of the analyte present in known standards can be used to determine unknown concentrations by measuring the amount of light they absorb. Hollow cathode lamps are mostly used in atomic absorption spectrometry as a radiation source. They contain a tungsten anode and a hollow cylindrical cathode made of the element to be analyzed. The lamps are unique to the element so, suitable lamp should be used for the analysis of the specific element (Yuan et al., 1995).

The most important advantage of using atomic absorption spectroscopy is the rapid and accurate determination of the concentration of a specific element in a sample alone with the single-element hollow cathode lamp. Early investigation on the analysis of minerals with atomic absorption spectroscopy showed that it was as accurate and sensitive as other available methods and it brought about the rapidity and freedom from extraneous elements (Garcia et al., 1972). Therefore, it is very suitable for the encapsulation efficiency measurements of emulsions containing mineral in their inner phase. In the study conducted by Bonnet et al. (2009), the release rate of magnesium entrapped in the double emulsion was measured by atomic absorption spectroscopy. Similarly, Bonnet et al. (2010) quantified the magnesium amount in the external phase with atomic absorption spectroscopy in their another study in which impact of sodium caseinate concentration and location was investigated. In addition, in the study of Herzi & Essafi (2018), the amount of magnesium released from inner aqueous phase to outer aqueous phase of the double emulsions based on crystallized oils was also measured with atomic absorption spectroscopy. Herzi & Essafi (2020) also used this method to analyze the magnesium release behavior of the double emulsions incorporated into the yogurt. Moreover, Weerasuriya et al. (2017) determined the encapsulation efficiency of the copper

containing anticancer drug encapsulated in the nanoparticles by atomic absorption spectroscopy method.

Atomic absorption spectroscopy was chosen as the encapsulation efficiency method in this study primarily due to the opportunity of measuring magnesium only apart from other constituents. It was important for our system containing many different compounds due to the complex structure of legume flours and the sodium chloride used for the balance the osmotic pressure. This structure handicaps the usage of other methods like conductivity and spectrophotometry while atomic absorption spectroscopy provide accurate measurement for the magnesium amount solely.

1.5.2 Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) is a phenomenon that results from interactions of nuclei with magnetic fields. The nuclei of atoms in compounds behave uniquely in response to magnetic fields in their environment. In NMR process, basically transition between states take place by a radio frequency signal of proper frequency. This leads to higher energy states and when radio frequency signal is switched off, spins go back to their lower state. Change in the energy states of spins and returning to equilibrium is called relaxation. Electromagnetic energy is retransmitted during relaxation and creates NMR signal. Relaxation combines two different mechanisms; longitudinal relaxation (T_1) and transverse relaxation (T_2). NMR relaxation spectrum, is the output of the NMR relaxometry assay, is obtained by applying the inverse Laplace method to the signal curve.

T_2 relaxometry and pfg-NMR relaxometry are useful and non-destructive techniques to investigate the enclosed water volume of vesicular dispersions (Sabatino et al., 2011). While T_2 relaxometry relies on differences in water relaxation behavior in the internal and external compartment upon application of an external water-soluble paramagnetic probe, pfg (pulsed-field gradient) NMR relaxometry discriminates internal and external water based on differences in diffusion behavior (Vermeir et al., 2014). With respect to emulsions, NMR offers a variety of unique insights into

both molecular orientation and transport kinetics within the various phases. Studies show that, it is possible to determine the particle size of inner water droplets and also to determine the enclosed water fraction by using NMR techniques in double emulsions (Vermeir et al., 2014; Wolf et al., 2009). Wolf et al. (2009) revealed the possibility of usage of NMR as a particle size determination method for the inner phase of water-in-oil-in-water double emulsions. The obtained droplet diameters from pfg-NMR were compared with the results from laser diffraction method and comparable results were obtained. Similarly, Vermeir et al. (2016), could determine the inner water droplet size of double emulsions by diffusion NMR. Moreover, Vermeir et al. (2014) suggested that NMR could be used to determine encapsulation efficiency of double emulsions by analyzing the enclosed water volume with the help of a paramagnetic agent. They stated that the main advantage of nuclear magnetic resonance spectroscopy is measuring emulsion as itself without any additional treatment, so it can be applied to any existing emulsion (Vermeir et al., 2014).

1.6 Objective of the Study

The main objective of this study is to encapsulate magnesium, as the water soluble active compound, in the water-in-oil-in-water double emulsion and use it in a food product to produce food enriched with magnesium. Magnesium was chosen as the active compound for various reasons. First of all, magnesium as a mineral takes place in many vital reactions in body and its malabsorption causes serious illnesses. With the increase in the consumption of processed foods, more people suffer from lower magnesium intake. Enrichment of foods with magnesium can reduce magnesium deficiency problem leading to a healthier life. In this study, double emulsion entrapment was chosen since it has an extra protective layer as compared to a single emulsion. Oil droplets in the outer aqueous phase can form a barrier and decrease the release rate. The two layered structure of double emulsion exhibits an extensive protection against external factors like oxygen and light and prevent oxidation and deterioration of magnesium. Therefore, double emulsion is more adequate for sensitive active compounds like magnesium.

Utilization of legume flours in the stabilization of the secondary layer of the double emulsions is also the aim of this study. There is an increasing movement towards using plant based ingredients as an alternative to animal based and allergenic sources. Legume flours have a great potential to be used as a hydrophilic emulsifier with their high protein content and good emulsifying properties beside their health benefits, sustainability and accessibility. However, there is no research in the literature on the usage of legume flours in double emulsion preparation.

The study also aims to analyze the effect of osmotic balance between aqueous phases on the stability of double emulsions. Another objective of this study is to analyze the effect of different homogenization methods, legume flour type and its concentration on the characteristics of double emulsion in terms of particle size, rheology, stability, efficiency, optical imaging and nuclear magnetic resonance analyses. The last objective of this study is to integrate the double emulsion containing magnesium into a food product to analyze its baking stability and in vitro digestion and also its effect on the quality of the product.

CHAPTER 2

MATERIALS & METHODS

2.1 Materials

Magnesium, the main ingredient of this study, was supplied from Sigma Aldrigh Chemical Co. (St. Louis, MO, USA) in the form of magnesium chloride. Sunflower oil, used in the double emulsion, was purchased from Yudum Gıda San. ve Tic. A.Ş (Balıkesir, Turkey). PGPR, the lipophilic emulsifier used in the preparation of the first emulsion, was supplied by Eti Gıda San. ve Tic. A.Ş. (Eskişehir, Turkey). Lecithin, another lipophilic emulsifier used together with PGPR, was bought from Lipoid Gmbh (Ludwigshafen, Germany). Sodium azide, an antimicrobial agent, and nitric acid were purchased from Sigma Aldrigh Chemical Co. (St. Louis, MO, USA). Lentil flour was supplied from Smart Chemical Trading Co. Inc (Turkey) and pea flour was obtained from Molar Chemical Materials Trading Co. Inc. (Turkey).

Wheat flour used for cake dough preparation was supplied from Söke Un (Ankara, Turkey). Sugar (Bal Küpü, Aksaray, Turkey), salt (Billur Tuz, Izmir, Turkey) and baking powder (Dr. Oetker, Izmir, Turkey) were purchased from local markets. Egg white powder was obtained from ETI Food Industry Co. Inc. (Eskişehir, Turkey). Pepsin and pancreatin enzymes, hydrochloric acid, sodium hydroxide and potassium phosphate monobasic were supplied from Sigma Aldrich Chemical Co. (St. Louis, MO, USA).

2.2 Preparation of Double Emulsions

In this study, water-in-oil-in-water (W/O/W) double emulsions were prepared by two step emulsification method. In the first step, primary emulsion was formed by mixing water phase with oil phase which contain lipophilic emulsifier. They were

homogenized under harsh conditions to produce stable droplets. In the second step, primary emulsion and outer water phase containing hydrophilic surfactant were homogenized under mild conditions.

2.2.1 Preparation of W/O Primary Emulsion

Firstly, inner aqueous phase was prepared by dissolving magnesium chloride (1.25 g $MgCl_2$ / 100 g primary emulsion) and sodium azide (0.05 g NaN_3 / 100 g primary emulsion) in the distilled water. The solution was homogenized with magnetic stirrer (Daihan Scientific Co., Ltd.) at 400 rpm for 15 min.

Oil phase was prepared by dissolving lipophilic emulsifiers in the oil. PGPR and lecithin were used as lipophilic emulsifiers. Oil phase was prepared by dissolving 2.5% PGPR and 2.5% lecithin in the oil and homogenizing by High Speed Homogenizer (IKA T25 Digital Ultra-Turrax, Selangor, Malaysia) at 10 000 rpm for 1 min. Inner water phase to oil phase ratio was chosen as 40% to 60% for the primary emulsion since water-oil ratio were found as the optimum for water in oil primary emulsion (Altuntas et al., 2017). Therefore 1.5 g PGPR and 1.5 g lecithin will be added into 60 g oil to obtain the desired ratio at the primary emulsion. After they were mixed and homogenized, the mixture was waited at water bath (GFL 1086, Burgwedel, Germany) at 50 ^{o}C for 15 min.

Finally, primary emulsion was formed by adding water and oil phases together. Water phase, containing magnesium chloride and sodium azide, (40%) was added into oil phase, containing PGPR and lecithin, (60%). The addition was drop by drop during homogenization with High Speed Homogenizer (IKA T25 Digital Ultra-Turrax, Selangor, Malaysia) at 18 000 rpm for 10 min. The prepared primary emulsions were kept at refrigerator at 4 ^{o}C for 18 hours to hydrate the solute completely.

For ultrasonic application, first the emulsion was formed and homogenized at High Speed Homogenizer (IKA T25 Digital Ultra-Turrax, Selangor, Malaysia) at 18 000 rpm for 5 min, then it was treated by Ultrasonic Homogenizer (Sonic Ruptor 400,

OMNI International the Homogenizer Company, Georgia, USA) at 160 W power, 20 kHz frequency and with 50% pulse for 10 min. Samples were kept in ice bath during ultrasonic homogenization to prevent increase in temperature. The prepared primary emulsions were kept at refrigerator at 4 ^{o}C until they were used in the double emulsion production.

2.2.2 Preparation of W/O/W Double Emulsion

Double emulsion preparation is the second step which covers the emulsification of outer water phase with primary emulsion. In this study, lentil flour with 56% carbohydrate, 23% protein and 1.7% fat content and pea flour with 55.3% carbohydrate, 21% protein, 1.6% fat content were used as surfactant for the double emulsion. The primary emulsion to outer water phase ratio was chosen as 40% primary emulsion and 60% outer water phase since it is found as the most stable (Yildirim et al., 2017).

In the preparation of outer water phase, four different concentrations (15%, 20%, 25%, 30%) were studied to analyze the effect of pea flour amount. As an example, for 20% flour containing outer water phase, 20 g flour was added into 100 g distilled water and they were mixed with high speed homogenizer at 10 000 rpm for 1 min. Then, in order to dissolve flour in aqueous phase and to achieve highly viscous solutions, 0.2 M NaOH was added drop by drop until solution pH equals to 12. The solution stirred at magnetic stirrer at 1000 rpm, during NaOH addition for correct measurement. This basic solution was then heated in water bath which was set to 90 ^{o}C for 30 min, with continuous shaking at 90 rpm. Heating was completed when the solution temperature reached to 75 ^{o}C.

The primary phase was kept at water bath (30 ^{o}C, 20 rpm) for 15 min to be heated up to warm temperatures. In the preparation of double emulsions, 40% primary emulsion and 60% outer water phase were mixed for all experiments. The outer water phase containing flour was placed into the mixing bowl of the food processor (Arçelik K-1190 Robolio, Turkey) and mixed at 600 rev/min for 1 min and then the

primary emulsion was added into the food processor drop by drop, at 800 rev/min for 7 min. The final emulsion continued to be mixed at 600 rev/min for 2 min. The produced double emulsions were kept at refrigerator at 4 ^{o}C for further analyses.

2.3 Analyses of Double Emulsion

2.3.1 Particle Size Analysis

Particle size of the oil droplets in the double emulsion and the distribution of particles was analyzed by a laser diffraction particle size analyzer (Mastersizer 3000, Malvern Instruments, Worcestershire, UK). The measurements of laser diffraction particle size analyzer were based on Mie Scattering Theory. The refractive index of dispersant was chosen as 1.33, considering the refractive index of water. Similarly, refractive index of dispersed was taken as 1.464, considering the refractive index of sunflower oil. Globule absorbance was set to 0.01 and the obscuration range was between 8% and 20%. The rotary speed was 1600 rpm during the measurement. The surface volume mean diameter, namely Sauter mean diameter (D(3,2)), and span of oil droplets were calculated via the software of particle size analyzer.

$$D_{32}=\frac{\sum n_i d_i^3}{\sum n_i d_i^2} \qquad \text{(Equation 1)}$$

$$\text{Span}=\frac{d(v,90)-d(v,10)}{d(v,50)} \qquad \text{(Equation 2)}$$

where n_i represents the number of related particles per unit volume, d_i represents the diameter of particles and d(v,10), d(v,50) and d(v,90) represents the diameter of particles that are inside the range of 10, 50, and 90%, respectively.

2.3.2 Rheology

Rheological measurements were done by a rheometer with cone and plate geometry equipment (Kinexus, Malvern Instruments, Worcestershire, UK) at 20 ^{o}C in the

range of 0.1 to 200 s^{-1}. Cone with angle of 4°, having 40 mm diameter and 0.001 mm gap was used during measurements. Apparent viscosity versus shear rate data was supplied from the software. Appearent viscosity of double emulsions were analyzed at 200 s^{-1}.

2.3.3 Instant Stability

Instant stability was determined according to sedimentation rate of emulsions by centrifugation method. Double emulsion samples were put into 15 mL centrifuge tubes and two centrifuge tubes containing sample were placed in centrifuge (Hettich Mikro 200/200R, Sigma Laborzentrifugen GmbH, Germany). Centrifugation last for 15 min at 5 000 rpm. After centrifugation, the infranatant part was measured to calculate sedimentation rate from the below formula;

$$S=\frac{h_s}{h_o}x100 \qquad \text{(Equation 3)}$$

Where hs is the height of infranatant part (m), ho is the height of initial emulsion (m) and S is instant stability (%).

2.3.4 Long Term Stability

Long term stability was determined by observing gravitational separation. Double emulsion samples were taken into centrifuge tubes and they were placed into two different temperatures; 4 °C and 20 °C for six months. Separated parts were measured each week and long term stability was calculated by using the same formula (Equation 3) with instant stability.

2.3.5 Encapsulation Efficiency

2.3.5.1 Sample Preparation

Atomic absorption spectroscopy is used in order to analyze the amount of magnesium, which transfer from inner aqueous phase to outer aqueous phase that is non-capsulated. Since the magnesium in the outer aqueous phase was analyzed, first step was the separation of two phases, outer aqueous phase and primary emulsion. Double emulsions were taken into 50 mL centrifuge tubes and they were centrifuged at 2000 rpm for 15 min. Double emulsions were separated softly at low rpm values in order not to destroy the primary emulsion. The primary emulsion was collected as supernatant and discarded. The remaining part was the outer aqueous phase and this was diluted with distilled water containing 2% nitric acid with a dilution rate of 1:2. The outer aqueous phase was again centrifuged at higher rpm values, 4000 rpm for 15 min, to discard remaing oil droplets and precipate the flour. After that centrifugation procedure, the water part containing magnesium was obtained and diluted once more with distilled water containing 2% nitric acid with a dilution rate of 1:1. Then, the solution was filtered through PTFE (Polytetrafluoroethylene) filter to remove remaining bigger particles. PTFE filter was chosen according to preliminary experiments. Both hydrophilic and hydrophobic filter types were tried in order to find the best one, which transfers the magnesium but exclude the residuals of flour. PTFE showed higher efficiency among the filter types thanks to its compatibility with nitric acid solutions.

2.3.5.2 Determination of the Digestion Procedure

The aqueous part should be digested before measurement in atomic absorption spectroscopy. Digestion procedure depends on the sample that will be analyzed. In order to find the best digestion method for double emulsions, three different wet digestion methods were tried. The most common reagent used in the digestion of atomic absorption spectroscopy samples, nitric acid, was used for all methods. For

the first method, nitric acid with the same amount of sample was added and they were heated for three hours with closed lid. For the second method, the mixture of sample and nitric acid (1:1) was heated for one hour. Then the sample and the nitric acid mixture was heated at 95 ^{o}C in the fume hood with open lid for one hour. For the third method, hydrogen peroxide, half of the amount of sample was added during secondary heating period of the second method. Since second method gave the best result considering the concentration result read by the atomic absorption spectrophotometer, it was chosen for this study.

Therefore, for the digestion procedure, 10 mL of nitric acid was added into 10 mL sample and the mixture was heated in the water bath (GFL 1086, Burgwedel, Germany) set to 90 ^{o}C for 1 hour with closed lid. Then the solution was heated at about 95 ^{o}C in the fume hood with open lid for one hour to evaporate nitric acid. After the solution was cooled down, it was completed to 50 mL with distilled water containing 2% nitric acid and filtered from PTFE filter to remove residuals of digestion. After the digestion process, the sample containing the aqueous part of the outer water phase and free of residuals was obtained. The sample was diluted with dilution rate of 1:10 with distilled water containing 2% nitric acid before atomic absorption spectroscopy analysis.

2.3.5.3 Atomic Absorption Spectrophotometer Measurement

Atomic absorption spectrophotometer measures the absorbance and converts this absorbance data into concentration data by the help of calibration curve. In order to generate the calibration cure, standard solutions with known concentrations should be read by the instrument. The instrument has upper and lower limits therfore, standards should be prepared so as not to exceed the limits. By considering these information, standard solutions were prepared before each analysis. The standard solutions were prepared by diluting the known reference standard (1000 ppm) at different ratios with distilled water containing 2% nitric acid.

Atomic absorption spectrometry analysis was conducted by atomic absorption spectrophotometer (Thermo Scientific iCE 3300, UK) at 285.2 nm using a magnesium lamp operated at 6 mA current and a 0.7 mm slit burner head for the air-acetylene flame. Absorbance values were read three times and the average of them was taken as the absorbance value of the sample. Then, the absorbance values were transformed into magnesium concentration values through calibration curve. Three replicates were used for each type of double emulsion. The result given by the equipment was the concentration of the sample analyzed in terms of ppm. The concentration of the analyzed sample was first converted to concentration of outer water phase considering the dilution factors during separation and digestion procedures. Then, the amount of magnesium in the outer phase was found using the concentration of the outer phase and the volume of the outer phase with the formula below;

$$N_{o,Mg} = C_{o,Mg} \times V_o \qquad \text{(Equation 4)}$$

where $C_{o,Mg}$ (mg/L) is the magnesium concentration of outer phase, V_o (L) is the volume of outer aqueous phase and $N_{o,Mg}$ (mg) is the amount of magnesium present in the outer phase.

The amount of magnesium remaining in the inner phase was found by subtracting the amount of magnesium that passed into the outer phase from the amount of magnesium added during emulsion preparation. Emulsion efficiency was calculated as the ratio of the amount of magnesium remaining in the inner phase to the amount of magnesium added while preparing the emulsion (Equation 5).

$$\text{Efficiency (\%)} = \frac{N_{i,Mg} - N_{o,Mg}}{N_{i,Mg}} \times 100 \qquad \text{(Equation 5)}$$

where $N_{o,Mg}$ (mg) is the amount of magnesium present in the outer phase and $N_{i,Mg}$ (mg) is the amount of magnesium initially encapsulated in the droplets.

Apart from encapsulation efficiency calculations, atomic absorption spectroscopy was also used to analyze the amount of magnesium in the lentil and pea flour. In order to detect the magnesium content of flours, 1 g of legume flour was dissolved

in 50 mL distilled water and mixed with high speed homogenizer at 7000 rpm. After 2 hours, the flour-water mixtures were taken into 50 mL centrifuge tubes and they were centrifuged at 3000 rpm for 10 min. The flour part participated and the aqueous part was collected as supernatant and taken into another 50 mL centrifuge tube. After the homogenized mixture was centrifuged at 3000 rpm for 10 min and separated from the residues of cake, the wet digestion procedure was applied and the samples were analyzed with atomic absorption spectrometer (Thermo Scientific iCE 3300, UK) with the same procedure above. The concentration of the analyzed sample, converted to concentration of the prepared solution considering the dilution factors during separation and digestion procedures. Then the concentration of magnesium in the legume flours was calculated.

2.3.6 Nuclear Magnetic Resonance Analysis

For NMR experiments, emulsions were put into tubes with 15 mm inner diameter and 15 mm height. Experiments were performed at a system operating at the ^{1}H frequency of 13.57 MHz (SpinTrack, Resonance Systems GmBH, Germany). CPMG pulse sequence was used to measure the T_2 relaxation times and the obtained data were analyzed with XPFit software (Softsonics, Israel).

For enclosed volume experiments, double emulsions were prepared for different flour concentrations. After the double emulsion preparation, 2% (v/w) from 10 mM $MnCl_2$ prepared with distilled water was added into the double emulsions and stored in the refrigerator for about 16 hours before measurements. Double emulsions with and without manganese chloride were analyzed with Carr Purcell Meiboom Gill (CPMG) pulse sequence to conduct T_2 relaxometry analysis.

In order to analyze the effect of $MgCl_2$ on lentil and pea flour proteins, NMR spectroscopy T_2 relaxometry was used. Firstly, 1% legume flour was dissolved in 50 mL water and mixed with high speed homogenizer at 10000 rpm. Then 1% $MgCl_2$ was added into 25 mL flour-water mixture. The solutions with and without $MgCl_2$ were put inside glass tubes having 15 mm inner diameter until they reach to height

of 15 mm and analyzed with a benchtop NMR (SpinTrack, Mary El, Yoshkar-Ola, Russia) with 13.57 MHz frequency. Carr Purcell Meiboom Gill (CPMG) pulse sequence was used for T_2 relaxometry experiments. The data obtained from the pulse sequences were analyzed by MATLAB program.

2.3.7 Optical Imaging

The morphological properties of double emulsions were analyzed under an inverted light microscope (PrimoVert, Zeiss, Jena, Germany). One drop of double emulsion sample was taken on to the glass slide and spread with another clean glass slide to form a thin layer before imaging in order to increase visibility. Images were extracted by microscope camera (Sony CCD Color Digital Video C-Mount Microscope Camera, Tokyo, Japan) at 40× magnification level and analyzed by TopView software program.

2.4 Incorporation of Double Emulsions into Cakes

2.4.1 Preparation of Cakes

Among the prepared double emulsions, the ones with the highest stability and efficiency were selected and added into the cake in order to measure the stability of double emulsions during heat treatment and to observe the effects of addition of double emulsion on quality parameters. For this purpose, the cake formulation determined by Sakiyan et al. (2007) was adapted. While preparing the cake, cake flour containing 8% dry gluten, 0.65% ash and 13.5% moisture was used. The cake batter was prepared to contain 100% flour, 100% sugar, 50% sunflower oil, 9% egg white powder, 3% salt and 5% baking powder and 90% water (all percentages are in weight of flour basis).

Cake dough was prepared to contain 15% double emulsion. Double emulsions were added into cake batter in such a way that balances the water, oil and flour content.

Two different control cakes were also prepared. For the control cake with magnesium, magnesium chloride was added directly into cake batter at an amount which was equal to the case when double emulsion was added. For the control cake with primary emulsion, primary emulsion of the double emulsion was added at an amount which was equal to the case when double emulsion was added. Both control cakes and cakes with double emulsion had the same amount of magnesium chloride. Thus, it could be possible to compare the effects of encapsulation.

For the preparation of cake batter, firstly, all dry ingredients (flour, sugar, egg white powder, baking powder and salt) were mixed thoroughly. Then, after adding oil and water to the dry substances, they were mixed with a mixer (Kitchen Aid, 5K45SS, USA) for 2 min at 85 rpm, 1 minute at 140 rpm and finally at 85 rpm for 2 more min. Cake batters of 100g were weighted into beakers having 7.5 cm diameter and they were baked at 175 ^{o}C, for 30 min in a preheated conventional oven (9411FT, Arçelik Inc. Co., Istanbul, Turkey).

2.4.2 Characterization of Cakes

2.4.2.1 Weight Loss

For the weight loss measurements, cake batters were weighted before baking and the cakes were weighted just after baking. The weight loss percentages were calculated by proportioning the difference in the weight to the weight of cake batter (Equation 6).

$$WL=\frac{W1-W2}{W1} \times 100 \qquad \text{(Equation 6)}$$

In the equation, WL refers to the percent weight loss (%), W1, the weight of the cake batter before baking (g), and W2, the weight of the cakes after baking (g).

2.4.2.2 Specific Volume

Specific volumes of the cakes were determined by rapeseed displacement method (Sahin, Sumnu, 2006).

2.4.2.3 Color

Crust color of the cakes were measured using Konica Minolta Spectrophotometer (CM-10, Japan) and the results were evaluated in terms of CIE color coordinate system, (L*, a*, and b*). Overall color change, $\Delta E*$, according to the formula below (Equation 7).

$$\Delta E^* = \sqrt{\left(L^* - L_o^*\right)^2 + (a^* - a_o^*)^2 + \left(b^* - b_o^*\right)^2} \qquad \text{(Equation 7)}$$

Where L*, a*, and b* refers the coordinates of the color of the cake and L_o^*, a_o^*, b_o^* refers the coordinates of the color of the reference. Barium sulphate was used as reference and its L_o^*, a_o^*, b_o^* values are 93.2; -1.4; 0.12 respectively.

2.4.2.4 Hardness

Hardness of the cakes were analyzed in order to evaluate the texture of the cakes. The hardness of the cakes was measured 1 hour after baking using a texture analyzer (Lloyd Instruments LR 30K, UK). Samples, cut in cubic shape with 25 mm on one side, was compressed up to 25% of their original thickness at a speed of 55 mm/min and analyzed using a cylindrical probe and a 50 N weight unit (Sevimli et al., 2005). The peak force of the first compression cycle was taken as the hardness value.

2.4.2.5 Sensory Analyses

Cakes were evaluated 1 hour after baking, using hedonic scale. A taste panel was arranged to compare the taste, hardness and color likeness of the cakes with double

emulsion, cakes with direct magnesium addition and cakes containing no magnesium. The cakes were cut into cubes (4×4×4 cm^3) and presented to the 30 panelists. Panelists were expected to evaluate the samples in terms of taste, hardness and color without any preliminary information. A sunlight colored lamp was used in the panel to determine the crust color. A scale of 9 was used to evaluate the samples and the samples were asked to be evaluated by giving a number between 1: dislike extremely and 9: like extremely (Wichchukit & O'Mahony, 2015).

2.5 Baking Stability

Atomic absorption spectroscopy was used in order to analyze the baking stability of double emulsions in the cakes. Heat can cause disintegration of double emulsion which cause leakage of the inner material. So, the magnesium transfer between aqueous phases after baking was evaluated to determine baking stability. The amount of magnesium transferred from inner water phase to outer water phase was calculated and the percent ratio of remaining magnesium amount to initially added magnesium amount was considered as the baking stability.

Moreover, double emulsions were subjected to heat treatment without integration into any food product. Their encapsulation efficiencies were also calculated to analyze the effect of heat on the double emulsion and the on the food complex separately.

In order to analyze the baking stability of double emulsions in the cakes, cakes were cooled down for 1 hour after baking. Then, 5 g of from each cake was dissolved in 50 mL double distilled water and they were homogenized at 15000 rpm for 5 min with high speed homogenizer (IKA T25 Digital Ultra-Turrax, Selangor, Malaysia), for complete dissolution. After the homogenized mixture was centrifuged at 5000 rpm for 10 min and separated from the residues of cake, the wet digestion procedure was applied and the samples were analyzed with atomic absorption spectrometer (Thermo Scientific iCE 3300, UK) with the same procedure explained in 2.3.5.2.

Similarly, double emulsions subjected to heat treatment were also analyzed with atomic absorption spectrometry. Firstly, double emulsions were heated in the water bath at 90 ^{o}C for 30 min and then they were cooled down for 1 hour. 5 gr from each double emulsion was dissolved in 50 mL double distilled water and the same procedure was applied directly for the double emulsions similar to explained in 2.3.5.2.

2.6 Simulated In-Vitro Digestion of Magnesium in the Cakes

Digestion of magnesium in the cakes were analyzed both in the gastric and intestinal media. To observe the digestion, simulated gastric juice and simulated intestinal juice were prepared and cakes were exposed to them for a certain period. Control cakes containing magnesium without any encapsulation and containing primary emulsion were also analyzed as a control and their digestion mechanisms were compared.

2.6.1 Simulated Gastric Digestion

For the preparation of simulated gastric fluid (SGF), 2 g sodium chloride and 3.2 g pure pepsin were mixed in the 7 mL of hydrochloric acid and they were completed to 1000 ml. Then the pH of the solution was adjusted to 1.2 with 3 M hydrochloric acid. (Setiasih et al, 2018).

Baked and cooled cake samples of 500 mg were dissolved in 7 mL SGF in the 50 mL centrifuge tubes. These centrifuge tubes were placed into the shaking water bath which was set to 37 ^{o}C and 80 rpm. After 2 hours of incubation, samples were centrifuged at 3000 rpm for 5 min. and 5 mL of aqueous sample was taken from each tube. pH of the solution was adjusted to 7 with 2 M sodium hydroxide in order to stop enzyme activity and then samples were diluted with distilled water containing 2% nitric acid with a dilution rate of 1:1. After that, wet digestion procedure for atomic absorption spectrometry was applied and the samples were analyzed with atomic absorption spectrometry as explained in section 2.3.5.2.

2.6.2 Simulated Intestinal Digestion

For the preparation of simulated intestinal fluid (SIF), 6.8 g potassium phosphate monobasic was dissolved in 250 mL distilled water. Then 77 mL 0.2 N sodium hydroxide and 500 mL distilled water were added into this mixture. Lastly, 10 g pancreatin was added slowly into the solution and mixed by magnetic stirrer at 1500 rpm for 1 hour. Obtained mixture was completed to 1000 mL with distilled water and pH was adjusted to 6.8 with 2 M sodium hydroxide (Ranjan et al, 2016).

Baked and cooled cake samples of 500 mg were dissolved in 12 mL SGF in the 50 mL centrifuge tubes. These centrifuge tubes were placed into the shaking water bath which was set to 37 ^{o}C and 80 rpm. After 2 hours of incubation, samples were centrifuged at 3000 rpm for 5 min. and 5 mL of aqueous sample was taken from each tube. Samples were diluted with distilled water containing 2% nitric acid with a dilution rate of 1:1. After that, wet digestion procedure for atomic absorption spectrometry was applied and the samples were analyzed with atomic absorption spectrometry as explained in section 2.3.5.2.

2.7 Statistical Analysis

All the results were analyzed by analysis of variance (ANOVA) in order to observe differences between variables with the help of Minitab program. Tukey's Multiple Comparison Test ($p \leq 0.05$) was used to compare samples whose results displays significant difference in ANOVA test. Data represented the mean values of at least two replications.

2.8 Experimental Design

Full factorial experimental design was used in the thesis. It can be seen in Table 2.1.

Table 2.1 Experimental Design of the Study

	Independent Variables	Dependent Variables
Magnesium Containing Double Emulsions	Legume Flour Type • Lentil • Pea Legume Flour Concentration • 15% • 20% • 25% • 30% Homogenization Method • HSH • UH	• Particle size and distribution • Rheology • Instant stability • Long term stability • Encapsulation efficiency • Optical Imaging • NMR Relaxometry Analysis
Magnesium Containing Cakes	Encapsulation Technique • Primary Emulsion • Double Emulsion Legume Flour Type • Lentil • Pea Legume Flour Concentration • 20% • 25% • 30%	• Weight loss • Specific volume • Color • Hardness • Sensory Analysis • Baking Stability • In vitro Digestion

CHAPTER 3

RESULTS & DISCUSSION

3.1 Effect of Osmotic Balance on Stability

As a preliminary experiment, the effect of salt addition in the outer water phase on the stability of double emulsion was investigated. Stability of double emulsions containing 20% lentil flour with sodium chloride addition to the outer aqueous phase to balance osmotic pressure and prevent dehydration of the inner aqueous phase (containing magnesium) was analyzed by centrifugation method during four weeks. The experiments were also repeated without sodium chloride addition to show the importance of osmotic balance for stability. The stability of double emulsions prepared with sodium chloride resulted in higher stability even at time equals to zero and the effect of salt addition on stability was more significant after one week (Figure 3.1). In literature, there are many studies that investigate the effects of salt addition on stability of emulsions. The study of Sapei et al. (2012), showed the effect of salt addition into inner water phase on the stability of double emulsions. They found that salt added emulsions displayed higher stability and encapsulation efficiency. They explained the reason as; increased emulsifier adsorption at the interphase and reduced interfacial tension resulted from the electrolytes. Since the first aim of this study is to encapsulate magnesium, the inner water phase included magnesium chloride. This study showed that addition of magnesium chloride attributes to emulsion stability besides being the active agent. Another study conducted by Pistel & Kissel (2000), suggested that addition of salt to outer phase could be a simple and efficient way to produce stable emulsion with high encapsulation efficiency and enhanced microsphere characteristics. Their results showed that salt addition into outer water phase decreased the microsphere surface area regardless of the salt concentration in the inner water phase and this led to better release profiles and encapsulation efficiencies. In addition, Kanouni et al. (2002), concluded that even

small quantities of salt to balance osmotic pressure greatly enhanced the long term stability of double emulsions. As a result, all the double emulsions in this study were prepared with the addition of sodium chloride to the outer aqueous phase to balance the osmotic pressure of the inner aqueous phase resulted from magnesium in it.

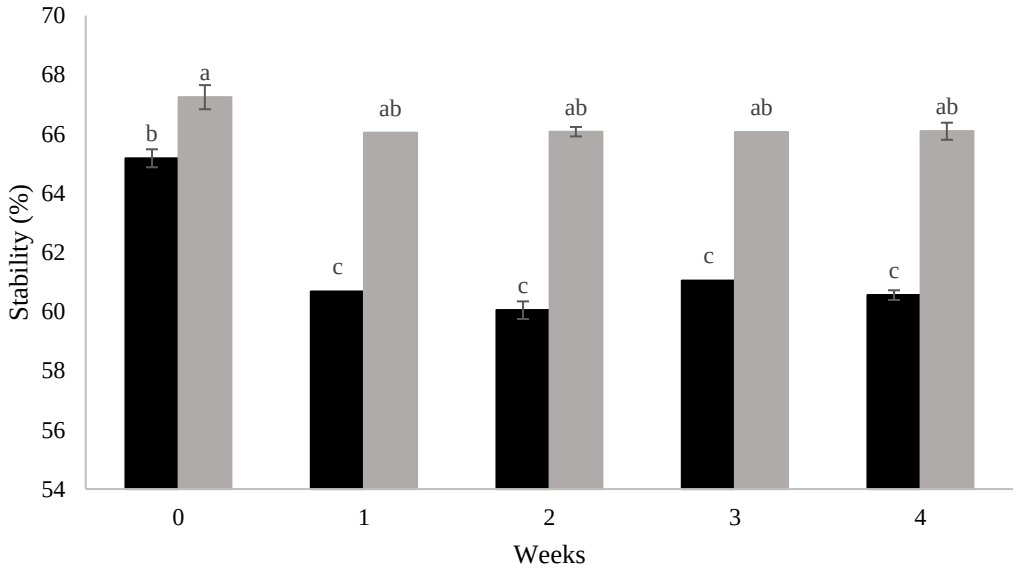

Figure 3.1. Stability (%) of double emulsions without NaCl; (■) and with NaCl; (■) in the outer aqueous phase

3.2 Analysis of Magnesium Amount in Legume Flours

The main purpose of this study is to increase magnesium intake by encapsulating magnesium into double emulsion and integrating double emulsions into food systems. Legume flours are used as the surfactant at the outer water phase of double emulsions. Legume flours have already magnesium in their structure, so the magnesium concentration of legume flours was calculated to determine the additional magnesium coming from legume flours at different concentrations.

At the end of calculations, magnesium contents of lentil and pea flours were found as 48.11 mg/100 g and 66.12 mg/100 g, respectively. According to a study conducted by Köse et al. (2018), the average magnesium amount in green lentil cultivar was

found as 71.9 mg/100 g. Also the magnesium content of raw lentil flour was found as 105.1 mg/100 g (Porres et al., 2004). The magnesium content of pea flour was calculated as 81.29 mg/100 g (Ohizua et al., 2017). The differences in magnesium concentrations could be resulted from the seed type, growth medium and soil content.

3.3 NMR T_2 Relaxometry to Analyze the Effect of Magnesium on the Solubility of Legume Proteins

The stability and solubility of proteins are directly affected from the solvent type and concentration due to interactions between the solvent and the protein. Salts as the solvent has a strong effect with their ionic species. Although some salts such as $CaCl_2$ and NaSCN are known to decrease stability and increase solubility of proteins, that is salting in effect, some salts such as $(NH_4)_2SO_4$ and Na_2SO_4 decrease solubility and precipitate or crystallize proteins, that is salting out effect (Arakawa et al., 1990).

The increase in T_2 value indicates protein precipitation. T_2 value did not change significantly after the addition of magnesium into solution prepared with lentil flour and water. Thus, it can be said that magnesium does not have a salting-in or salting-out effect on lentil flour. However, the T_2 values of solutions prepared with pea flour and water increased after the addition of magnesium. This indicates that magnesium interacts with pea proteins in such a way that it increases the stability of pea proteins and decreases the solubility of pea proteins in water. Then it can be concluded that, magnesium had a salting out effect on pea proteins.

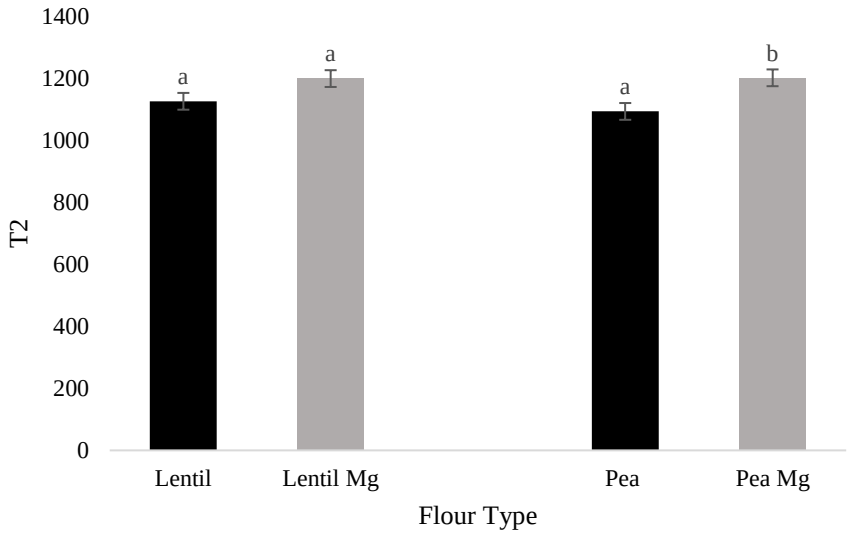

Figure 3.2. T_2 values of solutions prepared with lentil and pea flour with and without magnesium chloride

3.4 Characterization of Magnesium-Loaded Double Emulsions

In this study, magnesium was encapsulated with double emulsion method by using lentil and pea flour at different concentrations. Legume flours were preferred due to their potential use as a hydrophilic surfactant as an alternative to protein based and allergenic ones. Two different homogenization methods, high speed homogenization and ultrasonic homogenization were also studied. The effect of the legume flour type, concentration and homogenization method on the stability and release properties of double emulsion were analyzed.

3.4.1 Particle Size and Distribution

Particle (droplet) size and its distribution are one of the most important parameters for double emulsions since it affects stability and rheological properties directly. Smaller droplets are highly resistant against coalescence, aggregation and creaming.

In this study, after particle size distributions of double emulsions stabilized by lentil flour were analyzed, it was observed that both the ones prepared with high speed homogenizer and ultrasonic homogenization obeyed monomodal particle size distribution model (Figure 3.3-3.4). On the other hand, double emulsions prepared with pea flour showed bimodel particle size distribution for all flour concentrations and for both homogenization methods (Figure 3.5-3.6). Cofrades et al. (2013), stated that double emulsions could exhibit monomodal, bimodal or even trimodal particle size distribution. Double emulsions prepared with PGPR as lipophilic emulsifier and sodium caseinate or whey protein concentrate as hydrophilic emulsifier showed monomodal particle size distribution in their study. Yildirim et al. (2017), also observed monomodal particle size distribution for double emulsions prepared with PGPR and sodium caseinate, while the double emulsions containing whey protein isolate and xanthan gum had bimodal particle size distribution. They indicated that particle size distributions of emulsions were affected by composition, concentration and type of emulsion and by homogenization conditions. In this study, surfactant type determined the particle size distributions of double emulsions principally, due to the differences in their compositions, protein structures and emulsifying abilities, whereas the concentration of hydrophilic emulsifier and homogenization method did not change the particle size distribution model type. However, they had a significant effect on polydispersity and particle size of droplets.

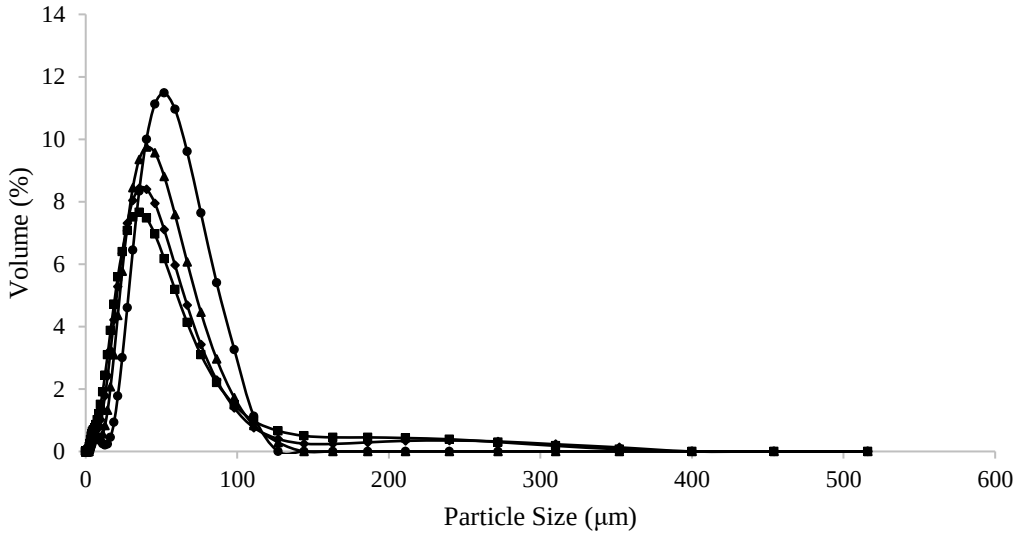

Figure 3.3. Particle size distribution of double emulsions prepared with high speed homogenization containing lentil flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30%

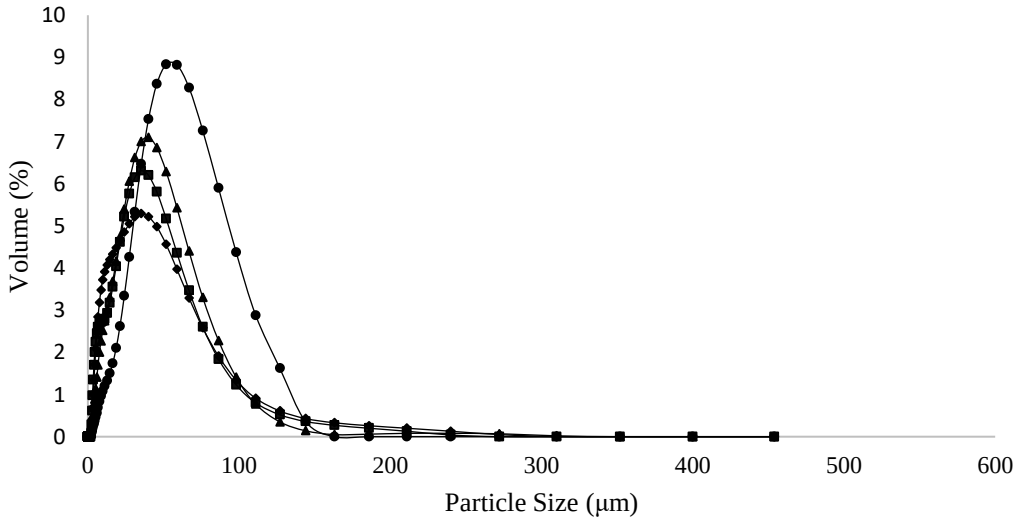

Figure 3.4. Particle size distribution of double emulsions prepared with ultrasonic homogenization containing lentil flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30%

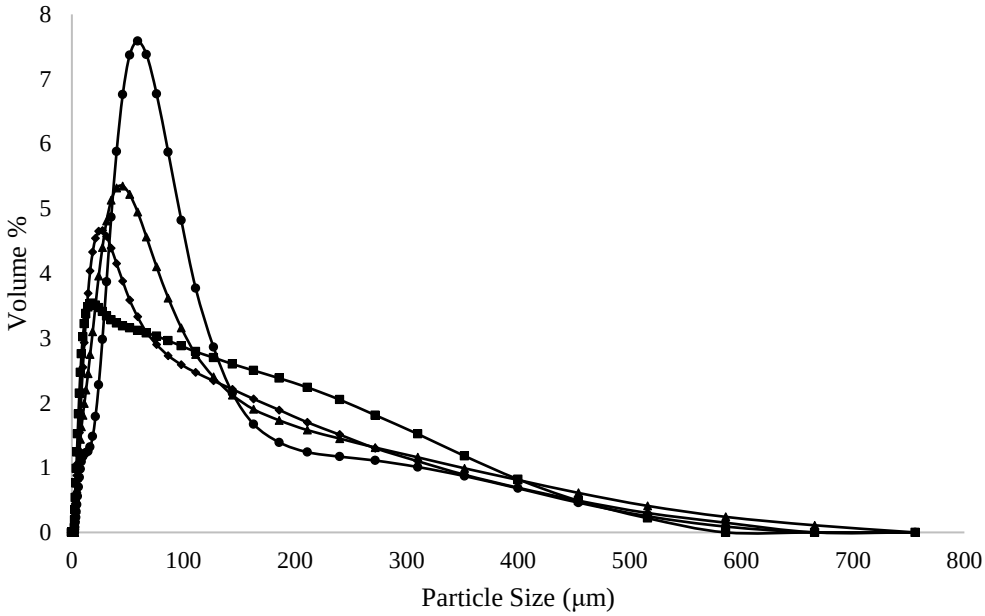

Figure 3.5. Particle size distribution graph of double emulsions prepared with high speed homogenization containing pea flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30%

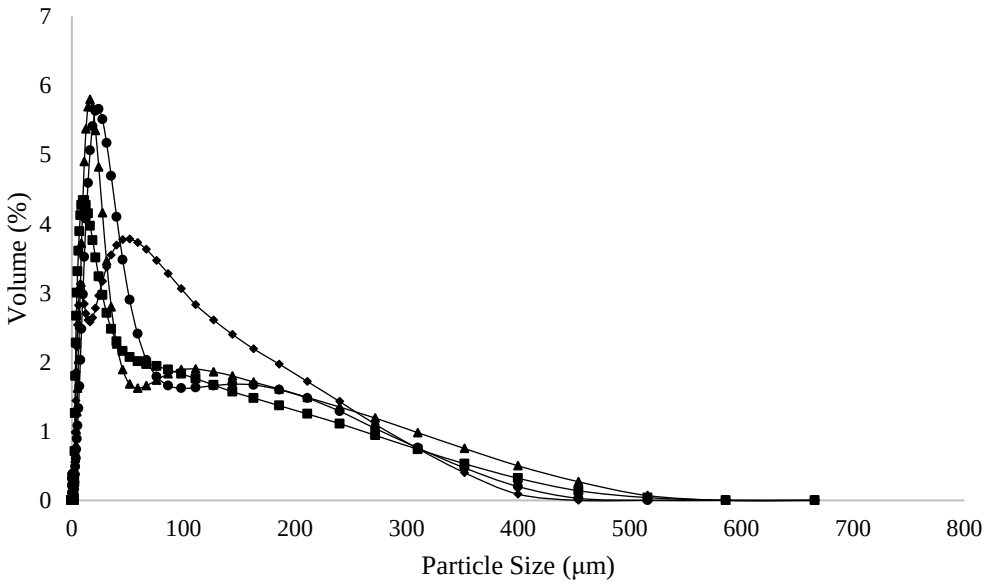

Figure 3.6. Particle size distribution graph of double emulsions prepared with ultrasonic homogenization containing pea flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30%

As can be seen from the figures, while particle size of double emulsions with lentil flour was in the range of 0-140 μm (Figure 3.3), double emulsions with pea flour had broader range between 0-600 μm (Figure 3.5). Therefore, the span value of double emulsions prepared with lentil flour was found to be 1.573, whereas those prepared with pea flour were calculated as 3.966. The higher span of double emulsions prepared with pea flour indicated that they had less homogeneous distribution.

For the double emulsions produced by ultrasonic homogenization, the span value of the double emulsions prepared with lentil flour was found to be 2.143, while those prepared with pea flour were calculated as 6.276. Since span is an indicator for polydispersity, the increase in the span values after ultrasonic homogenization indicated that ultrasonic homogenization produced emulsions with a wider range of different particle sizes. Normally, it is known that with the high energy input, ultrasonication leads to narrower particle size distribution (Hatkar & Gogate, 2012). The higher span value could be explained with the non-uniform mixing during homogenization. The oil droplets close to probe were exposed to more disruption causing a more polydisperse emulsion.

In order to analyze the particle size of double emulsions, surface-volume mean diameter, namely, Sauter mean diameter D[3,2] of samples were determined and shown in Table 3.1. When the Sauter mean diameters of the double emulsions were analyzed, it was observed that, legume flour type, legume flour concentration and homogenization method had a significant effect on the particle size of double emulsions ($p<0.05$).

Table 3.1 Particle size of double emulsions prepared with different lentil and pea flour concentrations and homogenization methods (HSH: high-speed homogenization, UH: ultrasonic homogenization)

Legume Flour Type	**Homogenization Method**	**Legume Flour Concentration (%)**	**D[3,2] (µm)**
Lentil	HSH	15	38.45±0.15[a]
	HSH	20	28.45±0.15[c]
	HSH	25	24.75±1.05[cd]
	HSH	30	21.65±0.95[de]
	UH	15	32.65±0.75[b]
	UH	20	18.00±1.20[ef]
	UH	25	15.45±0.15[f]
	UH	30	15.60±0.10[f]
Pea	HSH	15	34.05±1.05[a]
	HSH	20	27.85±1.15[b]
	HSH	25	22.05±0.65[c]
	HSH	30	19.55±1.85[cd]
	UH	15	9.83±0.37[f]
	UH	20	16.00±0.10[de]
	UH	25	16.15±0.15[de]
	UH	30	12.03±0.05[ef]

* Different letters in the same column represents significant difference ($p \leq 0.05$)

Double emulsions prepared with pea flour had smaller particles than the ones with lentil flour. Sauter mean diameter of the double emulsions with lentil flour were found about 10% larger than the ones with pea flour for high speed homogenization (Table 3.1). Since particle size of droplets is mostly affected from proteins instead of starch which could not place at the interface of emulsions, the difference observed between lentil and pea flour in the particle size of droplets might come from the

solubility and surface charge differences of lentil and pea proteins. As the proteins dissolve in the continuous phase well, they can easily locate to the water-oil interphase and produce smaller droplets (Karaca, et al., 2011). According to the study of Karaca et al. (2011) in which the emulsifying properties of legume flours at neutral pH (pH=7) were studied, pea protein had the lowest emulsion capacity and stability due to its low solubility, low surface charge and high hydrophobicity. In another study which also considered the effect of pH, it was observed that lentil proteins had slightly smaller droplets than pea proteins at pH=3, pH=5 and pH=7 (Chang et al., 2015). However, the study conducted by Jiang et al. (2017), revealed the great impact of extremely basic pH on the physicochemical properties of pea protein. The solubility of pea protein increased from 8% to 55% after shifting pH to 12, while no change was observed at pH=2, pH=4 and pH=10. Jiang et al. (2009) reported that extreme basic pH causes partial protein unfolding and formation of "molten globule" structure which result in lost in some molecular interactions and increased peptide chain flexibility. Although, higher ionic strength and higher solubility was also observed for lentil proteins especially below pH=2 and above pH=8 (Jarpa-Parra et al., 2014), the solubility and emulsifying properties have not been compared at pH=12 for lentil and pea proteins. However, the smaller particle size obtained with the use of pea proteins could be attributed to the higher surface charge, higher solubility and therefore higher emulsifying abilities of pea proteins with respect to lentil proteins at pH=12.

Legume flour concentration also had a significant effect on the Sauter mean diameter of double emulsions. The particle size of the double emulsions prepared with lentil flour decreased as concentration of flour increased (Figure 3.7). While the Sauter mean diameter was measured as 38.45 μm at 15% lentil flour concentration, 19.55 μm was obtained with 30% when high speed homogenization was applied. Particle size reduction was directly related with the protein content in double emulsions. In this study, lentil flour contained high protein content (23%). Proteins are good surfactants due to their ability to adsorb at the water-oil interface and form stabilizing layer around oil droplets. During emulsion formation, they are adsorbed at the surface of oil droplets in the form of a densely packed layer. They decrease the

interfacial tension and prevent coalescence by forming a physical barrier at the interphase (Karaca, et al., 2011). Therefore, as the amount of lentil flour increased, the amount of protein increased, so more protein became adsorbed at the interface and created smaller droplets. Ye (2008), observed in his study that particle size decreased as total protein concentration increased in emulsions prepared using sodium caseinate and whey protein concentrate. Thus, particle size of emulsions were larger at lower protein concentrations. Moreover, Hebishy et al. (2015), stated that emulsions containing 1% whey protein isolate had a larger particle size than those containing 2% and 4%.

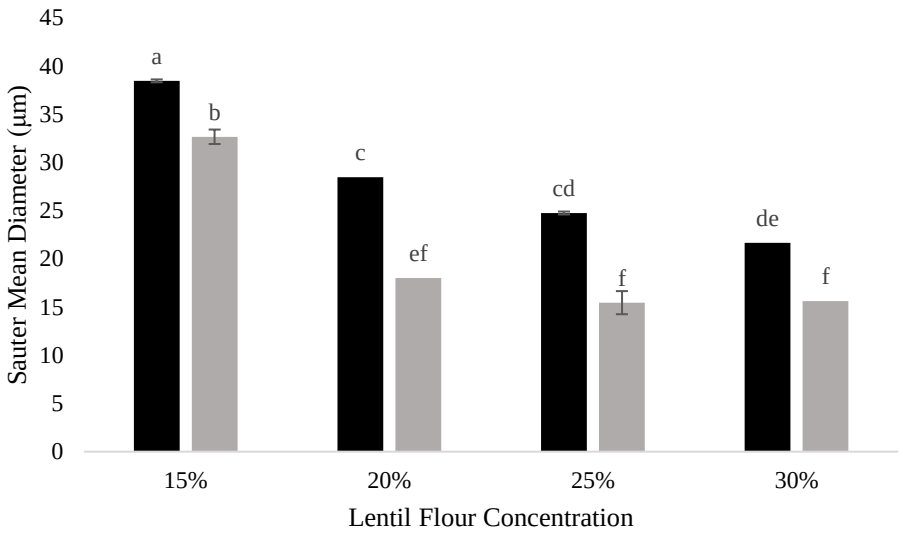

Figure 3.7. Sauter mean diameters of double emulsions prepared with different lentil flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■)

When the Sauter mean diameter of double emulsions prepared with high speed homogenization and ultrasonic homogenization were compared for the ones with lentil flour, a significant decrease was observed after ultrasonic homogenization (Figure 3.7) (from 38.45 to 32.65 µm, 28.45 to 18.00 µm, 24.75 to 15.45 µm and 21.65 to 15.6 µm). The decrease in particle size with ultrasonic homogenization was observed in many studies too (Schuch et al., 2014; Wischke & Borchert, 2006). It was proved that higher energy input by means of ultrasonication resulted in

producing emulsions with smaller particle size. According to Tal-Figiel (2007), in order to obtain emulsions with smaller droplet size, ultrasound should be applied as the second step, since typical rotational homogenizers did not provide sufficient energy to form mean droplets.

However, in the case of double emulsions with pea flour, particle size of the double emulsions decreased with increasing flour concentration in the secondary phase up to a critical flour concentration (Figure 3.8). Sauter mean diameter of double emulsions prepared with HSH decreased from 34.05 μm to 22.05 μm when flour concentration increased from 15% to 25%. The increase in the flour concentration from 25% to 30% did not significantly affect the particle size of double emulsions. Similarly, double emulsions prepared with US reached to critical point at 20% pea flour concentration with 16 μm droplet diameter (Table 3.1). In order to explain this behavior, it is important to indicate that protein and starch in the flour are the determinant components in the emulsion formation and particle size reduction. While proteins are adsorbed at the interface between aqueous and oil phases and form a viscoelastic film at the interfacial layer (Burger & Zhang, 2019), starch molecules mostly act as thickener and prevent coalescence by adsorbing to the surface of the oil droplet and forming a strong network (Paximada et al., 2016). Therefore, the decrease in the particle size with increasing flour concentration could be explained with the fact that more protein and starch molecule caused densely packed droplets surrounded by thick and bulky polymeric layers (Tamnak et al., 2016). However, after a specific amount of protein is already adsorbed at the droplet surface, increasing the flour concentration did not create a significant difference. Researches showed that, emulsions reached to their saturation at which all protein molecules covered the droplets efficiently and after that point excess proteins remained in the aqueous phase (Paximada et al., 2021). Delahaije et al. (2015), explained this phenomenon by referring as protein-poor regime and protein-rich regime. While at protein-poor regime, protein amount is insufficient the cover the interface of droplets for emulsification and is the determinant variable for droplet size, at protein-rich regime, droplet size is independent of protein content, since sufficient amount of protein is present already.

Moreover, ultrasonic homogenization provided double emulsions with lower particle size as compared to HSH (Figure 3.8). Ultrasonication is well known to be an effective homogenization method for producing small emulsion droplets by interfacial waves and shear forces caused by sound field application. The observation of the effect of ultrasonic homogenization on droplet size reduction is also consistent with other studies. Silva et al. (2020) observed the effect of ultrasonication both on particle size reduction and increased adsorption of protein at the interface. Leong et al. (2017), also showed that as the sonication power increased, disruption of droplets increased which led to smaller particle sizes.

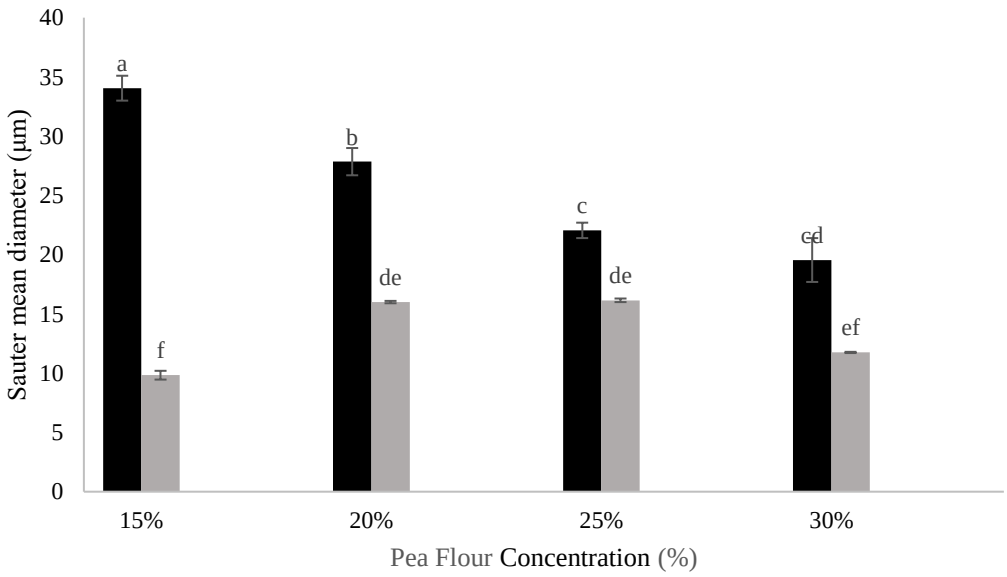

Figure 3.8. Sauter mean diameters of double emulsions prepared with different pea flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■)

3.4.2 Rheology

Rheological properties of double emulsions were analyzed to observe the effects of flour type, concentration and homogenization method. Double emulsions prepared with both legume flours at different concentrations and with both homogenization methods behaved as non-Newtonian fluid. Besides, for all double emulsions,

viscosity decreased with the increasing shear rate showing that they were shear thinning fluids (Figure 3.9-3.12). This is a typical behavior for double emulsions having weakly bonded droplets susceptible to deformation (Huang et al., 2021; Sridharan et al., 2020; Ying et al., 2021). According to study conducted by Garti & Bisperink (1998), double emulsions showed non-Newtonian and shear thinning behavior. Moreover, Murillo-Martínez et al. (2011), found that double emulsions showed shear thinning behavior at high shear rates. They explained this behavior by the structural deformation. As shear was applied, the structure of double emulsion was broken down and this led to a decrease in viscosity. In other words, at higher shear rates, droplets could separate from each other and could be aligned in the direction of flow and consequently, flow resistance decreases (Choi et al., 2018).

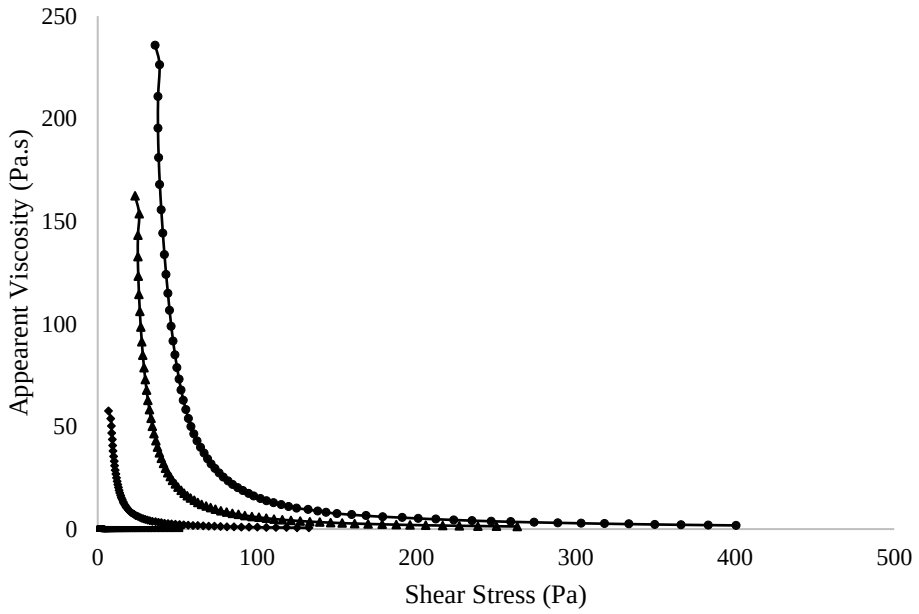

Figure 3.9. Apparent viscosity of double emulsions prepared with high speed homogenization containing lentil flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30%

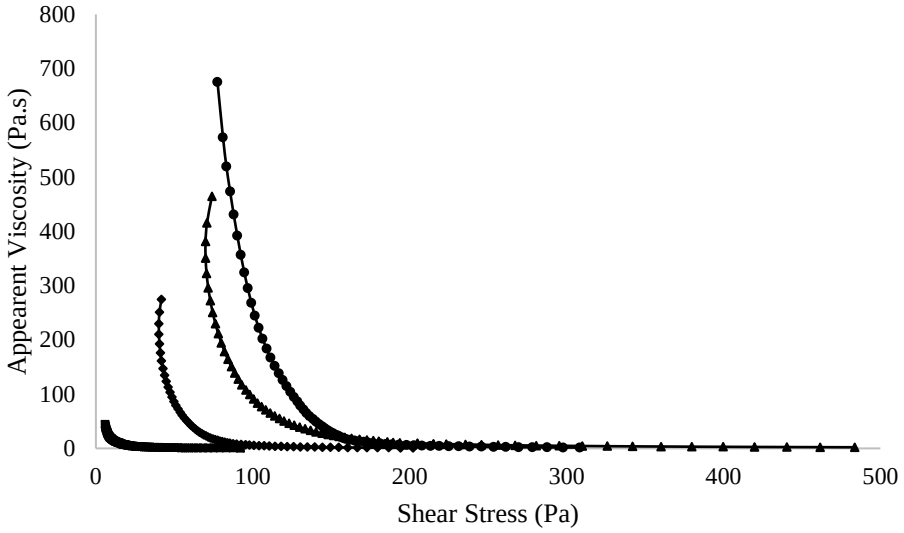

Figure 3.10. Apparent viscosity of double emulsions prepared with ultrasonic homogenization containing lentil flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30%

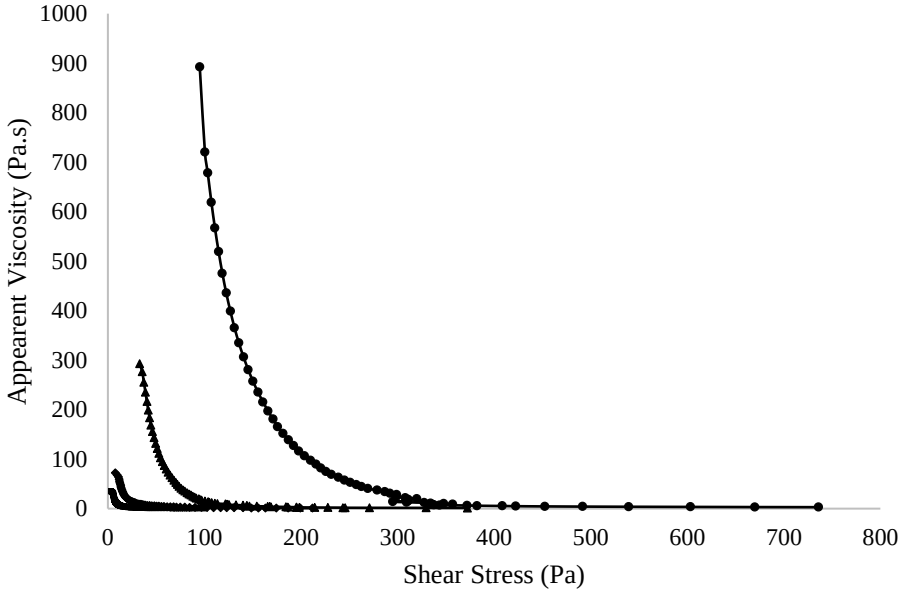

Figure 3.11. Apparent viscosity of double emulsions prepared with high speed homogenization containing pea flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30%

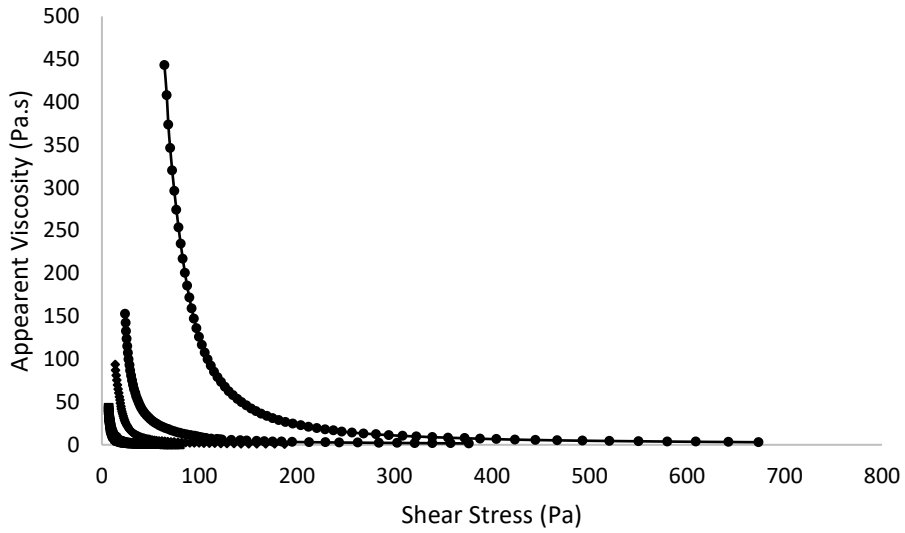

Figure 3.12. Apparent viscosity of double emulsions prepared with ultrasonic homogenization containing pea flour at different concentrations (●):15%, (▲):20%, (♦):25%, (■):30%

After deciding on the behavior, the model that best fits to the double emulsions was investigated. Power-law model, having the highest determination coefficient (R^2>0.95), was used as the behavior model. The power-law model formulation is shown in Equation 8.

$$\tau = k \times \gamma^n \qquad \text{(Equation 8)}$$

where τ represents shear stress (Pa), γ represents shear rate (s^{-1}), k represents consistency index ($Pa.s^n$) and n represents the flow behavior index (dimensionless). The corresponding values for double emulsions in that study are tabulated in Table 3.2.

Table 3.2 Consistency index and flow behavior index of double emulsions prepared with different lentil and pea flour concentrations and homogenization methods (HSH: high-speed homogenization, UH: ultrasonic homogenization)

Flour Type	**Homogenization Method**	**Legume Flour Concentration (%)**	**k (Pa.s^n)**	**n**	**R^2**
	HSH	15	0.09±0.01^{f}	0.527^{a}	0.9982
	HSH	20	16.23±0.80^{e}	0.378^{b}	0.9909
	HSH	25	39.93±0.38^{d}	0.335^{b}	0.9801
Lentil	HSH	30	60.19±1.62^{c}	0.308^{bc}	0.9690
	UH	15	13.19±0.70^{e}	0.359^{b}	0,9986
	UH	20	52.86±3.20^{cd}	0.195^{de}	0,9504
	UH	25	94.81±5.04^{b}	0.163^{e}	0,9771
	UH	30	117.44±1.84^{a}	0.256^{cd}	0,9658
	HSH	15	9.71±0.22^{e}	0.418^{a}	0.9899
	HSH	20	19.81±0.75^{de}	0.396^{ah}	0.9828
	HSH	25	70.78±7.69^{c}	0.249^{de}	0.9758
Pea	HSH	30	176.22±9.32^{a}	0.210^{e}	0.9623
	UH	15	11.52±0.14^{e}	0.338^{bc}	0,9882
	UH	20	25.61±0.47^{de}	0.337^{bc}	0,9858
	UH	25	43.15±0.34^{d}	0.367^{abc}	0,9832
	UH	30	107.43±1.11^{b}	0.312^{cd}	0,9847

* Different letters in the same column represents significant difference ($p \leq 0.05$)

According to statistical analyses, both the type and concentration of legume flour and homogenization type had a significant impact on the appearent viscosity of double emulsions. Regardless of the legume flour type and homogenization method, the apparent viscosity of the double emulsion increased with increasing flour concentration. The appearent viscosity of double emulsions prepared with HSH increased from 0.1 Pa.s^n to 60 Pa.s^n, increase was from 13 Pa.s^n to 117 Pa.s^n for the

ones prepared with US when lentil flour concentration increased from 15% to 30%. In the case of pea flour, the appearent viscosity of double emulsions prepared with both high speed homogenization (HSH) and ultrasonic homogenization (UH) increased with increasing flour concentration significantly after 20% pea flour concentration. While the consistency index value was determined as 19.81 $Pa.s^n$ for double emulsions containing 20% pea flour, it increased to 176.22 $Pa.s^n$ for the ones with 30% pea flour when HSH was used. The increase in consistency index was from 25.61 $Pa.s^n$ to 107.43 $Pa.s^n$ with the increase in pea flour concentration from 20% to 30%, for ultrasonic homogenization (Table 3.2). As the flour amount increased, the amount of protein and starch in the emulsion increased. Although starch is an important thickening agent, studies conducted by Sridharan et al. (2020), showed that starch molecules did not contribute to the viscosity increase since their bulky form prevented their interaction with oil droplets. Therefore, the main determinant factor in viscosity is the protein amount in the emulsion. At higher flour concentrations, more pea protein could interact with the components of emulsions and increase emulsification results in obtaining more homogenous and dense emulsions. Similarly, in the study of Moriano & Alamprese (2020), the increase in apparent viscosity was observed with increasing protein amount for both types of protein, whey protein concentrate and egg white powder. In addition, in the study conducted by Sathe & Salunkhe (1981), it was observed that as of the concentration of bean protein concentrate and bean protein isolate increased, emulsion viscosity increased. Moreover, Rani and Bhattacharya (1989) observed in their study with pasta containing rice flour that apparent viscosity increased as the concentration of rice flour increased. Moreover, Moriano & Alampres, (2020), also observed the significant increase in apparent viscosity of double emulsions with higher concentrations of protein powder.

Ultrasonication has long been used for reduction of viscosity of solutions beside homogenization. The effect of ultrasound on rheological properties was also observed in this study. For the double emulsions prepared with pea flour, ultrasonication did not change appearent viscosity significantly at low pea flour concentrations (15% and 20%), whereas, it caused a significant decrease in viscosity

(about 39%) at high pea flour concentrations (25% and 30%) (Figure 3.14). This is in accordance with the study of Zisu et al. (2013), who observed that the effect of sonication on viscosity decrease was improved with the solutions having higher starting viscosities. On the other hand, our results contradicts with the findings of Hamidi et al. (2014), who suggested that viscous liquids were more resistant to viscosity changes due to having stronger forces. The decrease in appearent viscosity with ultrasonic application is mainly due to ultrasonic cavitation, acoustic wave dissipation and boundary friction and the heat generated during these phenomena. Moreover, the breakdown of intermolecular and intramolecular bonds due to collapse of cavitation bubbles bring on disruption of macromolecules and lower the viscosity (Zisu et al., 2013). Behrend et al. (2000), claims that the effect of this coalescence is stronger for viscous fluids since higher viscosity slows down the separation of droplets and increases contact times which enhance the coalescence when ultrasonication is applied to emulsions.

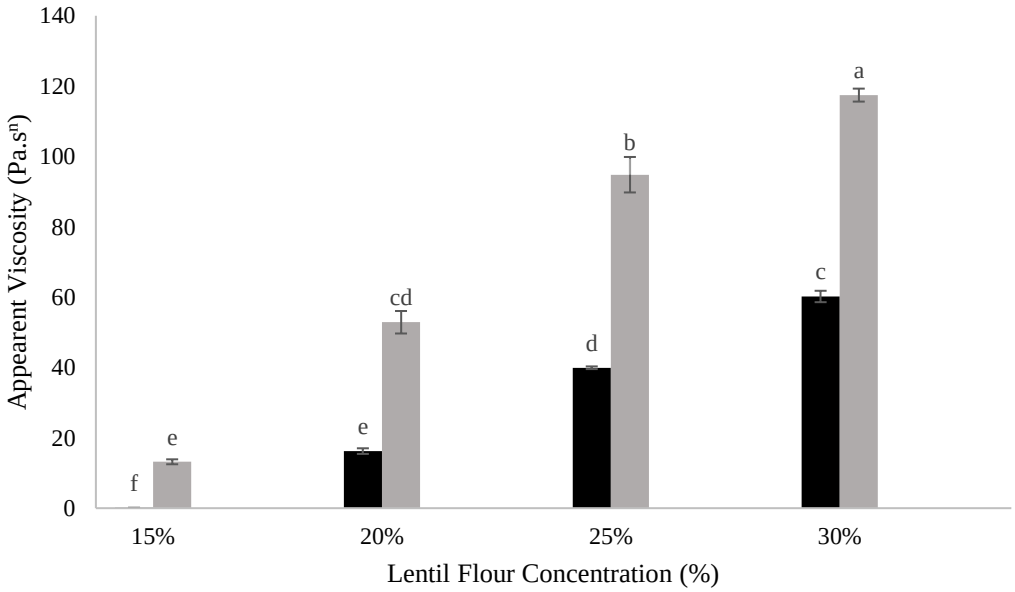

Figure 3.13. Appearent viscosity of double emulsions prepared with different lentil flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■)

On the other hand, incorporation of ultrasonic homogenization into the process increased appearent viscosity of double emulsions prepared with lentil flour (Figure 3.13). Double emulsions prepared by ultrasonic homogenization had higher viscosity than those prepared with high speed homogenization for all lentil flour concentrations. Hongyu et al. (2000), also observed an increase in viscosity after ultrasonic homogenization application in emulsions. In their study with yoghurt, they concluded that ultrasonic homogenization increased the volume fraction of available casein proteins which caused an increase in the viscosity. Similarly, in this study, the high energy input during ultrasonic homogenization might have enabled lentil proteins to release and increased their bonding capacity, which led to an increase in the viscosity of outer aqueous phase, thus the emulsion. Ultrasonic homogenization is a mechanical process used for mixing and homogenization purposes using sound energy. Ultrasonic waves were produced during ultrasonic homogenization and the pressure change created by these waves caused deformation of the particles (Tang et al., 2013).

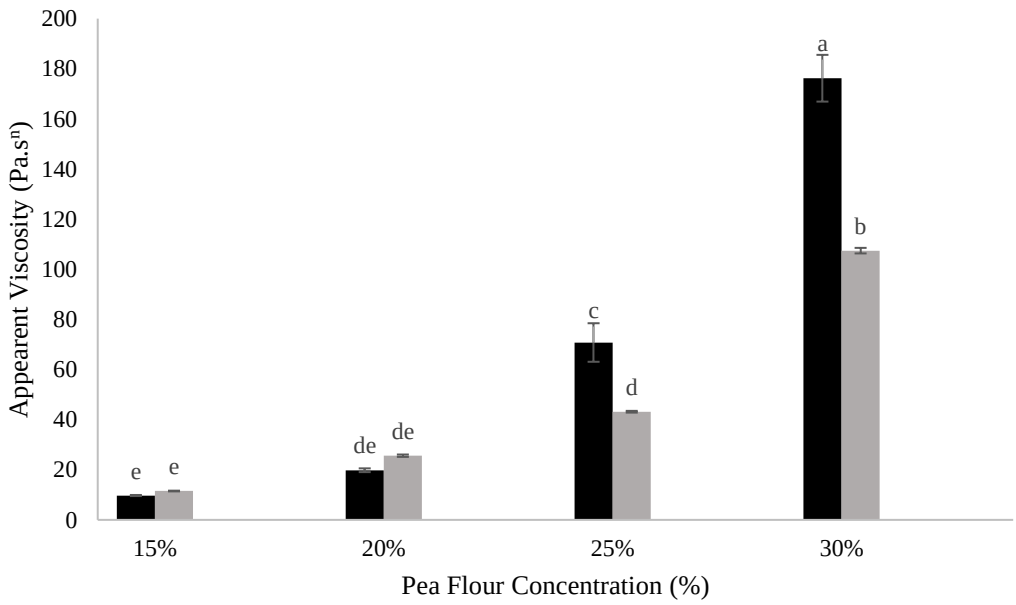

Figure 3.14. Appearent viscosity of double emulsions prepared with different pea flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■)

3.4.3 Instant Stability

Stability, the ability to resist changes, is another important quality parameter since it is directly related to the shelf life of double emulsions. Double emulsions are subjected to macroscopic (creaming, sedimentation) and microscopic (coalescence, flocculation) phase changes that play role in their stability (Fasinu et al., 2015). As separation of phases is the most common problem in the stability of double emulsions and occur faster, it was considered as the limiting factor and measured as the centrifugal separation. The phase separation was quantified by proportioning the volume of non-separated part to total volume centrifuged and demonstrated as percentage stability.

Phase separation involves migration of oil droplets upward and agglomeration at the top of emulsion and the rate of migration determines the stability. According to Stoke's law (Equation 8), the rate of migration is directly proportional to the particle size of droplets and inversely proportional to the viscosity of the media under the force of gravity (Fasinu et al., 2015). This explains the relation of stability with particle size and viscosity of the emulsions.

$$v = \frac{2gr^2(\rho_2 - \rho_1)}{9\mu_1} \qquad \text{Equation (8)}$$

where the v refers to the rate of the isolated rigid spherical droplet particles, r refers the particle radius, ρ2 and ρ1 refer the densities of the particle and the medium, g refers the gravitational acceleration and μ_1 refers the viscosity of the medium.

In this study, stabilities of double emulsions containing lentil flour were correlated with particle diameter and consistency index with correlation coefficient -0.826 (p=0.011) and 0.966 (p=0.000), respectively, for high speed homogenization and -0.987 (p=0.000) and 0.820 (p=0.013), for ultrasonic homogenization. The stabilities of double emulsions containing pea flour were correlated with particle diameter and consistency index with correlation coefficient -0.969 (p=0.000) and 0.923 (p=0.001) for high speed homogenization and -0.956 (p=0.003) and 0.993 (p=0.000), for ultrasonic homogenization.

Table 3.3 Instant stability, particle size and consistency index of double emulsions prepared with different lentil and pea flour concentrations and homogenization methods (HSH: high-speed homogenization, UH: ultrasonic homogenization)

Flour Type	Homogenization Method	Flour (%)	Instant Stability (%)	D[3,2] (µm)	k (Pa.s^n)
Lentil	HSH	15	67.57±0.27^{f}	38.45±0.15^{a}	0.09±0.01^{f}
	HSH	20	67.63±0.09^{f}	28.45±0.15^{c}	16.23±0.80^{e}
	HSH	25	73.22±0.15^{d}	24.75±1.05^{cd}	39.93±0.38^{d}
	HSH	30	75.99±0.05^{c}	21.65±0.95^{de}	60.19±1.62^{c}
	UH	15	68.49±0.09^{e}	32.65±0.75^{b}	13.19±0.70^{e}
	UH	20	99.23±0.10^{b}	18.00±1.20^{ef}	52.86±3.20^{cd}
	UH	25	99.95±0.05^{a}	15.45±0.15^{f}	94.81±5.04^{b}
	UH	30	99.66±0.06^{ab}	15.60±0.10^{f}	117.44±1.84^{a}
Pea	HSH	15	67.79±0.07^{f}	34.05±1.05^{a}	9.71±0.22^{e}
	HSH	20	71.68±0.41^{e}	27.85±1.15^{b}	19.81±0.75^{de}
	HSH	25	75.82±0.27^{d}	22.05±0.65^{c}	70.78±7.69^{c}
	HSH	30	79.71±0.22^{c}	19.55±1.85^{cd}	176.22±9.32^{a}
	UH	15	67.32±0.17^{f}	9.83±0.37^{f}	11.52±0.14^{e}
	UH	20	75.03±0.16^{d}	16.00±0.10^{de}	25.61±0.47^{de}
	UH	25	81.04±0.05^{b}	16.15±0.15^{de}	43.15±0.34^{d}
	UH	30	99.73±0.11^{a}	12.03±0.05^{ef}	107.43±1.11^{b}

* Different letters in the same column represents significant difference ($p \leq 0.05$)

Firstly, particle size had a great effect on emulsion stability. As the particle size of the droplets decreased, the movement rate of the droplets also decreased due to the gravitational forces. Therefore, it was seen that decreasing the particle size of the droplets increased the stability (Table 3.3). There are many studies proving that knowledge (Altuntas et al., 2017; Cofrades et al., 2013; Yildirim et al., 2017). The results obtained from this study are in accordance with the other studies. As the flour

concentration increased, emulsions with smaller particle sizes were obtained which increased the stability of the emulsions. The increase in flour concentration from 15% to 30%, resulted in the increase of instant stability from 67.57% to 75.99% for lentil flour and 67.79% to 79.71% for pea flour containing double emulsions prepared by HSH. High stabilities about 99.66% and 99.73% for lentil and pea flour stabilized emulsions were achieved at 30% legume flour concentration after ultrasonic homogenization. This is compatible with the expectation, since as the legume flour concentration increases, particle size of droplets decreases that results in reduction in migration rate and thus, stability is increased. In addition, as discussed before, the particle size of the double emulsions reduced significantly after ultrasonic homogenization due to the high energy input (Table 3.1). For this reason, the stability of double emulsions prepared by ultrasonic homogenization was found to be higher than those prepared by high speed homogenization for all legume flour concentrations except double emulsions prepared with 15% pea flour, whose stability stayed as the same.

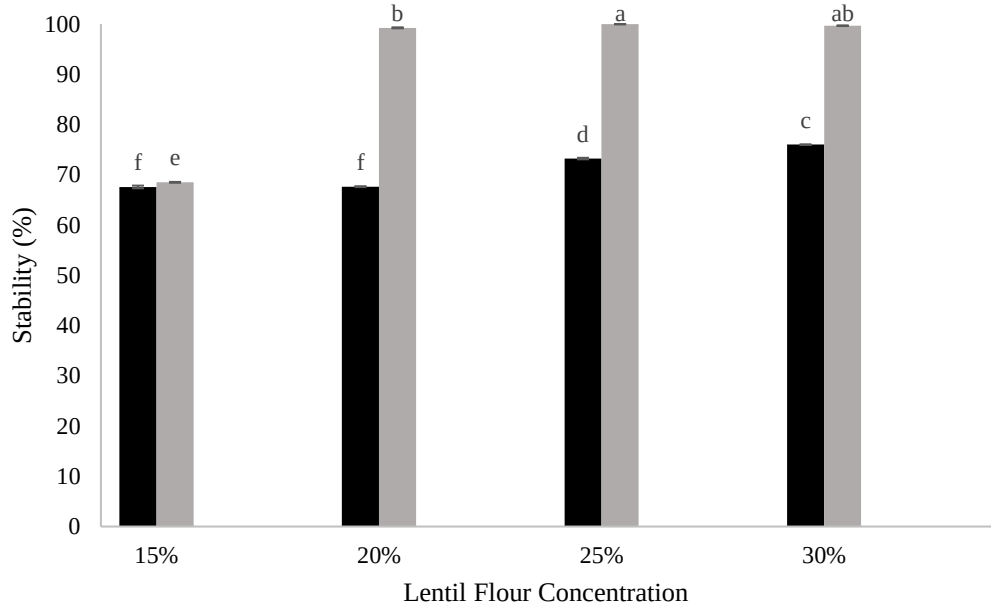

Figure 3.15. Instant stability of double emulsions prepared with different lentil flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■)

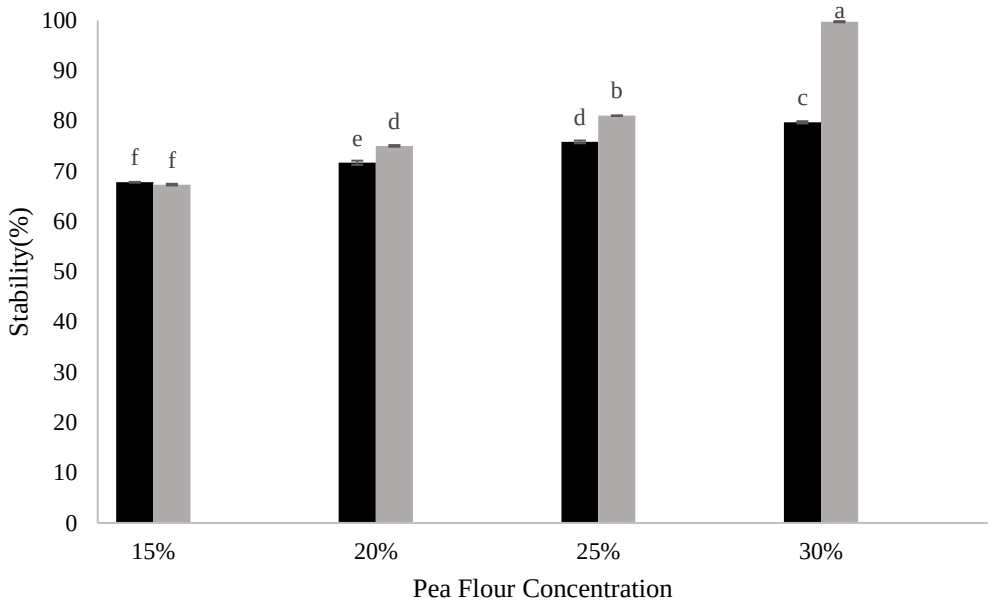

Figure 3.16. Instant stability of double emulsions prepared with different pea flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■)

Secondly, viscosity of outer phase is known to affect the emulsion stability significantly. In double emulsions with more viscous outer phase, the movement of oil droplets is restricted by surrounding viscous solution and the precipitation of the surfactant at the interphase is limited (Huang et al., 2001). In the study conducted by Benichou et al. (2007), more viscous solutions were obtained by changing the ionic charge and this provided an increase in emulsion stability. The increased viscosity led to a better coverage and decreased the release rate of active agent. Similarly, in this study, the emulsions prepared with both lentil and pea flour had higher viscosity at higher legume flour concentrations and this led to higher stability.

For the cases in which viscosity increased while droplet size remained unchanged (pea flour concentration>25% for HSH and pea four concentration>20% for UH), instant stability increased significantly proving the effect of viscosity alone. Higher viscosity decreased the movement ability of droplets and the probability of collisions resulting in greater stability (H. Huang et al., 2021). Moreover, again significant stability increase was observed for the cases in which particle size decreased and

viscosity did not change significantly (pea flour concentration<20% for HSH and pea four concentration<20% for UH). This result supports the knowledge that, the smaller the droplets, the lower the rate of movement due to external forces (Yildirim et al., 2017).

In addition, double emulsions prepared by ultrasonic homogenization had higher stability values than those prepared with high speed homogenization (Figure 3.15-3.16). Especially, the ultra high stabilities (>99%) of double emulsions having lentil flour more and equal to 20% concentration could be explained by the combined effect of their smaller particle sizes and larger viscosities. Canselier et al. (1999), investigated the effects of ultrasonic homogenization and high speed homogenization methods on emulsions and observed that emulsions obtained by ultrasonic homogenization had much lower particle size and higher stability. They stated that the most important reason for the destabilization of emulsions was due to the coalescence of particles and thus phase separation. Although the stabilities of the emulsions produced by ultrasonic homogenization were also higher at the beginning, the difference between the stabilities of the emulsions produced by the two methods increased over time. Much faster phase separation rate was observed in emulsions produced with high speed homogenization. The use of ultrasonic homogenization had been proposed in this study to produce stable emulsions.

3.4.4 Long Term Stability

Long term stability is a measure for endurance of double emulsion against time. Although double emulsions are thermodynamically unstable systems, there are many approaches to produce more stable emulsions. In this study, in order to measure long term stability, samples were placed into tubes and exposed to two different environmental temperatures; 4 ^{o}C and 20 ^{o}C. The long term stability was reported as the percentage of volume of non-separated part to the total volume.

The stability of double emulsions containing lentil and pea flour except the concentration of 15%, did not change during 6 months. Double emulsions with 20%,

25% and 30% flour content remained stable, without any phase separation for 6 months. However, samples prepared with 15% lentil flour were observed to separate starting from the first week at both temperatures. The stabilities of double emulsions containing 15% lentil flour that were exposed to 4 ^{o}C was higher than the ones that were exposed to 20 ^{o}C at the beginning, but the difference decreased and they had almost the same stability value at the end of one month (Figure 3.17). Stabilities of double emulsions exposed to 4 ^{o}C and 20 ^{o}C decreased from 80.772% to 66.375% and 75.564% to 66.139%, respectively. This was because they reached to their maximum phase separation.

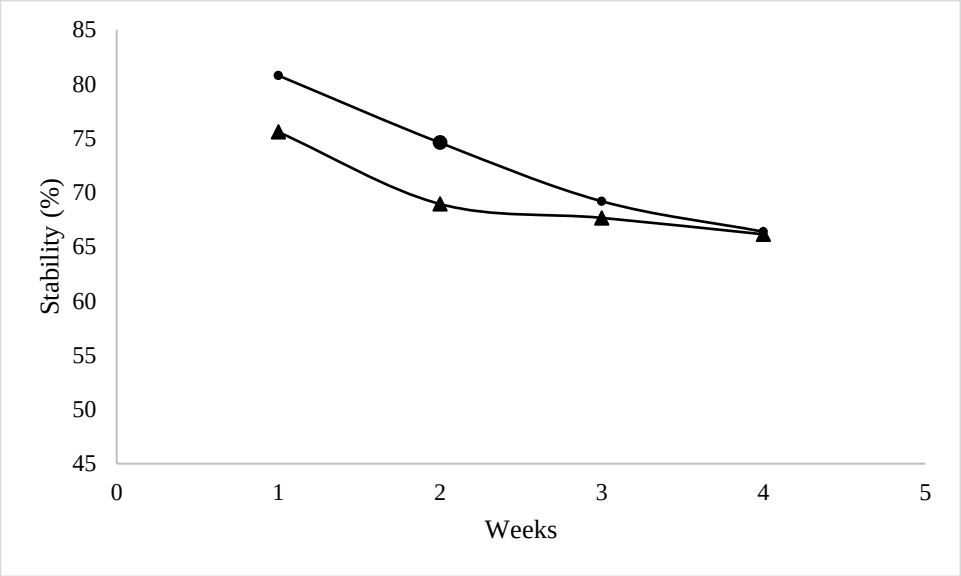

Figure 3.17. Long term stability of double emulsions containing 15% lentil flour at different environments; (●):4 ^{o}C, (▲):20 ^{o}C

Although double emulsions containing 15% pea flour did not exposed to phase separation, they did not remain stable. The oil droplets aggregated and coalescence occurred. The oil droplet clusters were visible but the coalescence was not measured numerically.

The lower stability of double emulsions with lower flour content might be due to their low viscosity and larger particle size. As can be seen in Table 3.2, the average apparent viscosity of the samples containing 15% lentil flour was approximately 200 times lower than the apparent viscosity samples containing 20% lentil flour. Since

the outer phase viscosity of the samples containing lentil flour at the lowest concentration (15%) was very low, the oil droplets could not be wrapped properly and the emulsion was subjected to phase separation due to gravitational force. Moreover, higher particle size diameter of double emulsions at 15% lentil and pea flour concentration could indicate the inefficient droplet covering by proteins to stabilize water-oil interphase of the emulsions. The insufficient surfactant caused formation of emulsions with lower stability.

When the long-term stabilities of double emulsions prepared by ultrasonic homogenization were analyzed, double emulsions with lentil and pea flour showed 100% stability in both environments. No phase separation was observed in the double emulsions analyzed for 6 months. This high stability value was achieved due to the smaller particle size of the double emulsions prepared by ultrasonic homogenization. In the system produced, the viscous outer phase was able to surround the small particles properly, thus preventing the aggregation of oil droplets and keeping the emulsions stable without phase separation.

3.4.5 Optical Imaging

Optical images of double emulsions were observed by inverted light microscope in order to analyze the effects homogenization type on the morphological properties of double emulsions. The inner aqueous phase regions in the oil droplets and the surrounding outer aqueous phase were observed distinctly from the images (Figure 3.18-3.25). The oil droplets in the double emulsion prepared with HSH had spherical shapes. Pea flour stabilized double emulsions had a perfect sphere shape at all pea flour concentrations. This is the result of high emulsifying capacity of pea proteins proving their ability to form stable emulsions. The amphiphilic nature of pea proteins increased their adsorption at the interface and decreased interfacial tension which enhanced the emulsifying properties of pea proteins (Burger & Zhang, 2019).

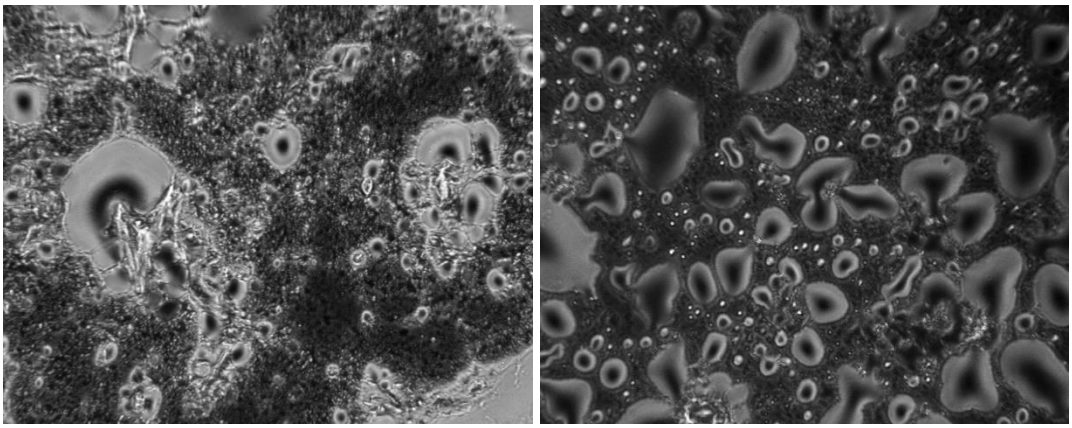

Figure 3.18. Optical images of double emulsion with 15% lentil flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image)

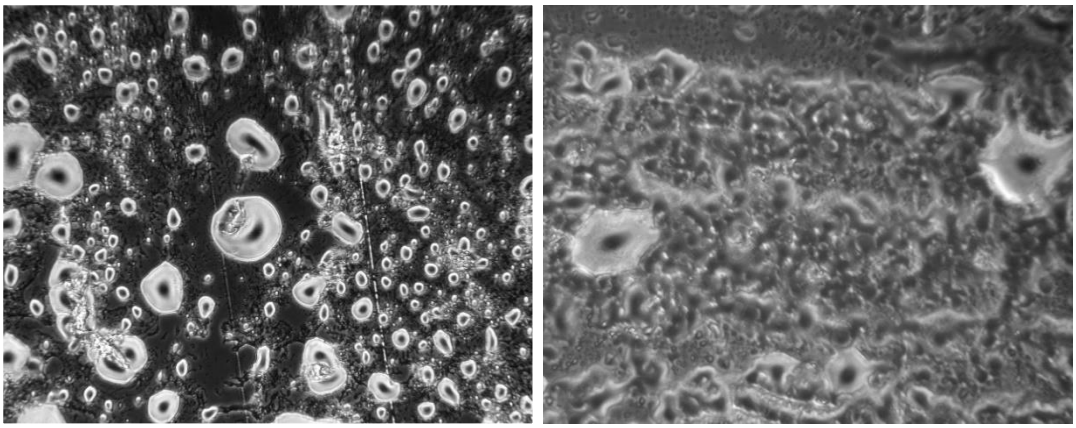

Figure 3.19. Optical images of double emulsion with 20% lentil flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image)

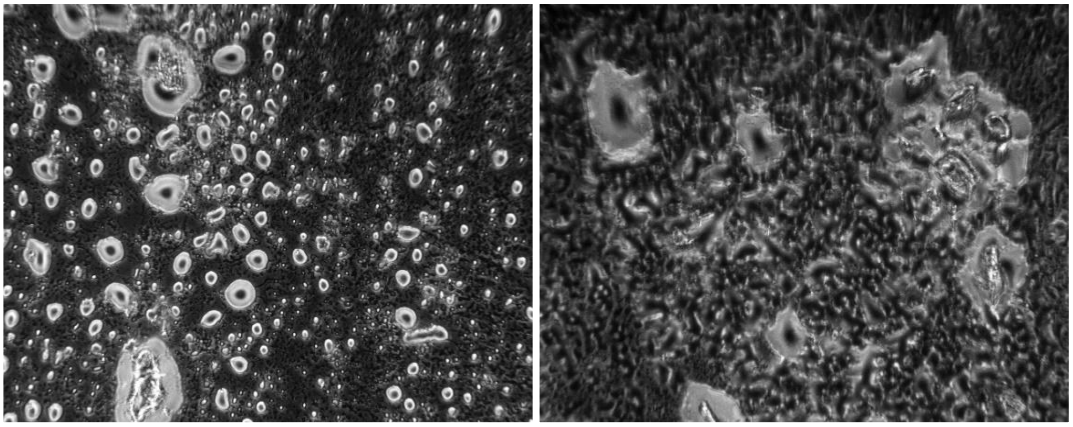

Figure 3.20. Optical images of double emulsion with 25% lentil flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image)

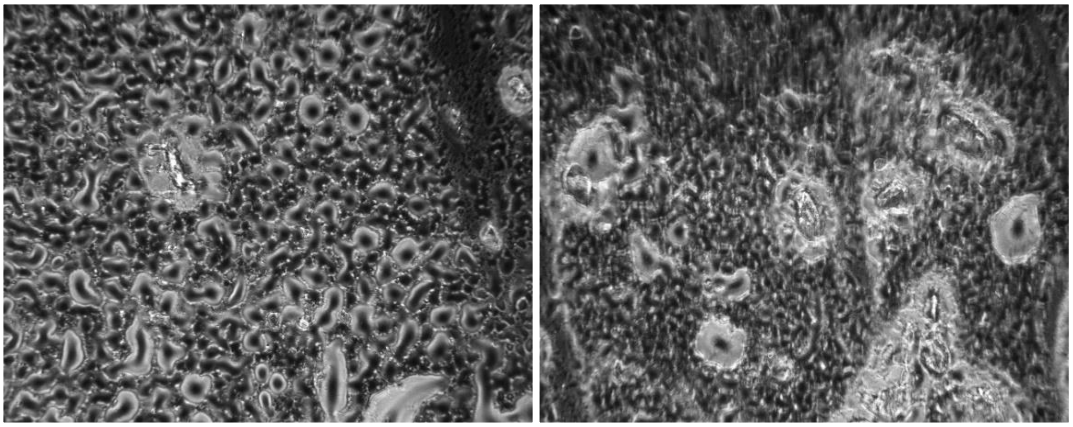

Figure 3.21. Optical images of double emulsion with 30% lentil flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image)

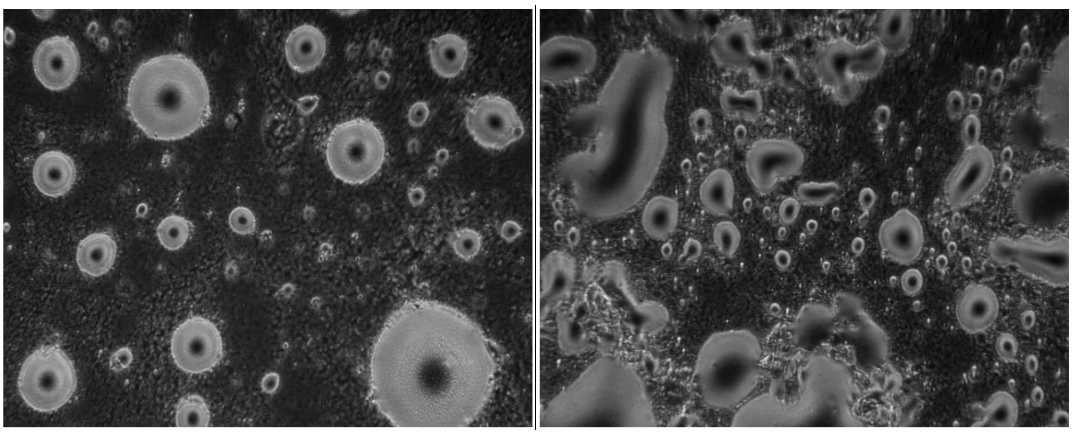

Figure 3.22. Optical images of double emulsion with 15% pea flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image)

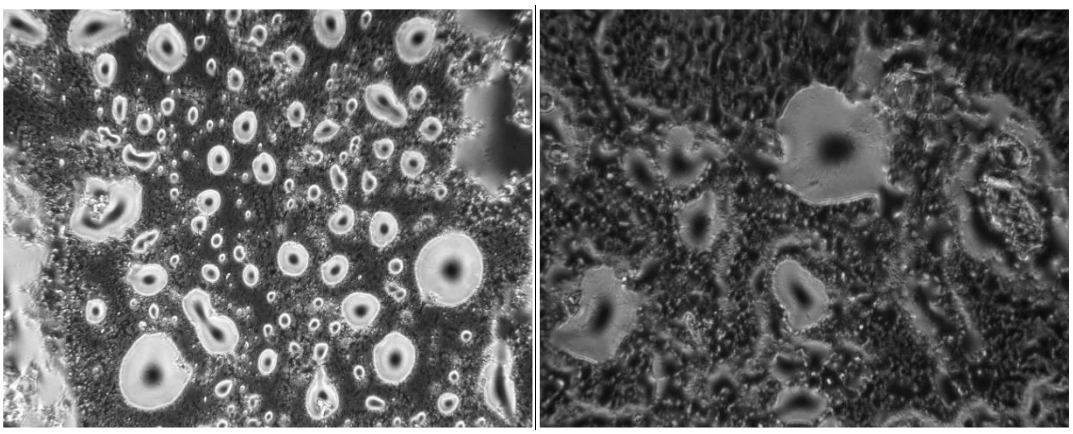

Figure 3.23. Optical images of double emulsion with 20% pea flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image)

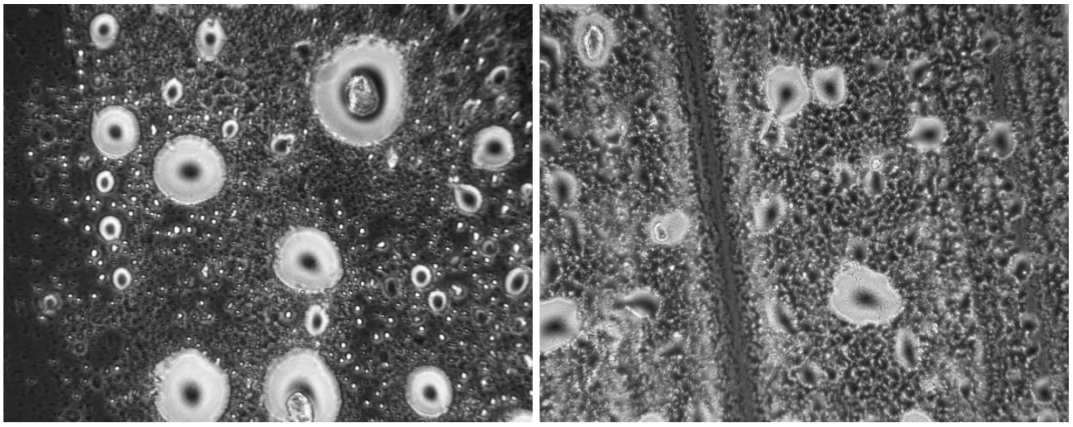

Figure 3.24. Optical images of double emulsion with 25% pea flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image)

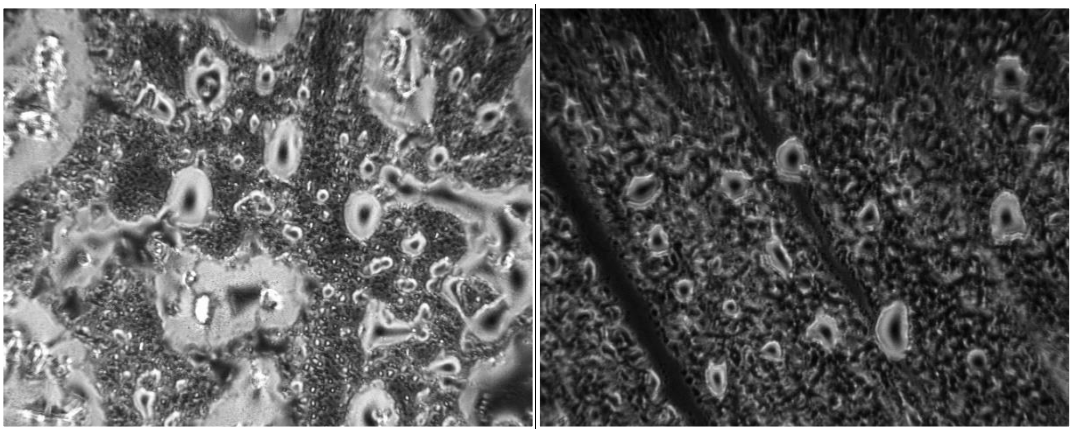

Figure 3.25. Optical images of double emulsion with 30% pea flour prepared with high speed homogenization (left image) and ultrasonic homogenization (right image)

When the images of the double emulsions were analyzed, it was observed that, homogenization method had a great effect on the morphology of double emulsions. The shape of the particles of double emulsions prepared with high speed homogenization were mainly round while the particles of double emulsions prepared with ultrasonic homogenization had irregular shapes. This could be the result of high energy intake during ultrasonic homogenization process (Tang et al., 2013). The hydrodynamic shear and turbulence generated during ultrasound caused disruption of weak forces and decrease in interfacial tension (O'Sullivan et al., 2016), which

resulted in obtaining smaller and non-spherical droplets. The images of the double emulsions showed parallel results of particle size and distribution. The decrease in particle size after ultrasonic homogenization could also be observed from the images.

3.4.6 Encapsulation Efficiency

Encapsulation efficiency of double emulsion is a measure for the percentage of inner phase that is successfully entrapped into the double emulsion system. Although it is one of the most important parameters for quality of double emulsion, it is hard to be measured due to the length and complexity of the analysis methods. To determine the encapsulation efficiency, the amount of internal water phase transferred to the external water phase is analyzed and the percentage of magnesium encapsulated was calculated.

In this study, the efficiencies of double emulsions were calculated by proportioning the amount of magnesium enclosed in the emulsion to the amount of magnesium added into the emulsion during preparation. In order to measure the amount of magnesium trapped in the emulsion, the amount of magnesium transferred to the outer phase of the emulsion was measured and calculations were made considering the total magnesium amount. A magnesium lamp was used to measure the magnesium amount in the outer phase by atomic absorption spectroscopy. In this way, only the concentration of the magnesium was determined without the influence of other minerals.

When the results of atomic absorption spectrometry were analyzed, it could be seen that all the parameters, legume flour type, legume flour concentration and homogenization type significantly affected the release properties of double emulsions. Double emulsions prepared with pea flour displayed higher encapsulation efficiencies than the ones with lentil flour. The average encapsulation efficiencies were calculated as 95.5% and 94.7% for pea flour and lentil flour stabilized double emulsions, respectively. The higher encapsulation efficiency of double emulsions with pea flour could be explained with their higher emulsifying abilities of pea

proteins at extremely basic pH values. The lower surface hydrophobicity of pea protein ensured the high solubility and better migration at the oil-water interface which led to higher encapsulation efficiencies (Burger & Zhang, 2019).

The legume flour concentration had an inverse effect on the encapsulation efficiencies of double emulsions. Higher efficiency values were obtained at lower flour concentrations (Figure 3.26-3.27). This could be explained with their higher droplet sizes. According to (Schuch et al., 2014), encapsulation efficiency was inversely correlated with particle size of emulsions. As particle size decreased, total interface area between oils droplets and outer water phase increased, which led to higher possibility of transfer from inner phase to outer phase, so efficiency decreased. The decrease in the particle size of droplets and the increase in the oil-water interphase could also be observed from the optical images of double emulsions (Figure 3.18-3.25).

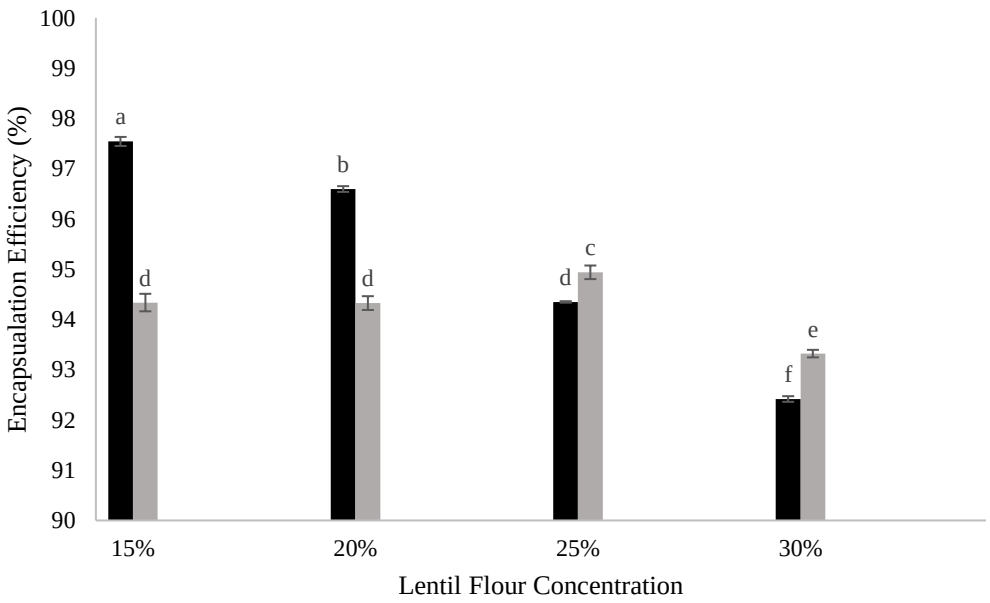

Figure 3.26. Encapsulation efficiency of double emulsions prepared with different lentil flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■)

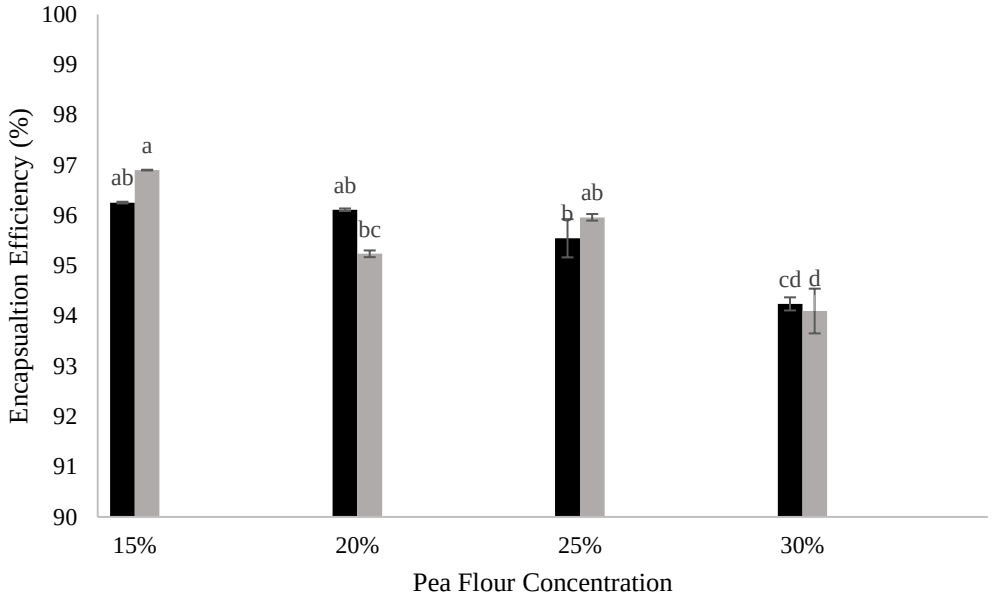

Figure 3.27. Encapsulation efficiency of double emulsions prepared with different pea flour concentrations and homogenization methods; high-speed homogenization: (■) and ultrasonic homogenization: (■)

When the encapsulation efficiencies were analyzed with great extent of scope, it was observed that, the increase in pea flour concentration did not affect encapsulation efficiencies of double emulsions at lower concentrations, whereas a decrease was observed in efficiency when pea flour concentration was increased from 25% to 30% for both homogenization types. It is well known that encapsulation efficiencies of double emulsions are mostly affected by their particle sizes. In contrast to stability, encapsulation efficiency decreases with decreasing particle sizes. Smaller particle sizes present larger interface areas which increase the transfer of valuable material from inner to outer phase (Schuch et al., 2014). On the contrary, encapsulation efficiencies of double emulsions were not observed to be dependent on particle size in our study. Encapsulation efficiencies of double emulsions remained constant with the increasing flour concentration up to 25% pea flour and a significant decrease in encapsulation efficiency was observed at 30% pea flour. Similar findings were reported also in the study of Ying et al. (2021). As Herzi et al. (2014) stated, there are two mechanisms that trigger the release of inner materials, coalescence and

migration. In their study in which they analyzed the effect of inner droplet fraction on release rate of magnesium, coalescence was insignificant and migration was the reason for release. On the other hand, coalescence could also diminish the efficiency of double emulsions as Ying et al. (2021) observed in their study. Considering the non-increasing particle sizes, coalescence of droplets may be neglected, since migration plays bigger role in the release of magnesium in our study. The lowest encapsulation efficiency at the highest pea flour concentration could be explained by the fact that excess low molecular weight hydrophilic surfactant could diffuse into inner phase which endanger to the stability of primary emulsion (Herzi et al., 2014). The damage in the primary emulsion results in the leakage of magnesium and promote the migration to outer phase, causing a lower encapsulation efficiency.

The homogenization method affected encapsulation efficiency of double emulsions prepared with lentil flour significantly. Encapsulation efficiencies of double emulsions prepared with ultrasonic homogenization were lower than those prepared with high speed homogenization (Figure 3.26). This result could be explained with their increased energy levels as well as their lower particle sizes. Khadem & Sheibat-Othman (2019) used ultrasonic homogenization in the outer phase of the double emulsions and examined its effects on emulsion properties. As a result of their studies, it was observed that as the ultrasonication time during the preparation of the external phase increased, the transfer between phases increased and thus the efficiency decreased. The kinetic energy and surface energy gained by the particles at each breakage event caused inner droplets to leak towards the outer droplets. Thus, to achieve higher efficiency, they suggested having larger particles in the outer phase as long as physically stability of emulsions was ensured.

Different from the double emulsions with lentil flour, encapsulation efficiencies of double emulsions with pea flour did not change significantly after ultrasonic homogenization (Figure 3.27). Since the high shear force applied during ultrasonic homogenization causes droplet collision and produces smaller droplets, ultrasonication may increase the possibility of coalescence. Higher coalescence and smaller particle size of droplets can cause the release of inner phase to outer phase

(Leong et al., 2017). On the other hand, in this study the use of ultrasonication together with high speed homogenization and at moderate sonication power prevented negative effects of ultrasonication on efficiency for double emulsions containing pea flour and similar encapsulation efficiencies were achieved.

Table 3.4 Encapsulation efficiency of double emulsions prepared with different lentil and pea flour concentrations and homogenization methods (HSH: high-speed homogenization, UH: ultrasonic homogenization)

Legume Flour Type	**Homogenization Method**	**Legume Flour Concentration (%)**	**Encapsulation Efficiency (%)**
Lentil	HSH	15	97.54 ± 0.09^{a}
	HSH	20	96.59 ± 0.06^{b}
	HSH	25	94.35 ± 0.02^{d}
	HSH	30	92.42 ± 0.06^{f}
	UH	15	94.34 ± 0.17^{d}
	UH	20	94.36 ± 0.14^{d}
	UH	25	94.94 ± 0.14^{c}
	UH	30	93.32 ± 0.08^{e}
Pea	HSH	15	$96.26 \pm 0.02^{ab*}$
	HSH	20	96.11 ± 0.03^{ab}
	HSH	25	95.55 ± 0.38^{b}
	HSH	30	94.24 ± 0.13^{cd}
	UH	15	96.90 ± 0.01^{a}
	UH	20	95.24 ± 0.07^{bc}
	UH	25	95.96 ± 0.07^{ab}
	UH	30	94.09 ± 0.45^{d}

* Different letters in the same column represents significant difference ($p \leq 0.05$)

If we make a general assessment, all the double emulsions regardless of flour type, flour concentration and homogenization type showed quite high encapsulation efficiencies (>92%) (Table 3.4). They had higher encapsulation efficiencies as compared to results of similar studies. Vélez-Erazo et al. (2020) coated polyunsaturated fatty acids with a mixture of maltodextrin and modified starch by ultrasonic homogenization and found that the yields of emulsions varied between 30.23% and 93.53%. In addition, Silva et al. (2020), created double emulsions by ultrasonic homogenization and mixing casein and whey proteins in different proportions. The highest encapsulation efficiency was measured as 71% for emulsions prepared with whey protein only. In the study on encapsulation of oleuropein within double emulsion, encapsulation efficiency of 91% was achieved when optimum conditions were satisfied by arranging surfactant, WPC and pectin content, inner to outer phase ratio and pH (Gharehbeglou et al., 2019). However, in this study, the encapsulation efficiencies of double emulsions prepared using different legume flour concentrations and different homogenization methods varied between 92.42% and 97.54%. The high efficiency results showed that double emulsions prepared using legume flour were highly protective on magnesium. The structure of legume flours played a vital role in obtaining emulsions with high encapsulation efficiencies. Protein-polysaccharide complexes together are known to enhance encapsulation efficiency of double emulsions by surrounding the oil droplets and restraining the release of inner phases (Huang et al., 2021). Thus, the high starch and protein content of lentil and pea flour helped to keep magnesium inside. Moreover, it is known that amphiphilic polymers stabilize emulsions and improve encapsulation efficiency (Meng et al., 2004). Amphiphilic character of proteins in the lentil and pea flours also helped to obtain emulsions with high encapsulation efficiencies.

3.4.7 Nuclear Magnetic Resonance Relaxometry

Nuclear magnetic resonance relaxometry is a fast and convenient analytical technique, which is used in many fields. It has been used for emulsion characterization, specifically to investigate the oil and water proton pools and understand the stability and the formation (Bernewitz et al., 2013; Hughes et al., 2013; Vermeir, Balcaen, Sabatino, Dewettinck, & Van der Meeren, 2014).

When the T_2 relaxation times of the double emulsions were examined, it was seen that the double emulsions had two proton pools, approximately around 32 ms and 182 ms (Table 3.5). The contributions of these pools to the overall signal were determined as 75.5% and 24.5%, respectively. These fractions were compatible with the ratio of water and oil phases of the prepared double emulsions. The double emulsions consisted of 16% internal water phase, 24% oil phase and 60% external water phase. Therefore, the total water percentage in the double emulsion, which was 76%, was consistent with the T_2 measurements (75.5%). The T_2 relaxation time of pure water is about ~2seconds (Hills et al., 1990) and is much higher than the relaxation time of an oil. However, with the addition of flour with high carbohydrate and protein content, the mobility of water protons were restricted and the relaxation times shortened. Relaxation time of water protons that was strongly associated with lentil flour constituents was observed as approximately 32 ms. Then, it can be concluded that, the signal around 180 ms with about 24.5% was related to the oil phase of the double emulsion which was also consistent with ‘oil relaxation’ times observed in previous studies (Lewis et al., 2015).

Table 3.5 Relaxation time and fraction of integrated peak area of double emulsions prepared with lentil and pea flour at different concentrations

Flour Type	Flour Concentration	τ (ms)	B (%)
	15%	46.2	75.2
		194.0	24.8
	20%	28.7	74.8
Lentil Flour		173.3	25.2
	25%	28.0	77.0
		190.0	23.0
	30%	25.3	76.0
		172.5	24.1
	15%	41.2	74.2
		183.3	25.8
	20%	32.2	76.3
Pea Flour		188.3	23.7
	25%	26.0	75.3
		160.0	24.7
	30%	23.5	74.6
		165	25.4

When the signals of double emulsions prepared with lentil flours at different concentrations were examined, as the flour concentration increased, the relaxation time of the water phase decreased. This might be due to the decrease in free water with the increase in the amount of starch and protein.

T_2 analysis of the primary emulsion was also performed to observe signals from the primary emulsion only, without the external water phase. Two peaks were obtained from primary emulsion; at 100 and 1100 ms (Table 3.6). The percent integrated peak areas were 60.8% and 39.2% for signal at 100 and 1100 ms, respectively. The primary emulsion was prepared with 40% water and 60% oil phases and the proportions of the measured peak areas were found to represent these percentages

very well. Since the internal water phase in the primary emulsion consisted of distilled water and magnesium only, the relaxation time of the water was found to be approximately 1,100 ms as expected. The relaxation time of the oil in the primary emulsion was measured around 100 ms, which was less than the relaxation time of the oil phase in the double emulsion. This could be explained by the emulsification of the oil phase with the external water phase. Due to the increase in the interaction of the oil phase with water molecules during formation of the double emulsion, an increase in the relaxation time of the oil phase was observed. In conclusion, ratio of water and oil phases could be observed with NMR results accurately, which is important to show that the content of any prepared emulsion could be analyzed with NMR without separating it into phases. In addition emulsification of the oil could have been determined for the legume flour as confirmed in previous studies (Kirtil & Oztop, 2016; Pocan et al., 2019) for different hydrocolloids.

Table 3.6 Relaxation time and fraction of integrated peak are of primary emulsion

	τ (ms)	**B (%)**
Primary Emulsion	100	60.8
	1100	39.2

Vermeir et al (2014), suggests that, efficiency of a double emulsion can also be characterized by enclosed water volume, that is, fraction of the water volume which form droplets in the oil phase. According to their study, it is possible to calculate enclosed water volume of a double emulsion with the help of a paramagnetic agent, manganese dichloride. When the double emulsion itself is analyzed with NMR, the inner and outer water phases give signals together. However, with the addition of manganese dichloride, the signal of the outer water phase is largely affected and shifted to a lower relaxation time, while the signal of the inner water phase remains almost same. This distinction in signals of inner and outer water phases enables calculation of enclosed water volume of double emulsions. In order to obtain encapsulation efficiency via NMR, similar procedure was applied to double emulsions. After T_2 relaxometry analysis of double emulsions, manganese chloride

is added to the double emulsions to calculate enclosed water volume of double emulsions. It was expected that, after manganese addition, there will be a shift in the relaxation time and a decrease in the fraction of integrated peak area of double emulsions. However, the relaxation time did not shift significantly (Table 3.7). Moreover, while the fraction of integrated area of some double emulsions slightly decrease or remain constant, even an increase was observed in double emulsion prepared with 15% lentil flour.

Table 3.7 Relaxation time and fraction of integrated peak are of double emulsions prepared with lentil and pea flour at different concentrations with addition of 2%, 10 mM $MnCl_2$ addition

Flour Type	**Flour Concentration**	**τ (ms)**	**B (%)**
Lentil Flour	15%	32.3	77.6
		188.6	22.4
	20%	26.4	72.6
		145.7	27.4
	25%	21.1	70.7
		149.5	29.3
	30%	22.3	75.7
		172.5	24.4
Pea Flour	15%	33.7	69.4
		180.0	30.6
	20%	25.0	74.5
		180.0	25.5
	25%	19.8	66.1
		115.0	33.9
	30%	21.3	74.3
		228.8	25.7

In order to observe whether the manganese chloride interacts with the legume proteins to create salting-out effect or not, the experiments were conducted with outer water phase and manganese chloride. Manganese chloride added into the outer water phase at different concentrations (2% and 4%) and as the manganese chloride increased, T_2 value of samples also decreased. This means that manganese chloride did not have salting-out effect on legume proteins and it decreased signal due to its paramagnetic properties.

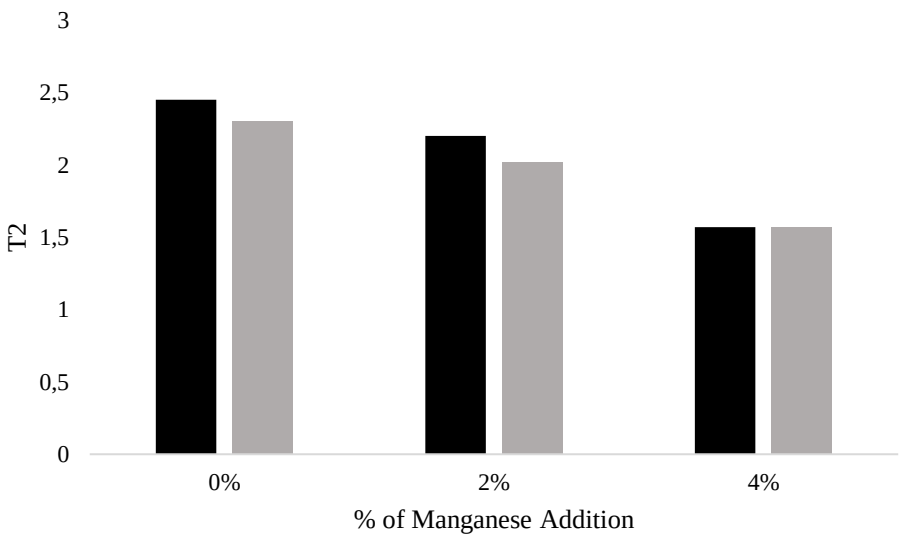

Figure 3.28. T_2 results of outer water phases prepared with lentil (■) and pea (■) flour at 20% concentration with addition of manganese at different concentrations

Since legume flours are complex structures having different macromolecules, they make it difficult to differentiate signals and observe the effect of manganese chloride. Therefore, for the enclosed volume calculations, the signal coming from the outer water phase tried to be completely eliminated upon addition of manganese chloride. It was aimed to determine the amount of manganese chloride that is necessary to eliminate all the signals coming from the outer water phase with the experiments conducted with outer water phase only and then, to add the required amount of manganese into the double emulsion. However, the elimination of signals coming from lentil and pea flours could not be achieved due to the complex structure of flours and their high starch content. These findings demonstrated that, NMR is not a

suitable encapsulation efficiency method for double emulsions prepared with flour as a hydrophilic surfactant.

3.5 Characterization of Magnesium-Loaded Cakes

In order to analyze the effects of addition of encapsulated magnesium on quality of foods, stable double emulsions were chosen and added into cakes. A baked food, cake, was chosen as the model food product to observe the effects of baking (a heat treatment) on stability of encapsulated capsules. Double emulsions prepared with 20%, 25% and 30% lentil flour and by ultrasonic homogenization method were selected for baking analysis, considering their high stability. Double emulsions were added to the cake dough and the quality values of the cakes after baking were evaluated by means of hardness, specific volume, weight loss, color and sensory analysis. For comparison, control cakes, containing magnesium without any coating, and cakes containing magnesium encapsulated within the primary emulsion were also prepared and analyzed.

3.5.1 Hardness

The hardness results obtained from texture analyzer showed that cakes prepared with primary and double emulsions, containing both lentil and pea flour at all flour concentrations, had similar hardness value, whereas the cakes with direct magnesium addition were significantly harder than the others (Table 3.8). Salts are known to cause stronger bonds by increasing protein-protein interactions which results in obtaining tighter dough (Lee et al., 2020). The uncoated magnesium took part in the dough structure and caused an increase in protein-protein interaction leading to a harder texture. Tan et al. (2018), also observed that salt added noodles had harder structure than those without salt. Increased hardness is an undesirable property for cakes, and this result supports the idea that magnesium chloride should be encapsulated since direct addition causes undesirable textural properties.

Table 3.8 Hardness (N) of cakes containing double emulsions prepared with lentil and pea flour

Emulsion Type	Legume Flour Type	Legume Flour Concentration (%)	Hardness (N)
Control (Mg)	-	-	1.65±0.05[a]
Primary Emulsion	-	-	1.12±0.04[b]
Double Emulsion	Lentil	20	1.14±0.07[b]
Double Emulsion	Lentil	25	1.10±0.01[b]
Double Emulsion	Lentil	30	1.07±0.03[b]
Double Emulsion	Pea	20	1.06±0.07[b]
Double Emulsion	Pea	25	1.09±0.04[b]
Double Emulsion	Pea	30	1.04±0.01[b]

* Different letters in the same column represents significant difference ($p \leq 0.05$)

3.5.2 Specific Volume

The specific volumes of both control cakes and cakes containing double emulsion were not statistically different. Moreover, legume flour type did not have any impact on the specific volume of the cakes (Table 3.9). This result showed that the integration of double emulsions into cakes did not significantly change the volume of cakes.

Table 3.9 Specific volume (ml/g) of cakes containing double emulsions prepared with lentil and pea flour

Emulsion Type	Legume Flour Type	Legume Flour Concentration (%)	Specific Volume (ml/g)
Control (Mg)	-	-	1.64±0.01[a]
Primary Emulsion	-	-	1.67±0.01[a]
Double Emulsion	Lentil	20	1.66±0.00[a]
Double Emulsion	Lentil	25	1.66±0.02[a]
Double Emulsion	Lentil	30	1.64±0.00[a]
Double Emulsion	Pea	20	1.67±0.07[a]
Double Emulsion	Pea	25	1.66±0.02[a]
Double Emulsion	Pea	30	1.68±0.00[a]

* Different letters in the same column represents significant difference ($p \leq 0.05$)

3.5.3 Weight Loss

Baking process involves emigration of water vapor from inside to outside during heat transfer and this led to weight loss of products. Weight loss can affect hardness and volume, so, it is one of the important quality parameters for cakes. In this study, weight loss of cakes prepared with double emulsions containing lentil and pea flour at different concentrations did not show a significant difference (Table 3.10).

Moreover, the weight loss of cakes containing with primary emulsions were similar with the cakes containing double emulsions. When we analyze the weight loss of cakes prepared with direct magnesium addition, they exhibited lower weight loss than the ones prepared with primary emulsion. This could be explained by the high affinity of salts with water. Higher non-capsulated salt amount in the cake increased retention of water, thus decreased weight loss. Lynch et al. (2009), also concluded in their studies that the addition of salt could reduce water mobility and therefore the transfer of water from the inner part to the outer part.

Table 3.10 Weight loss (%) of cakes containing double emulsions prepared with lentil and pea flour

Emulsion Type	**Legume Flour Type**	**Legume Flour Concentration (%)**	**Weight Loss (%)**
Control (Mg)	-	-	6.09 ± 0.10^{b}
Primary Emulsion	-	-	6.90 ± 0.10^{a}
Double Emulsion	Lentil	20	6.73 ± 0.03^{ab}
Double Emulsion	Lentil	25	6.68 ± 0.48^{ab}
Double Emulsion	Lentil	30	6.98 ± 0.08^{a}
Double Emulsion	Pea	20	6.42 ± 0.12^{ab}
Double Emulsion	Pea	25	6.60 ± 0.06^{ab}
Double Emulsion	Pea	30	6.49 ± 0.25^{ab}

* Different letters in the same column represents significant difference ($p \leq 0.05$)

3.5.4 Color

The crust color of the cakes prepared with direct magnesium addition, primary emulsion, and double emulsion did not differ from each other significantly (Table 3.11). So, it could be concluded that, the main mechanism that determines the crust color is Maillard browning and the color differences coming from lentil flour in the outer water phase of double emulsions remain insignificant.

Table 3.11 Color difference in crust of cakes containing double emulsions prepared with lentil and pea flour

Emulsion Type	**Legume Flour Type**	**Legume Flour Concentration (%)**	**ΔE***
Control (Mg)	-	-	30.15±0.28[a]
Primary Emulsion	-	-	30.43±0.11[a]
Double Emulsion	Lentil	20	32.39±0.23[a]
Double Emulsion	Lentil	25	33.73±2.03[a]
Double Emulsion	Lentil	30	33.34±0.22[a]
Double Emulsion	Pea	20	30.88±0.25[a]
Double Emulsion	Pea	25	32.00±2.65[a]
Double Emulsion	Pea	30	32.65±0.22[a]

* Different letters in the same column represents significant difference ($p \leq 0.05$)

3.5.5 Sensory Analysis

In order to examine the acceptability of the cakes produced, sensory analysis were made and the cakes were evaluated in terms of taste, hardness and color. The sensory analysis results showed that the taste scores of cakes with direct magnesium addition were significantly lower than the others (Table 3.12). It is known that adding magnesium to foods causes chemical spoilage and protein agglomeration, thus causing an unacceptable change in taste (Anastasova et al., 2020). Sensory analysis results in this study were in accordance with this information. The low acceptability results of cakes prepared with uncoated magnesium proved the idea of encapsulation of magnesium before adding into foods. Moreover, the cakes containing magnesium encapsulated in double emulsion and the cakes prepared without any magnesium addition did not show significant difference. This result showed that, thanks to the high protection supplied from double emulsion, magnesium enriched cake production having similar taste with magnesium free cakes could be achieved. The hardness results of sensory analysis were consistent with the results obtained from texture analyzer (Table 3.8 and Table 3.12). Cakes with direct magnesium addition were found to be harder than others by the panelists. When the colors of the cakes were examined, it was found that the cakes with direct magnesium addition and the cakes encapsulated with double emulsion were both similar to the control cakes in terms of appearance. In addition, both double emulsions with lentil and pea flour resulted in similar taste, hardness and color of cakes according to the panelists, indicating the possible use of both legume flours as a surfactant without quality loss in the food product.

Table 3.12 Sensory analysis of cakes

Cake Content	Taste	Hardness	Color
Magnesium Free	7.57±0.48[a]	7.71±0.67[a]	8.14±0.26[a]
Uncoated Magnesium	2.57±0.37[b]	5.29±0.36[b]	7.88±0.23[a]
Magnesium in Double Emulsion with 20% Lentil Flour	7.71±0.36[a]	7.86±0.18[a]	8.29±0.29[a]
Magnesium in Double Emulsion with 20% Pea Flour	7.57±0.37[a]	7.57±0.37[a]	8.29±0.21[a]

* Different letters in the same column represents significant difference ($p \leq 0.05$)

3.6 Baking Stability and In-Vitro Digestion

3.6.1 Baking Stability

The baking stabilities of the double emulsions in the cakes were evaluated by examining the release of magnesium after heat treatment. Baking stability results were quite promising for usage of double emulsions in baked foods since the huge amount of magnesium remained in the inner phase after baking process. The results showed that the concentration of lentil flour in the outer phase of the double emulsions did not bring out a significant difference in baking stability, while the double emulsion containing 20% pea flour had higher baking stability than higher concentrations of pea flour (Table 3.13). However, it was observed that double emulsions protected magnesium much better than primary emulsions. Double emulsions have an additional layer compared to primary emulsions and this additional layer provides more protection by reducing the release rate of the active substance placed in the inner phase (Garti and Lutz, 2004). In this study, it was observed that double emulsions with different lentil flour concentrations preserved magnesium by 79%-83% and provided almost 12.5% more protection than the

primary emulsion. Similarly, double emulsions with pea flour preserved magnesium at about 86% - 93%, which was about 24% more than the protection of primary emulsions. The double emulsions prepared with 20% pea flour kept about 93% of the magnesium, causing only a very small increase (2.2%) in magnesium release due to heat treatment. The ones with 25% and 30% pea flour showed almost the same baking stability of 86.52%, which was still 17% higher than the protection of primary emulsion. The increase in the protection of magnesium in the internal phase with double emulsion showed the importance of using double emulsion in encapsulation of active agents such as magnesium.

Table 3.13 Baking stability of cakes

Emulsion Type	**Legume Flour Type**	**Legume Flour Concentration (%)**	**Baking Stability (%)**
Control (Mg)	-	-	0.817 ± 0.028^{e}
Primary Emulsion	-	-	71.671 ± 0.943^{d}
Double Emulsion	Lentil	20	82.861 ± 1.203^{bc}
Double Emulsion	Lentil	25	79.931 ± 1.044^{c}
Double Emulsion	Lentil	30	79.065 ± 0.476^{c}
Double Emulsion	Pea	20	93.157 ± 1.158^{a}
Double Emulsion	Pea	25	87.004 ± 0.901^{b}
Double Emulsion	Pea	30	86.033 ± 0.112^{b}

* Different letters in the same column represents significant difference ($p \leq 0.05$)

Moreover, the heat stability of double emulsions were also analyzed without integration into cakes. The heating stabilities of double emulsions prepared with lentil and pea flour did not differ significantly. However, the magnesium release of heated double emulsions was significantly lower than double emulsions in the baked cakes. Therefore, heated double emulsions showed higher encapsulation efficiencies. Baking is a complex process containing mainly gelatinization of starch, denaturation of proteins and evaporation of water. During baking, at temperatures 80-110 ^{o}C, proteins dentaure and unfolded proteins form interactions with other unfolded proteins which cause formation of a new structure (Donovan, 1977). Although double emulsion contained only legume proteins, cakes containing double emulsions had wheat proteins and egg white proteins beside legume proteins. The interactions between these proteins and newly formed bonds could cause more protein agglomeration and distortion of the double emulsion structure in the baked cakes.

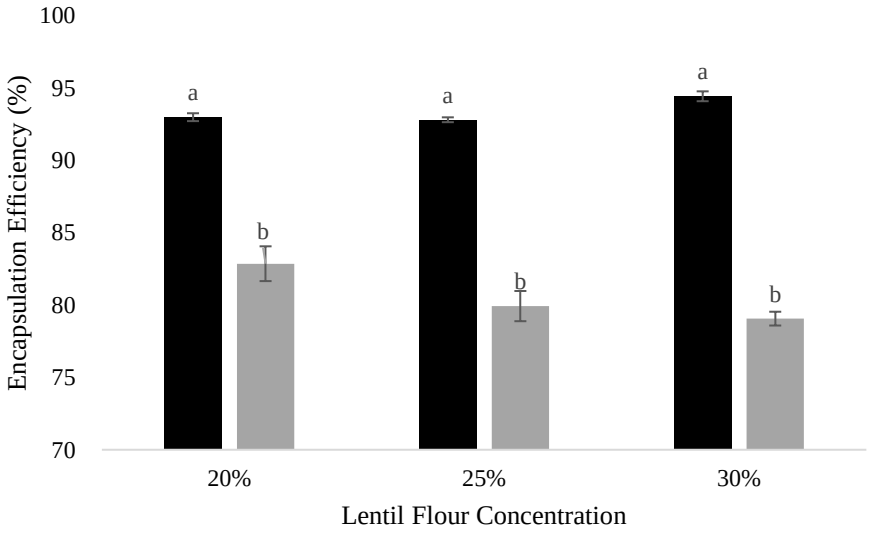

Figure 3.29. Heat stability of double emulsions prepared with lentil flour (■) and cakes containing double emulsions prepared with lentil flour (■)

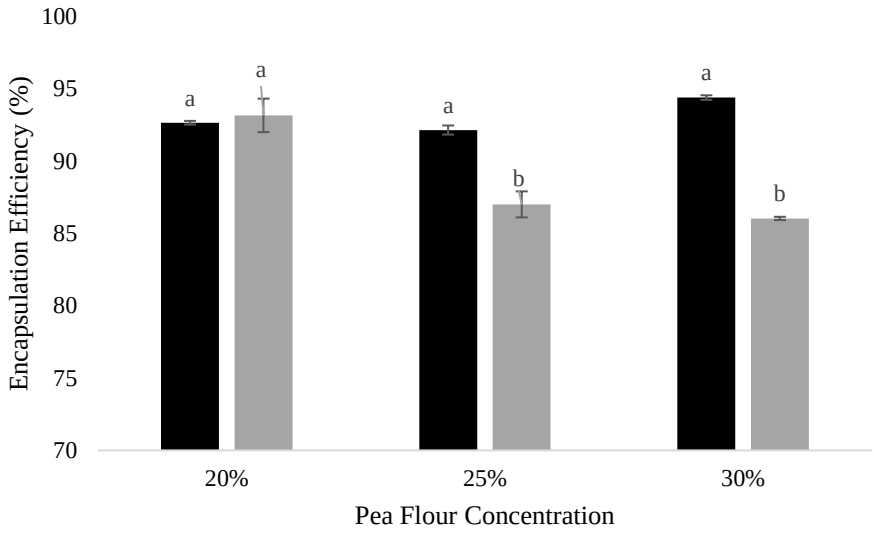

Figure 3.30. Heat stability of double emulsions prepared with pea flour (■) and cakes containing double emulsions prepared with pea flour (■)

Emulsions are known to be directly affected from heat treatments due to their thermodynamically unstable structure. As Sarkar et al. (2016) observed, high temperatures triggers heat induced droplet aggregation causing increase in droplet size. They explained that at about denaturation temperatures of proteins that stabilize emulsions, surface hydrophobicity of droplets increases and the released hydrophobic sites leads to an increase in protein-protein interaction which results droplet aggregation. On the other hand, despite the aggregation, Fechner et al. (2007) achieved to produce heat stable emulsions by mixing dextran as the polysaccharide within sodium caseinate. They demonstrated the importance of the used stabilizing agent to form stable interfacial film and to retard the release of inner material. When a sufficient amount of hydrophobic emulsifier was used in the inner phase, no increase was observed after heat treatment in the release amount of vitamin B12, which is encapsulated in the inner phase for the double emulsions prepared with sodium caseinate and dextran conjugates. (Fechner et al., 2007).

Similarly, the double emulsions stabilized with PGPR and sodium caseinate exhibited quite high resistance to heat which showed magnesium release lower than 6% for all oil types (Bonnet et al., 2009). In our study, the production of heat resistant

double emulsions was carried out by using natural, nutritious and plant based emulsifiers, lentil and pea flour.

3.6.2 In-Vitro Digestion

Within the scope of this study, it was aimed to add magnesium to foods in order to eliminate magnesium deficiency. Magnesium was encapsulated to eliminate the negative effects of magnesium on the quality properties of foods. In order to observe the digestion mechanism of the encapsulated magnesium in the human body, the cakes containing double emulsion were analyzed in simulated in-vitro gastric and intestinal juices and the amount of magnesium released out was measured with an atomic absorption spectrometer.

The analysis of the process of double emulsions through digestive system is important to recognize the changes in microstructure of emulsions during digestion and the release and absorption of magnesium. Studies showed that digestion profiles of emulsions were strongly affected from the emulsifier used in the outer phase (Frank et al., 2012) and the volume of the dispersed phase (Xu et al., 2018). Cakes containing double emulsion exhibited similar release percentages with the cakes containing uncoated magnesium both in simulated gastric and intestinal juices. (Table 3.14-3.15). The similar results of the cakes containing coated (with lentil and pea flour) and uncoated magnesium indicated that the coating material was digested in gastric and intestinal juices and did not prevent magnesium to release and demonstrated the disintegration of double emulsion structure under the effect of pepsin enzyme, primarily. In contrast, Xu et al. (2018) observed that the digestion of protein at the interfaces of double emulsion during gastric digestion caused collapse of double emulsion and release of primary emulsion, keeping the inner material inside until intestinal digestion. On the other hand, Xiao et al. (2017), proved the overwhelming effect of the pepsin digestion than the lipid digestion for the emulsions stabilized by protein. They observed that during gastric digestion, with the protein degradation by pepsin enzyme, the double emulsion droplets formed aggregates and the newly generated large droplets collapsed releasing the inner material. Frank et

al. (2012), explained the agglomeration of droplets of double emulsions stabilized with protein-carbohydrate conjugates in the gastric media with the "bridging flocculation", that was formation of polymer bridges due to polysaccharide chains.

Table 3.14 Release of magnesium in gastric juice when directly added, encapsulated within primary emulsion and double emulsion containing 20% legume flour

Emulsion Type	**Flour Type**	**Release in Gastric Juice (%)**
Control (direct Mg)	-	66.569±0.305[a]
Primary Emulsion	-	63.852±1.704[a]
Double Emulsion	lentil flour	59.222±2.870[a]
Double Emulsion	pea flour	58.122±1.504[a]

* Different letters in the same column represents significant difference ($p \leq 0.05$)

Table 3.15 Release of magnesium in intestinal juice when directly added, encapsulated within primary emulsion and double emulsion containing 20% legume flour

Emulsion Type	**Flour Type**	**Release in Intestinal Juice (%)**
Control (direct Mg)	-	85.052±0.666[ab]
Primary Emulsion	-	85.457±2.058[a]
Double Emulsion	lentil flour	74.500±2.769[b]
Double Emulsion	pea flour	75.781±1.191[ab]

* Different letters in the same column represents significant difference ($p \leq 0.05$)

Flours are structures containing high levels of starch and protein and are affected by heat treatment. During baking, proteins subjected to heat-induced protein breakdown, thermal protein aggregation and cross-linking and Maillard-type reactions. Pasini et al. (2001), examined the digestion of wheat flour proteins in detail in their study. According to the results of their study, they concluded that the protein aggregation that takes place in the interior of the baked product mainly involves SS bond formation and hydrophobic interactions. For this reason, lower degradability of proteins by pepsin enzyme in the crumb resulted from the decrease in the accessibility of the protease to the domains, deriving from protein aggregation. Magnesium digestion in gastric juice was around 60% for the cakes in this study and the fact that all magnesium did not release after pepsin treatment could be the reason of the reduced accessibility of pepsin enzyme.

Moreover, it was also observed that when the crumb samples were treated with pancreatin after pepsin digestion, the breakdown of proteins were nearly as much as the unbaked dough (Pasini et al., 2001). Pepsin and pancreatin are two digestive enzymes leading to protein degradation differently. While pepsin enzyme is an endopeptidase which breaks peptide bonds within protein molecules, pancreatin is a mixture of various proteases both containing endopeptidases and exopeptidases. Hence, pancreatin has an improved effect on protein aggregates. Therefore, the higher breakdown of the double emulsions in the cake within the intestinal fluid compared to the gastric juice could be explained with the effect of the pancreatin enzyme. Frank et al. (2012) suggested that despite the structural changes in gastric digestion, there were still oil droplets keeping the inner phase and the complete digestion was carried out after intestinal incubation. Moreover, Xu et al. (2018), confirmed the collapse of primary emulsions due to hydrolysis of oil under the effect of pancreatin. The high release percentage (about 75%) observed after intestinal digestion, exhibited the possibility of the well-absorption of magnesium through human gastrointestinal track and supported the usage of magnesium enriched cake formulations in order to prevent magnesium deficiency.

CHAPTER 4

CONCLUSION

This study aimed to produce stable and efficient double emulsions using lentil and pea flour as hydrophilic surfactant and to take advantage of them in the encapsulation of magnesium. The effect of legume flour type (lentil and pea flour), legume flour concentration (15%, 20%, 25%, 30%) and homogenization method (high speed homogenization and ultrasonic homogenization) on the emulsion properties and the release of magnesium were investigated. The produced double emulsions were analyzed in terms of particle size and distribution, rheology, instant stability, long term stability, encapsulation efficiency, morphology and nuclear magnetic resonance (NMR) experiments.

Pea flour was found to have better emulsifying properties under the applied conditions (pH=12), with lower particle size, higher stability and higher encapsulation efficiency. It was observed that as the concentration of flour used in the outer phase of the double emulsion increased, the particle size decreased, while the viscosity and stability increased. The double emulsions produced with ultrasonic homogenization were more stable than those produced by high speed homogenization. Emulsions produced with lentil flour had a stability over 99% for flour concentrations of 20% and above, while those produced with pea flour had similar stability at 30% flour concentration after the ultrasonic homogenization. All double emulsions showed magnesium encapsulation efficiency of over 92%. As a result, emulsions containing 20% lentil flour and 30% pea flour and produced by ultrasonic homogenization can be recommended for use in foods due to their high efficiency and stability. The resulting double emulsions were integrated into cake batter and their effect on the quality characteristics of the cake was investigated. It has been found that, coating of magnesium with double emulsion removed the negative effects coming from the direct addition of magnesium. It has been observed

that double emulsions preserved magnesium at a high rate during heat treatment, but they can be disintegrated during digestion, which allowed the absorption of magnesium. Therefore, double emulsions produced with lentil and pea flour can be recommended to be used in the coating of reactive ingredients such as magnesium and in the production of functional foods.

As a future work, double emulsions prepared with legume flours could be analyzed with a method like Fourier transform infrared (FTIR) spectroscopy to monitor the structural changes of proteins and polysaccharides, which provides a deeper understanding for the behavior of double emulsions under the changing conditions. Oxidative stability analysis could be applied to determine the effect of lipid oxidation on the stability of double emulsions. Furthermore, the different active agents such as antioxidants, polyphenols and vitamins could be encapsulated in the double emulsions stabilized with legume flours to produce functional foods. Moreover, future studies could be carried out in model animals to evaluate the in-vivo digestion mechanism.

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APPENDICES

A. Statistical Analaysis

Table 5.1 Two way Anova and Tukey's Comparison Test for stability of double emulsions containing 20% lentil flour with and without NaCl addition into the outer aqueous phase

General Linear Model: Stability versus salt; week

```
Factor  Type   Levels  Values
salt    fixed       2  with salt; without salt
week    fixed       5  0; 1; 2; 3; 4


Analysis of Variance for Stability, using Adjusted SS for Tests

Source     DF   Seq SS   Adj SS   Adj MS       F      P
salt        1  115,375  115,375  115,375  424,79  0,000
week        4   27,130   27,130    6,782   24,97  0,000
salt*week   4    9,908    9,908    2,477    9,12  0,002
Error      10    2,716    2,716    0,272
Total      19  155,128


S = 0,521156   R-Sq = 98,25%   R-Sq(adj) = 96,67%


Grouping Information Using Tukey Method and 95,0% Confidence

salt           N  Mean  Grouping
with salt    10  66,3  A
without salt  10  61,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

week  N  Mean  Grouping
0     4  66,2  A
3     4  63,6    B
1     4  63,4    B
4     4  63,3    B
2     4  63,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

salt          week  N  Mean  Grouping
with salt    0     2  67,2  A
```

Table 5.1 (Continued)

```
with salt    4     2  66,1  A B
with salt    2     2  66,1  A B
with salt    3     2  66,1  A B
with salt    1     2  66,0  A B
without salt  0     2  65,2    B
without salt  3     2  61,0      C
without salt  1     2  60,7      C
without salt  4     2  60,6      C
```

Table 5.2 Three way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil and pea flour concentrations

General Linear Model: Particle Size versus Flour Type

```
Factor      Type   Levels  Values
Flour Type  fixed       2  Lentil; Pea


Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source      DF   Seq SS   Adj SS  Adj MS     F      P
Flour Type   1   174,10   174,10  174,10  2,73  0,109
Error       30  1913,17  1913,17   63,77
Total       31  2087,26


S = 7,98575   R-Sq = 8,34%   R-Sq(adj) = 5,29%
```

General Linear Model: Particle Size versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       4  15; 20; 25; 30


Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source         DF   Seq SS   Adj SS  Adj MS     F      P
Concentration   3   593,13   593,13  197,71  3,71  0,023
Error          28  1494,14  1494,14   53,36
Total          31  2087,26


S = 7,30493   R-Sq = 28,42%   R-Sq(adj) = 20,75%


Unusual Observations for Particle Size

     Particle
Obs      Size      Fit  SE Fit  Residual  St Resid
 25   10,2000  28,7450  2,5827  -18,5450     -2,71 R
 26    9,4600  28,7450  2,5827  -19,2850     -2,82 R

R denotes an observation with a large standardized residual.
```

Table 5.2 (Continued)

General Linear Model: Particle Size versus Process

```
Factor   Type   Levels  Values
Process  fixed       2  HSH; UH


Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source   DF   Seq SS   Adj SS  Adj MS      F      P
Process   1   818,51   818,51  818,51  19,35  0,000
Error    30  1268,76  1268,76   42,29
Total    31  2087,26


S = 6,50322   R-Sq = 39,21%   R-Sq(adj) = 37,19%


Unusual Observations for Particle Size

     Particle
Obs      Size      Fit  SE Fit  Residual  St Resid
  9   33,4000  16,9850  1,6258   16,4150      2,61 R
 10   31,9000  16,9850  1,6258   14,9150      2,37 R

R denotes an observation with a large standardized residual
```

General Linear Model: Particle Size versus Flour; Conc; Process

```
Factor   Type   Levels  Values
Flour    fixed       2  Lentil; Pea
Conc     fixed       4  15; 20; 25; 30
Process  fixed       2  HSH; UH


Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source              DF   Seq SS  Adj SS  Adj MS       F      P
Flour                1   174,10  174,10  174,10  130,36  0,000
Conc                 3   593,13  593,13  197,71  148,04  0,000
Process              1   818,51  818,51  818,51  612,86  0,000
Flour*Conc           3   216,87  216,87   72,29   54,13  0,000
Flour*Process        1    39,25   39,25   39,25   29,39  0,000
Conc*Process         3    86,04   86,04   28,68   21,47  0,000
Flour*Conc*Process   3   138,00  138,00   46,00   34,44  0,000
Error               16    21,37   21,37    1,34
Total               31  2087,26


S = 1,15566   R-Sq = 98,98%   R-Sq(adj) = 98,02%


Unusual Observations for Particle Size

     Particle
Obs      Size      Fit  SE Fit  Residual  St Resid
 23   21,4000  19,5500  0,8172    1,8500      2,26 R
 24   17,7000  19,5500  0,8172   -1,8500     -2,26 R
```

Table 5.2 (Continued)

```
Grouping Information Using Tukey Method and 95,0% Confidence

Flour    N  Mean  Grouping
Lentil  16  24,4  A
Pea     16  19,7    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Conc  N  Mean  Grouping
15    8  28,7  A
20    8  22,6    B
25    8  19,6      C
30    8  17,3        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process   N  Mean  Grouping
HSH      16  27,1  A
UH       16  17,0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Conc  N  Mean  Grouping
Lentil  15    4  35,5  A
Lentil  20    4  23,2    B
Pea     15    4  21,9    B C
Pea     20    4  21,9    B C D
Lentil  25    4  20,1      C D E
Pea     25    4  19,1        D E
Lentil  30    4  18,6          E F
Pea     30    4  15,9            F

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Process  N  Mean  Grouping
Lentil  HSH      8  28,3  A
Pea     HSH      8  25,9    B
Lentil  UH       8  20,4      C
Pea     UH       8  13,5        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Conc  Process  N  Mean  Grouping
15    HSH      4  36,3  A
20    HSH      4  28,1    B
```

Table 5.2 (Continued)

```
25    HSH      4  23,4      C
15    UH       4  21,2      C
30    HSH      4  20,6      C
20    UH       4  17,0        D
25    UH       4  15,8        D E
30    UH       4  13,9          E

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Conc  Process  N  Mean  Grouping
Lentil  15    HSH      2  38,4  A
Pea     15    HSH      2  34,0  A B
Lentil  15    UH       2  32,6    B C
Lentil  20    HSH      2  28,5      C D
Pea     20    HSH      2  27,9        D
Lentil  25    HSH      2  24,8        D E
Pea     25    HSH      2  22,1          E F
Lentil  30    HSH      2  21,6          E F
Pea     30    HSH      2  19,6            F G
Lentil  20    UH       2  18,0            F G
Pea     25    UH       2  16,1              G H
Pea     20    UH       2  16,0              G H
Lentil  30    UH       2  15,6              G H
Lentil  25    UH       2  15,5              G H
Pea     30    UH       2  12,2                H I
Pea     15    UH       2   9,8                  I

Means that do not share a letter are significantly different.
```

Table 5.3 Two way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil flour concentrations

General Linear Model: Particle Size versus Conc; Process

```
Factor   Type   Levels  Values
Conc     fixed       4  15; 20; 25; 30
Process  fixed       2  HSH; UH


Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source        DF  Seq SS  Adj SS  Adj MS       F      P
Conc           3  710,16  710,16  236,72  231,80  0,000
Process        1  249,64  249,64  249,64  244,45  0,000
Conc*Process   3   16,29   16,29    5,43    5,32  0,026
Error          8    8,17    8,17    1,02
Total         15  984,27


S = 1,01057   R-Sq = 99,17%   R-Sq(adj) = 98,44%
```

Table 5.3 (Continued)

```
Grouping Information Using Tukey Method and 95,0% Confidence

Conc  N  Mean  Grouping
15    4  35,5  A
20    4  23,2    B
25    4  20,1      C
30    4  18,6      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process  N  Mean  Grouping
HSH      8  28,3  A
UH       8  20,4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Conc  Process  N  Mean  Grouping
15    HSH      2  38,4  A
15    UH       2  32,6    B
20    HSH      2  28,5      C
25    HSH      2  24,8      C D
30    HSH      2  21,7        D E
20    UH       2  18,0          E F
30    UH       2  15,6            F
25    UH       2  15,5            F

Means that do not share a letter are significantly different.
```

Table 5.4 Two way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% pea flour concentrations

General Linear Model: Particle Size versus Conc; Process

```
Factor   Type   Levels  Values
Conc     fixed       4  15; 20; 25; 30
Process  fixed       2  HSH; UH


Analysis of Variance for Particle Size, using Adjusted SS for Tests

Source        DF  Seq SS  Adj SS  Adj MS       F      P
Conc           3   99,83   99,83   33,28   20,17  0,000
Process        1  608,12  608,12  608,12  368,59  0,000
Conc*Process   3  207,75  207,75   69,25   41,97  0,000
Error          8   13,20   13,20    1,65
Total         15  928,90


S = 1,28446   R-Sq = 98,58%   R-Sq(adj) = 97,34%
```

Table 5.4 (Continued)

```
Unusual Observations for Particle Size

     Particle
Obs      Size      Fit  SE Fit  Residual  St Resid
  7   21,4000  19,5500  0,9083    1,8500      2,04 R
  8   17,7000  19,5500  0,9083   -1,8500     -2,04 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Conc  N  Mean  Grouping
15    4  21,9  A
20    4  21,9  A
25    4  19,1  A
30    4  15,9    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process  N  Mean  Grouping
HSH      8  25,9  A
UH       8  13,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Conc  Process  N  Mean  Grouping
15    HSH      2  34,0  A
20    HSH      2  27,8    B
25    HSH      2  22,1      C
30    HSH      2  19,6      C D
25    UH       2  16,2        D E
20    UH       2  16,0        D E
30    UH       2  12,2          E F
15    UH       2   9,8            F

Means that do not share a letter are significantly different.
```

Table 5.5 Three way ANOVA and Tukey's Comparison Test for consistency index of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil and pea flour concentrations

General Linear Model: Consistency versus Flour

```
Factor  Type   Levels  Values
Flour   fixed       2  Lentil; Pea


Analysis of Variance for Consistency, using Adjusted SS for Tests
```

Table 5.5 (Continued)

```
Source  DF  Seq SS  Adj SS  Adj MS     F      P
Flour    1     604     604     604  0,25  0,618
Error   30   71495   71495    2383
Total   31   72098


S = 48,8176   R-Sq = 0,84%   R-Sq(adj) = 0,00%


Unusual Observations for Consistency

Obs  Consistency     Fit  SE Fit  Residual  St Resid
 23      185,545  58,029  12,204   127,517      2,70 R
 24      166,901  58,029  12,204   108,872      2,30 R

R denotes an observation with a large standardized residual.
```

General Linear Model: Consistency versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       4  15; 20; 25; 30


Analysis of Variance for Consistency, using Adjusted SS for Tests

Source         DF  Seq SS  Adj SS  Adj MS      F      P
Concentration   3   47931   47931   15977  18,51  0,000
Error          28   24167   24167     863
Total          31   72098


S = 29,3787   R-Sq = 66,48%   R-Sq(adj) = 62,89%


Unusual Observations for Consistency

Obs  Consistency      Fit  SE Fit  Residual  St Resid
 23      185,545  109,663  10,387    75,883      2,76 R
 24      166,901  109,663  10,387    57,238      2,08 R

R denotes an observation with a large standardized residual.
```

General Linear Model: Consistency versus Process

```
Factor   Type   Levels  Values
Process  fixed       2  HSH; UH


Analysis of Variance for Consistency, using Adjusted SS for Tests

Source   DF  Seq SS  Adj SS  Adj MS     F      P
Process   1     667     667     667  0,28  0,601
Error    30   71431   71431    2381
Total    31   72098


S = 48,7959   R-Sq = 0,93%   R-Sq(adj) = 0,00%
```

Table 5.5 (Continued)

```
Unusual Observations for Consistency

Obs  Consistency     Fit  SE Fit  Residual  St Resid
 23      185,545  49,120  12,199   136,426      2,89 R
 24      166,901  49,120  12,199   117,781      2,49 R

R denotes an observation with a large standardized residual.
```

General Linear Model: Consistency versus Flour; Concentration; Process

```
Factor         Type   Levels  Values
Flour          fixed       2  Lentil; Pea
Concentration  fixed       4  15; 20; 25; 30
Process        fixed       2  HSH; UH


Analysis of Variance for Consistency, using Adjusted SS for Tests

Source                      DF   Seq SS   Adj SS   Adj MS       F      P
Flour                        1    603,7    603,7    603,7   25,26  0,000
Concentration                3  47931,3  47931,3  15977,1  668,43  0,000
Process                      1    667,0    667,0    667,0   27,90  0,000
Flour*Concentration          3   8928,0   8928,0   2976,0  124,51  0,000
Flour*Process                1   7855,1   7855,1   7855,1  328,63  0,000
Concentration*Process        3   2172,4   2172,4    724,1   30,30  0,000
Flour*Concentration*Process  3   3558,5   3558,5   1186,2   49,63  0,000
Error                       16    382,4    382,4     23,9
Total                       31  72098,3


S = 4,88899   R-Sq = 99,47%   R-Sq(adj) = 98,97%


Unusual Observations for Consistency

Obs  Consistency      Fit  SE Fit  Residual  St Resid
 21       63,086   70,777   3,457    -7,691     -2,22 R
 22       78,469   70,777   3,457     7,691      2,22 R
 23      185,545  176,223   3,457     9,322      2,70 R
 24      166,901  176,223   3,457    -9,322     -2,70 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour    N  Mean  Grouping
Pea     16  58,0  A
Lentil  16  49,3    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N   Mean  Grouping
```

Table 5.5 (Continued)

```
30             8  109,7  A
25             8   67,8    B
20             8   28,6      C
15             8    8,6        D

Means that do not share a letter are significantly different.
Grouping Information Using Tukey Method and 95,0% Confidence

Process   N  Mean  Grouping
UH       16  58,3  A
HSH      16  49,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Concentration  N   Mean  Grouping
Pea     30             4  141,8  A
Lentil  25             4   78,7    B
Lentil  30             4   77,5    B
Pea     25             4   57,0      C
Lentil  20             4   34,5        D
Pea     20             4   22,7        D
Pea     15             4   10,6          E
Lentil  15             4    6,6          E

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Process  N  Mean  Grouping
Lentil  UH       8  69,6  A
Pea     HSH      8  69,1  A
Pea     UH       8  46,9    B
Lentil  HSH      8  29,1      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  Process  N   Mean  Grouping
30             HSH      4  118,2  A
30             UH       4  101,1    B
25             UH       4   80,3      C
25             HSH      4   55,4        D
20             UH       4   39,2          E
20             HSH      4   18,0            F
15             UH       4   12,4            F G
15             HSH      4    4,9              G

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Concentration  Process  N   Mean  Grouping
Pea     30             HSH      2  176,2  A
```

Table 5.5 (Continued)

```
Lentil  25            UH       2  117,4    B
Pea     30            UH       2  107,4    B C
Lentil  30            UH       2   94,8      C
Pea     25            HSH      2   70,8        D
Lentil  30            HSH      2   60,2        D E
Lentil  20            UH       2   52,9        D E F
Pea     25            UH       2   43,1          E F G
Lentil  25            HSH      2   39,9            F G
Pea     20            UH       2   25,6              G H
Pea     20            HSH      2   19,8                H
Lentil  20            HSH      2   16,2                H I
Lentil  15            UH       2   13,2                H I
Pea     15            UH       2   11,5                H I
Pea     15            HSH      2    9,7                H I
Lentil  15            HSH      2    0,1                  I

Means that do not share a letter are significantly different.
```

Table 5.6 Two way ANOVA and Tukey's Comparison Test for consistency index of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil flour concentrations

General Linear Model: Consistency versus Concentration; Process

```
Factor         Type   Levels  Values
Concentration  fixed       4  15; 20; 25; 30
Process        fixed       2  HSH; UH


Analysis of Variance for Consistency, using Adjusted SS for Tests

Source                 DF   Seq SS   Adj SS  Adj MS       F      P
Concentration           3  14783,9  14783,9  4928,0  458,85  0,000
Process                 1   6550,0   6550,0  6550,0  609,88  0,000
Concentration*Process   3   2170,5   2170,5   723,5   67,37  0,000
Error                   8     85,9     85,9    10,7
Total                  15  23590,2


S = 3,27716   R-Sq = 99,64%   R-Sq(adj) = 99,32%


Unusual Observations for Consistency

Obs  Consistency     Fit  SE Fit  Residual  St Resid
 15       99,853  94,809   2,317     5,044      2,18 R
 16       89,765  94,809   2,317    -5,044     -2,18 R
R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
25             4  78,7  A
30             4  77,5  A
```

Table 5.6 (Continued)

```
20             4  34,5    B
15             4   6,6      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process  N  Mean  Grouping
UH       8  69,6  A
HSH      8  29,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  Process  N   Mean  Grouping
25             UH       2  117,4  A
30             UH       2   94,8    B
30             HSH      2   60,2      C
20             UH       2   52,9      C D
25             HSH      2   39,9        D
20             HSH      2   16,2          E
15             UH       2   13,2          E
15             HSH      2    0,1            F

Means that do not share a letter are significantly different.
```

Table 5.7 Two way ANOVA and Tukey's Comparison Test for consistency index of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% pea flour concentrations

General Linear Model: Consistency versus Concentration; Process

```
Factor         Type   Levels  Values
Concentration  fixed       4  15; 20; 25; 30
Process        fixed       2  HSH; UH


Analysis of Variance for Consistency, using Adjusted SS for Tests

Source                 DF   Seq SS   Adj SS   Adj MS       F      P
Concentration           3  42075,4  42075,4  14025,1  378,40  0,000
Process                 1   1972,1   1972,1   1972,1   53,21  0,000
Concentration*Process   3   3560,4   3560,4   1186,8   32,02  0,000
Error                   8    296,5    296,5     37,1
Total                  15  47904,4


S = 6,08809   R-Sq = 99,38%   R-Sq(adj) = 98,84%


Unusual Observations for Consistency
```

Table 5.7 (Continued)

```
Obs  Consistency      Fit  SE Fit  Residual  St Resid
  7      185,545  176,223   4,305     9,322      2,17 R
  8      166,901  176,223   4,305    -9,322     -2,17 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N   Mean  Grouping
30             4  141,8  A
25             4   57,0    B
20             4   22,7      C
15             4   10,6      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process  N  Mean  Grouping
HSH      8  69,1  A
UH       8  46,9    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  Process  N   Mean  Grouping
30             HSH      2  176,2  A
30             UH       2  107,4    B
25             HSH      2   70,8      C
25             UH       2   43,1        D
20             UH       2   25,6        D E
20             HSH      2   19,8        D E
15             UH       2   11,5          E
15             HSH      2    9,7          E

Means that do not share a letter are significantly different.
```

Table 5.8 Three way ANOVA and Tukey's Comparison Test for flow behavior index of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil and pea flour concentrations

General Linear Model: n versus Flour

```
Factor  Type   Levels  Values
Flour   fixed       2  Lentil; Pea


Analysis of Variance for n, using Adjusted SS for Tests

Source  DF    Seq SS    Adj SS    Adj MS     F      P
Flour    1  0,001404  0,001404  0,001404  0,16  0,690
```

Table 5.8 (Continued)

```
Error   30  0,259068  0,259068  0,008636
Total   31  0,260473


S = 0,0929280   R-Sq = 0,54%   R-Sq(adj) = 0,00%


Unusual Observations for n

Obs         n       Fit    SE Fit  Residual  St Resid
  1  0,522900  0,315088  0,023232  0,207813      2,31 R
  2  0,530200  0,315088  0,023232  0,215112      2,39 R

R denotes an observation with a large standardized residual.
```

General Linear Model: n versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       4  15; 20; 25; 30


Analysis of Variance for n, using Adjusted SS for Tests

Source         DF    Seq SS    Adj SS    Adj MS     F      P
Concentration   3  0,097765  0,097765  0,032588  5,61  0,004
Error          28  0,162708  0,162708  0,005811
Total          31  0,260473


S = 0,0762299   R-Sq = 37,53%   R-Sq(adj) = 30,84%


Unusual Observations for n

Obs         n       Fit    SE Fit   Residual  St Resid
 12  0,171400  0,326525  0,026951  -0,155125     -2,18 R

R denotes an observation with a large standardized residual.
```

General Linear Model: n versus Process

```
Factor   Type   Levels  Values
Process  fixed       2  HSH; UH


Analysis of Variance for n, using Adjusted SS for Tests

Source   DF    Seq SS    Adj SS    Adj MS     F      P
Process   1  0,030678  0,030678  0,030678  4,00  0,054
Error    30  0,229795  0,229795  0,007660
Total    31  0,260473


S = 0,0875205   R-Sq = 11,78%   R-Sq(adj) = 8,84%


Unusual Observations for n
```

Table 5.8 (Continued)

Obs	n	Fit	SE Fit	Residual	St Resid
1	0,522900	0,352675	0,021880	0,170225	2,01 R
2	0,530200	0,352675	0,021880	0,177525	2,09 R

R denotes an observation with a large standardized residual.

General Linear Model: n versus Flour; Concentration; Process

Factor	Type	Levels	Values
Flour	fixed	2	Lentil; Pea
Concentration	fixed	4	15; 20; 25; 30
Process	fixed	2	HSH; UH

Analysis of Variance for n, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Flour	1	0,001404	0,001404	0,001404	4,06	0,061
Concentration	3	0,097765	0,097765	0,032588	94,17	0,000
Process	1	0,030678	0,030678	0,030678	88,65	0,000
Flour*Concentration	3	0,027425	0,027425	0,009142	26,42	0,000
Flour*Process	1	0,053956	0,053956	0,053956	155,91	0,000
Concentration*Process	3	0,032145	0,032145	0,010715	30,96	0,000
Flour*Concentration*Process	3	0,011563	0,011563	0,003854	11,14	0,000
Error	16	0,005537	0,005537	0,000346		
Total	31	0,260473				

S = 0,0186029 R-Sq = 97,87% R-Sq(adj) = 95,88%

Grouping Information Using Tukey Method and 95,0% Confidence

Flour	N	Mean	Grouping
Pea	16	0,3	A
Lentil	16	0,3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	N	Mean	Grouping		
15	8	0,4	A		
20	8	0,3		B	
25	8	0,3			C
30	8	0,3			C

Means that do not share a letter are significantly different.

Table 5.8 (Continued)

Grouping Information Using Tukey Method and 95,0% Confidence

Process	N	Mean	Grouping
HSH	16	0,4	A
UH	16	0,3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Flour	Concentration	N	Mean	Grouping		
Lentil	15	4	0,4	A		
Pea	15	4	0,4		B	
Pea	20	4	0,4		B	
Pea	25	4	0,3			C
Lentil	20	4	0,3			C D
Lentil	30	4	0,3			C D
Pea	30	4	0,3			D
Lentil	25	4	0,2			D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Flour	Process	N	Mean	Grouping		
Lentil	HSH	8	0,4	A		
Pea	UH	8	0,3		B	
Pea	HSH	8	0,3		B	
Lentil	UH	8	0,2			C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Concentration	Process	N	Mean	Grouping		
15	HSH	4	0,5	A		
20	HSH	4	0,4		B	
15	UH	4	0,3		B	
25	HSH	4	0,3			C
30	UH	4	0,3			C
20	UH	4	0,3			C
25	UH	4	0,3			C
30	HSH	4	0,3			C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Flour	Concentration	Process	N	Mean	Grouping			
Lentil	15	HSH	2	0,5	A			
Pea	15	HSH	2	0,4		B		
Pea	20	HSH	2	0,4		B	C	
Lentil	20	HSH	2	0,4		B	C	D
Pea	25	UH	2	0,4		B	C	D
Lentil	15	UH	2	0,4		B	C	D
Pea	15	UH	2	0,3			C	D

Table 5.8 (Continued)

```
Pea     20            UH       2   0,3      C D
Lentil  25            HSH      2   0,3      C D
Pea     30            UH       2   0,3        D E
Lentil  30            HSH      2   0,3        D E
Lentil  30            UH       2   0,3          E F
Pea     25            HSH      2   0,2          E F
Pea     30            HSH      2   0,2            F G
Lentil  20            UH       2   0,2            F G
Lentil  25            UH       2   0,2              G

Means that do not share a letter are significantly different.
```

Table 5.9 Two way ANOVA and Tukey's Comparison Test for flow behavior index of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% pea flour concentrations

General Linear Model: n versus Concentration; Process

```
Factor         Type   Levels  Values
Concentration  fixed       4  15; 20; 25; 30
Process        fixed       2  HSH; UH


Analysis of Variance for n, using Adjusted SS for Tests

Source                 DF    Seq SS    Adj SS    Adj MS       F      P
Concentration           3  0,089928  0,089928  0,029976   78,45  0,000
Process                 1  0,083002  0,083002  0,083002  217,22  0,000
Concentration*Process   3  0,011286  0,011286  0,003762    9,85  0,005
Error                   8  0,003057  0,003057  0,000382
Total                  15  0,187272


S = 0,0195474   R-Sq = 98,37%   R-Sq(adj) = 96,94%


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
15             4   0,4  A
20             4   0,3    B
30             4   0,3    B
25             4   0,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process  N  Mean  Grouping
HSH      8   0,4  A
UH       8   0,2    B

Means that do not share a letter are significantly different.
```

Table 5.9 (Continued)

```
Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  Process  N  Mean  Grouping
15             HSH      2   0,5  A
20             HSH      2   0,4    B
15             UH       2   0,4    B
25             HSH      2   0,3    B
30             HSH      2   0,3    B C
30             UH       2   0,3      C D
20             UH       2   0,2        D E
25             UH       2   0,2          E

Means that do not share a letter are significantly different.
```

Table 5.10 Two way ANOVA and Tukey's Comparison Test for flow behavior index of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% pea flour concentrations

General Linear Model: n versus Concentration; Process

```
Factor         Type   Levels  Values
Concentration  fixed       4  15; 20; 25; 30
Process        fixed       2  HSH; UH


Analysis of Variance for n, using Adjusted SS for Tests

Source                 DF     Seq SS     Adj SS     Adj MS      F      P
Concentration           3  0,0352619  0,0352619  0,0117540  37,91  0,000
Process                 1  0,0016322  0,0016322  0,0016322   5,26  0,051
Concentration*Process   3  0,0324221  0,0324221  0,0108074  34,86  0,000
Error                   8  0,0024803  0,0024803  0,0003100
Total                  15  0,0717965


S = 0,0176079   R-Sq = 96,55%   R-Sq(adj) = 93,52%


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
15             4   0,4  A
20             4   0,4  A
25             4   0,3    B
30             4   0,3      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process  N  Mean  Grouping
UH       8   0,3  A
HSH      8   0,3  A
```

Table 5.10 (Continued)

```
Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  Process  N  Mean  Grouping
15             HSH      2   0,4  A
20             HSH      2   0,4  A B
25             UH       2   0,4  A B C
15             UH       2   0,3    B C
20             UH       2   0,3    B C
30             UH       2   0,3      C D
25             HSH      2   0,2        D E
30             HSH      2   0,2          E

Means that do not share a letter are significantly different.
```

Table 5.11 Three way ANOVA and Tukey's Comparison Test for instant stability of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil and pea flour concentrations

General Linear Model: IS versus Flour

```
Factor  Type   Levels  Values
Flour   fixed       2  Lentil; Pea


Analysis of Variance for IS, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS     F      P
Flour    1   141,4   141,4   141,4  0,89  0,354
Error   30  4783,2  4783,2   159,4
Total   31  4924,6


S = 12,6269   R-Sq = 2,87%   R-Sq(adj) = 0,00%
```

General Linear Model: IS versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       4  15; 20; 25; 30


Analysis of Variance for IS, using Adjusted SS for Tests

Source         DF  Seq SS  Adj SS  Adj MS     F      P
Concentration   3  1866,0  1866,0   622,0  5,69  0,004
Error          28  3058,6  3058,6   109,2
Total          31  4924,6


S = 10,4515   R-Sq = 37,89%   R-Sq(adj) = 31,24%
```

Table 5.11 (Continued)

```
Unusual Observations for IS

Obs      IS     Fit  SE Fit  Residual  St Resid
 11  99,134  78,390   3,695    20,744      2,12 R
 12  99,328  78,390   3,695    20,938      2,14 R

R denotes an observation with a large standardized residual.
```

General Linear Model: IS versus Process

```
Factor   Type   Levels  Values
Process  fixed       2  HSH; UH


Analysis of Variance for IS, using Adjusted SS for Tests

Source   DF  Seq SS  Adj SS  Adj MS      F      P
Process   1  1541,0  1541,0  1541,0  13,66  0,001
Error    30  3383,6  3383,6   112,8
Total    31  4924,6


S = 10,6201   R-Sq = 31,29%   R-Sq(adj) = 29,00%
```

General Linear Model: IS versus Flour; Concentration; Process

```
Factor         Type   Levels  Values
Flour          fixed       2  Lentil; Pea
Concentration  fixed       4  15; 20; 25; 30
Process        fixed       2  HSH; UH


Analysis of Variance for IS, using Adjusted SS for Tests

Source                       DF   Seq SS   Adj SS   Adj MS         F
P
Flour                         1   141,39   141,39   141,39   2294,29
0,000
Concentration                 3  1866,02  1866,02   622,01  10093,23
0,000
Process                       1  1540,98  1540,98  1540,98  25005,24
0,000
Flour*Concentration           3   202,52   202,52    67,51   1095,41
0,000
Flour*Process                 1   375,47   375,47   375,47   6092,72
0,000
Concentration*Process         3   534,55   534,55   178,18   2891,33
0,000
Flour*Concentration*Process   3   262,67   262,67    87,56   1420,76
0,000
Error                        16     0,99     0,99     0,06
Total                        31  4924,57


S = 0,248246   R-Sq = 99,98%   R-Sq(adj) = 99,96%
```

Table 5.11 (Continued)

```
Unusual Observations for IS

Obs      IS     Fit  SE Fit  Residual  St Resid
 19  72,089  71,675   0,176     0,414      2,36 R
 20  71,261  71,675   0,176    -0,414     -2,36 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour    N  Mean  Grouping
Lentil  16  81,5  A
Pea     16  77,3    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
30             8  88,8  A
25             8  82,5    B
20             8  78,4      C
15             8  67,8        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process   N  Mean  Grouping
UH       16  86,3  A
HSH      16  72,4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Concentration  N  Mean  Grouping
Pea     30             4  89,7  A
Lentil  30             4  87,8    B
Lentil  25             4  86,6      C
Lentil  20             4  83,4        D
Pea     25             4  78,4          E
Pea     20             4  73,4            F
Lentil  15             4  68,0              G
Pea     15             4  67,6              G

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Process  N  Mean  Grouping
Lentil  UH       8  91,8  A
Pea     UH       8  80,8    B
Pea     HSH      8  73,7      C
Lentil  HSH      8  71,1        D
```

Table 5.11 (Continued)

```
Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  Process  N  Mean  Grouping
30             UH       4  99,7  A
25             UH       4  90,5    B
20             UH       4  87,1      C
30             HSH      4  77,9        D
25             HSH      4  74,5          E
20             HSH      4  69,7            F
15             UH       4  67,9              G
15             HSH      4  67,7              G

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Concentration  Process  N   Mean  Grouping
Lentil  25             UH       2  100,0  A
Pea     30             UH       2   99,7  A
Lentil  30             UH       2   99,7  A
Lentil  20             UH       2   99,2  A
Pea     25             UH       2   81,0    B
Pea     30             HSH      2   79,7      C
Lentil  30             HSH      2   76,0        D
Pea     25             HSH      2   75,8        D
Pea     20             UH       2   75,0        D
Lentil  25             HSH      2   73,2          E
Pea     20             HSH      2   71,7            F
Lentil  15             UH       2   68,5              G
Pea     15             HSH      2   67,8              G H
Lentil  20             HSH      2   67,6              G H
Lentil  15             HSH      2   67,6              G H
Pea     15             UH       2   67,3                H

Means that do not share a letter are significantly different.
```

Table 5.12 Two way ANOVA and Tukey's Comparison Test for instant stability of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil flour concentrations

General Linear Model: IS versus Concentration; Process

```
Factor         Type   Levels  Values
Concentration  fixed       4  15; 20; 25; 30
Process        fixed       2  HSH; UH


Analysis of Variance for IS, using Adjusted SS for Tests

Source                 DF   Seq SS   Adj SS   Adj MS         F      P
Concentration           3  1003,98  1003,98   334,66  10592,24  0,000
```

Table 5.12 (Continued)

```
Process                 1  1718,88  1718,88  1718,88  54403,89  0,000
Concentration*Process   3   555,51   555,51   185,17   5860,84  0,000
Error                   8     0,25     0,25     0,03
Total                  15  3278,62


S = 0,177749   R-Sq = 99,99%   R-Sq(adj) = 99,99%


Unusual Observations for IS

Obs      IS     Fit  SE Fit  Residual  St Resid
  1  67,309  67,574   0,126    -0,265     -2,11 R
  2  67,838  67,574   0,126     0,265      2,11 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
30             4  87,8  A
25             4  86,6    B
20             4  83,4      C
15             4  68,0        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process  N  Mean  Grouping
UH       8  91,8  A
HSH      8  71,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  Process  N   Mean  Grouping
25             UH       2  100,0  A
30             UH       2   99,7  A B
20             UH       2   99,2    B
30             HSH      2   76,0      C
25             HSH      2   73,2        D
15             UH       2   68,5          E
20             HSH      2   67,6            F
15             HSH      2   67,6            F

Means that do not share a letter are significantly different.
```

Table 5.13 Two way ANOVA and Tukey's Comparison Test for instant stability of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% pea flour concentrations

General Linear Model: IS versus Concentration; Process

```
Factor         Type   Levels  Values
Concentration  fixed       4  15; 20; 25; 30
Process        fixed       2  HSH; UH


Analysis of Variance for IS, using Adjusted SS for Tests

Source                 DF   Seq SS   Adj SS  Adj MS        F      P
Concentration           3  1064,56  1064,56  354,85  3871,52  0,000
Process                 1   197,57   197,57  197,57  2155,54  0,000
Concentration*Process   3   241,70   241,70   80,57   878,99  0,000
Error                   8     0,73     0,73    0,09
Total                  15  1504,57


S = 0,302750   R-Sq = 99,95%   R-Sq(adj) = 99,91%


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
30             4  89,7  A
25             4  78,4    B
20             4  73,4      C
15             4  67,6        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process  N  Mean  Grouping
UH       8  80,8  A
HSH      8  73,7    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  Process  N  Mean  Grouping
30             UH       2  99,7  A
25             UH       2  81,0    B
30             HSH      2  79,7      C
25             HSH      2  75,8        D
20             UH       2  75,0        D
20             HSH      2  71,7          E
15             HSH      2  67,8            F
15             UH       2  67,3            F

Means that do not share a letter are significantly different.
```

Table 5.14 Three way ANOVA and Tukey's Comparison Test for encapsulation efficiency of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil and pea flour concentrations

General Linear Model: Efficiency versus Flour

```
Factor  Type   Levels  Values
Flour   fixed       2  Lentil; Pea


Analysis of Variance for Efficiency, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS     F      P
Flour    1   7,998   7,998   7,998  4,56  0,038
Error   46  80,642  80,642   1,753
Total   47  88,640


S = 1,32404   R-Sq = 9,02%   R-Sq(adj) = 7,05%


Unusual Observations for Efficiency

Obs  Efficiency      Fit  SE Fit  Residual  St Resid
  1     97,4094  94,7273  0,2703    2,6821      2,07 R
  2     97,7134  94,7273  0,2703    2,9861      2,30 R
  3     97,5011  94,7273  0,2703    2,7737      2,14 R

R denotes an observation with a large standardized residual.
```

General Linear Model: Efficiency versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       4  15; 20; 25; 30


Analysis of Variance for Efficiency, using Adjusted SS for Tests

Source         DF  Seq SS  Adj SS  Adj MS      F      P
Concentration   3  48,802  48,802  16,267  17,97  0,000
Error          44  39,838  39,838   0,905
Total          47  88,640


S = 0,951533   R-Sq = 55,06%   R-Sq(adj) = 51,99%


Unusual Observations for Efficiency

Obs  Efficiency      Fit  SE Fit  Residual  St Resid
 13     94,4151  96,2583  0,2747   -1,8433     -2,02 R
 14     94,0023  96,2583  0,2747   -2,2561     -2,48 R

R denotes an observation with a large standardized residual.
```

Table 5.14 (Continued)

General Linear Model: Efficiency versus Process

```
Factor   Type   Levels  Values
Process  fixed       2  HSH; UH


Analysis of Variance for Efficiency, using Adjusted SS for Tests

Source   DF  Seq SS  Adj SS  Adj MS     F      P
Process   1   2,907   2,907   2,907  1,56  0,218
Error    46  85,733  85,733   1,864
Total    47  88,640


S = 1,36519   R-Sq = 3,28%   R-Sq(adj) = 1,18%


Unusual Observations for Efficiency

Obs  Efficiency      Fit  SE Fit  Residual  St Resid
 10     92,3972  95,3816  0,2787   -2,9844     -2,23 R
 11     92,5221  95,3816  0,2787   -2,8596     -2,14 R
 12     92,3305  95,3816  0,2787   -3,0511     -2,28 R

R denotes an observation with a large standardized residual.
```

General Linear Model: Efficiency versus Flour; Concentration; Process

```
Factor         Type   Levels  Values
Flour          fixed       2  Lentil; Pea
Concentration  fixed       4  15; 20; 25; 30
Process        fixed       2  HSH; UH


Analysis of Variance for Efficiency, using Adjusted SS for Tests

Source                      DF   Seq SS   Adj SS   Adj MS       F      P
Flour                        1   7,9979   7,9979   7,9979   93,31  0,000
Concentration                3  48,8015  48,8015  16,2672  189,79  0,000
Process                      1   2,9073   2,9073   2,9073   33,92  0,000
Flour*Concentration          3   2,1394   2,1394   0,7131    8,32  0,000
Flour*Process                1   3,0404   3,0404   3,0404   35,47  0,000
Concentration*Process        3  10,6268  10,6268   3,5423   41,33  0,000
Flour*Concentration*Process  3  10,3838  10,3838   3,4613   40,38  0,000
Error                       32   2,7427   2,7427   0,0857
Total                       47  88,6398


S = 0,292761   R-Sq = 96,91%   R-Sq(adj) = 95,46%


Unusual Observations for Efficiency

Obs  Efficiency      Fit  SE Fit  Residual  St Resid
 31     94,7845  95,5450  0,1690   -0,7605     -3,18 R
```

Table 5.14 (Continued)

```
 46     94,6495  94,0974  0,1690    0,5520      2,31 R
 47     93,2170  94,0974  0,1690   -0,8804     -3,68 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour    N  Mean  Grouping
Pea     24  95,5  A
Lentil  24  94,7    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration   N  Mean  Grouping
15             12  96,3  A
20             12  95,6    B
25             12  95,2      C
30             12  93,5        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process   N  Mean  Grouping
HSH      24  95,4  A
UH       24  94,9    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Concentration  N  Mean  Grouping
Pea     15             6  96,6  A
Lentil  15             6  95,9    B
Pea     25             6  95,8    B
Pea     20             6  95,7    B
Lentil  20             6  95,5    B
Lentil  25             6  94,6      C
Pea     30             6  94,2      C
Lentil  30             6  92,9        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Process   N  Mean  Grouping
Pea     UH       12  95,5  A
Pea     HSH      12  95,5  A B
Lentil  HSH      12  95,2    B
Lentil  UH       12  94,2      C

Means that do not share a letter are significantly different.
```

Table 5.14 (Continued)

```
Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  Process  N  Mean  Grouping
15             HSH      6  96,9  A
20             HSH      6  96,4  A
15             UH       6  95,6    B
25             UH       6  95,4    B C
25             HSH      6  94,9      C D
20             UH       6  94,8        D
30             UH       6  93,7          E
30             HSH      6  93,3          E

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Concentration  Process  N  Mean  Grouping
Lentil  15             HSH      3  97,5  A
Pea     15             UH       3  96,9  A B
Lentil  20             HSH      3  96,6    B C
Pea     15             HSH      3  96,3    B C D
Pea     20             HSH      3  96,1    B C D E
Pea     25             UH       3  96,0      C D E
Pea     25             HSH      3  95,5        D E F
Pea     20             UH       3  95,2          E F
Lentil  25             UH       3  94,9            F G
Lentil  25             HSH      3  94,3              G
Lentil  15             UH       3  94,3              G
Lentil  20             UH       3  94,3              G
Pea     30             HSH      3  94,2              G
Pea     30             UH       3  94,1              G H
Lentil  30             UH       3  93,3                H
Lentil  30             HSH      3  92,4                  I

Means that do not share a letter are significantly different.
```

Table 5.15 Two way ANOVA and Tukey's Comparison Test for encapsulation efficiency of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% lentil flour concentrations

General Linear Model: Efficiency versus Concentration; Process

```
Factor         Type   Levels  Values
Concentration  fixed       4  15; 20; 25; 30
Process        fixed       2  HSH; UH


Analysis of Variance for Efficiency, using Adjusted SS for Tests

Source                 DF   Seq SS   Adj SS   Adj MS       F      P
Concentration           3  32,7982  32,7982  10,9327  332,22  0,000
Process                 1   5,9470   5,9470   5,9470  180,72  0,000
Concentration*Process   3  18,9387  18,9387   6,3129  191,84  0,000
Error                  16   0,5265   0,5265   0,0329
```

Table 5.15 (Continued)

```
Total                  23  58,2104


S = 0,181405   R-Sq = 99,10%   R-Sq(adj) = 98,70%


Unusual Observations for Efficiency

Obs  Efficiency      Fit  SE Fit  Residual  St Resid
 14     94,0023  94,3355  0,1047   -0,3332     -2,25 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
15             6  95,9  A
20             6  95,5    B
25             6  94,6      C
30             6  92,9        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process   N  Mean  Grouping
HSH      12  95,2  A
UH       12  94,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  Process  N  Mean  Grouping
15             HSH      3  97,5  A
20             HSH      3  96,6    B
25             UH       3  94,9      C
25             HSH      3  94,3        D
15             UH       3  94,3        D
20             UH       3  94,3        D
30             UH       3  93,3          E
30             HSH      3  92,4            F

Means that do not share a letter are significantly different.
```

Table 5.16 Two way ANOVA and Tukey's Comparison Test for encapsulation efficiency of double emulsions prepared by HSH and UH; for 15%, 20%, 25% and 30% pea flour concentrations

General Linear Model: Efficiency versus Concentration; Process

```
Factor         Type   Levels  Values
Concentration  fixed       4  15; 20; 25; 30
Process        fixed       2  HSH; UH


Analysis of Variance for Efficiency, using Adjusted SS for Tests

Source                 DF   Seq SS   Adj SS  Adj MS      F      P
Concentration           3  18,1427  18,1427  6,0476  43,66  0,000
Process                 1   0,0007   0,0007  0,0007   0,01  0,942
Concentration*Process   3   2,0719   2,0719  0,6906   4,99  0,012
Error                  16   2,2162   2,2162  0,1385
Total                  23  22,4316


S = 0,372170   R-Sq = 90,12%   R-Sq(adj) = 85,80%


Unusual Observations for Efficiency

Obs  Efficiency      Fit  SE Fit  Residual  St Resid
 31     94,7845  95,5450  0,2149   -0,7605     -2,50 R
 47     93,2170  94,0974  0,2149   -0,8804     -2,90 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
15             6  96,6  A
25             6  95,8    B
20             6  95,7    B
30             6  94,2      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Process   N  Mean  Grouping
UH       12  95,5  A
HSH      12  95,5  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  Process  N  Mean  Grouping
15             UH       3  96,9  A
15             HSH      3  96,3  A B
20             HSH      3  96,1  A B
```

Table 5.16 (Continued)

```
25             UH       3  96,0  A B
25             HSH      3  95,5    B
20             UH       3  95,2    B C
30             HSH      3  94,2      C D
30             UH       3  94,1        D

Means that do not share a letter are significantly different.
```

Table 5.17 Two way ANOVA and Tukey's Comparison Test for hardness of cakes prepared with double emulsions containing 20%, 25% and 30% lentil and pea flour concentrations

General Linear Model: Hardness versus Flour; Concentration

```
Factor         Type   Levels  Values
Flour          fixed       2  Lentil; Pea
Concentration  fixed       3  20; 25; 30


Analysis of Variance for Hardness, using Adjusted SS for Tests

Source               DF    Seq SS    Adj SS    Adj MS     F      P
Flour                 1  0,005617  0,005617  0,005617  0,65  0,435
Concentration         2  0,003649  0,003649  0,001825  0,21  0,812
Flour*Concentration   2  0,003899  0,003899  0,001949  0,23  0,800
Error                12  0,103183  0,103183  0,008599
Total                17  0,116347


S = 0,0927284   R-Sq = 11,31%   R-Sq(adj) = 0,00%


Unusual Observations for Hardness

Obs  Hardness      Fit   SE Fit  Residual  St Resid
  7   0,96086  1,12529  0,05354  -0,16443     -2,17 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   N  Mean  Grouping
Lentil  9   1,1  A
Pea     9   1,1  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
20             6   1,1  A
25             6   1,1  A
30             6   1,1  A
```

Table 5.17 (Continued)

```
Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Concentration  N  Mean  Grouping
Lentil  20             3   1,1  A
Pea     25             3   1,1  A
Lentil  25             3   1,1  A
Lentil  30             3   1,1  A
Pea     20             3   1,1  A
Pea     30             3   1,0  A

Means that do not share a letter are significantly different.
```

Table 5.18 One way ANOVA and Tukey's Comparison Test for hardness of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% lentil flour concentrations

General Linear Model: Hardness versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  Control-Mg; Control-PE; Lentil 20%; Lentil
25%;
                              Lentil 30%


Analysis of Variance for Hardness, using Adjusted SS for Tests

Source         DF   Seq SS   Adj SS   Adj MS      F      P
Concentration   4  0,73409  0,73409  0,18352  20,34  0,000
Error          10  0,09022  0,09022  0,00902
Total          14  0,82431


S = 0,0949846   R-Sq = 89,06%   R-Sq(adj) = 84,68%


Unusual Observations for Hardness

Obs  Hardness      Fit   SE Fit  Residual  St Resid
  7   0,96086  1,12529  0,05484  -0,16443     -2,12 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
Control-Mg     3   1,7  A
Lentil 20%     3   1,1    B
Control-PE     3   1,1    B
Lentil 25%     3   1,1    B
Lentil 30%     3   1,1    B

Means that do not share a letter are significantly different.
```

Table 5.19 One way ANOVA and Tukey's Comparison Test for hardness of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% pea flour concentrations

General Linear Model: Hardness versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  Control-Mg; Control-PE; Pea 20%; Pea 25%;
Pea 30%


Analysis of Variance for Hardness, using Adjusted SS for Tests

Source         DF   Seq SS   Adj SS   Adj MS      F      P
Concentration   4  0,81133  0,81133  0,20283  32,82  0,000
Error          10  0,06181  0,06181  0,00618
Total          14  0,87313


S = 0,0786166   R-Sq = 92,92%   R-Sq(adj) = 90,09%


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
Control-Mg     3   1,7  A
Control-PE     3   1,1    B
Pea 25%        3   1,1    B
Pea 20%        3   1,1    B
Pea 30%        3   1,0    B

Means that do not share a letter are significantly different.
```

Table 5.20 Two way ANOVA and Tukey's Comparison Test for specific volume of cakes prepared with double emulsions containing 20%, 25% and 30% lentil and pea flour concentrations

General Linear Model: Volume versus Flour; Concentration

```
Factor         Type   Levels  Values
Flour          fixed       2  Lentil; Pea
Concentration  fixed       3  20; 25; 30


Analysis of Variance for Volume, using Adjusted SS for Tests

Source               DF    Seq SS    Adj SS    Adj MS     F      P
Flour                 1  0,000656  0,000656  0,000656  0,35  0,578
Concentration         2  0,000039  0,000039  0,000019  0,01  0,990
Flour*Concentration   2  0,000665  0,000665  0,000333  0,18  0,843
Error                 6  0,011396  0,011396  0,001899
Total                11  0,012756
```

Table 5.20 (Continued)

```
S = 0,0435821   R-Sq = 10,66%   R-Sq(adj) = 0,00%


Unusual Observations for Volume

Obs   Volume      Fit   SE Fit  Residual  St Resid
 11  1,73689  1,66712  0,03082   0,06976      2,26 R
 12  1,59736  1,66712  0,03082  -0,06976     -2,26 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   N  Mean  Grouping
Pea     6   1,7  A
Lentil  6   1,7  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
25             4   1,7  A
20             4   1,7  A
30             4   1,7  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Concentration  N  Mean  Grouping
Pea     30             2   1,7  A
Pea     20             2   1,7  A
Pea     25             2   1,7  A
Lentil  25             2   1,7  A
Lentil  20             2   1,7  A
Lentil  30             2   1,6  A

Means that do not share a letter are significantly different.
```

Table 5.21 One way ANOVA and Tukey's Comparison Test for specific volume of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% lentil flour concentrations

General Linear Model: Volume versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  Control-Mg; Control-PE; Lentil 20%; Lentil
25%;
                              Lentil 30%
```

Table 5.21 (Continued)

```
Analysis of Variance for Volume, using Adjusted SS for Tests

Source         DF     Seq SS     Adj SS     Adj MS     F      P
Concentration   4  0,0015682  0,0015682  0,0003920  2,03  0,228
Error           5  0,0009641  0,0009641  0,0001928
Total           9  0,0025323


S = 0,0138860   R-Sq = 61,93%   R-Sq(adj) = 31,47%


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
Control-PE     2   1,7  A
Lentil 25%     2   1,7  A
Lentil 20%     2   1,7  A
Lentil 30%     2   1,6  A
Control-Mg     2   1,6  A

Means that do not share a letter are significantly different.
```

Table 5.22 One way ANOVA and Tukey's Comparison Test for specific volume of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% pea flour concentrations

General Linear Model: Volume versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  Control-Mg; Control-PE; Pea 20%; Pea 25%;
Pea 30%


Analysis of Variance for Volume, using Adjusted SS for Tests

Source         DF    Seq SS    Adj SS    Adj MS     F      P
Concentration   4  0,001634  0,001634  0,000408  0,18  0,939
Error           5  0,011325  0,011325  0,002265
Total           9  0,012958


S = 0,0475913   R-Sq = 12,61%   R-Sq(adj) = 0,00%


Unusual Observations for Volume

Obs   Volume      Fit   SE Fit  Residual  St Resid
 11  1,73689  1,66712  0,03365   0,06976      2,07 R
 12  1,59736  1,66712  0,03365  -0,06976     -2,07 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence
```

Table 5.22 (Continued)

```
Concentration  N  Mean  Grouping
Pea 30%        2   1,7  A
Control-PE     2   1,7  A
Pea 20%        2   1,7  A
Pea 25%        2   1,7  A
Control-Mg     2   1,6  A

Means that do not share a letter are significantly different.
```

Table 5.23 Two way ANOVA and Tukey's Comparison Test for weight loss of cakes prepared with double emulsions containing 20%, 25% and 30% lentil and pea flour concentrations

General Linear Model: WL versus Flour; Concentration

```
Factor         Type   Levels  Values
Flour          fixed       2  Lentil; Pea
Concentration  fixed       3  20; 25; 30


Analysis of Variance for WL, using Adjusted SS for Tests

Source               DF   Seq SS   Adj SS   Adj MS     F      P
Flour                 1  0,38423  0,38423  0,38423  4,32  0,060
Concentration         2  0,07942  0,07942  0,03971  0,45  0,650
Flour*Concentration   2  0,12171  0,12171  0,06085  0,68  0,523
Error                12  1,06763  1,06763  0,08897
Total                17  1,65298


S = 0,298277   R-Sq = 35,41%   R-Sq(adj) = 8,50%


Unusual Observations for WL

Obs       WL      Fit   SE Fit  Residual  St Resid
 10  7,19291  6,67891  0,17221   0,51401      2,11 R
 12  6,14695  6,67891  0,17221  -0,53195     -2,18 R
 24  6,99301  6,49351  0,17221   0,49950      2,05 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   N  Mean  Grouping
Lentil  9   6,8  A
Pea     9   6,5  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
```

Table 5.23 (Continued)

```
30             6   6,7  A
25             6   6,6  A
20             6   6,6  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Concentration  N  Mean  Grouping
Lentil  30             3   7,0  A
Lentil  20             3   6,7  A
Lentil  25             3   6,7  A
Pea     25             3   6,6  A
Pea     30             3   6,5  A
Pea     20             3   6,4  A

Means that do not share a letter are significantly different.
```

Table 5.24 One way ANOVA and Tukey's Comparison Test for weight loss of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% lentil flour concentrations

General Linear Model: WL versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  Control-Mg; Control-PE; Lentil 20%; Lentil
25%;
                              Lentil 30%


Analysis of Variance for WL, using Adjusted SS for Tests

Source         DF   Seq SS   Adj SS   Adj MS     F      P
Concentration   4  1,44715  1,44715  0,36179  5,15  0,016
Error          10  0,70270  0,70270  0,07027
Total          14  2,14985


S = 0,265086   R-Sq = 67,31%   R-Sq(adj) = 54,24%


Unusual Observations for WL

Obs       WL      Fit   SE Fit  Residual  St Resid
 10  7,19291  6,67891  0,15305   0,51401      2,37 R
 12  6,14695  6,67891  0,15305  -0,53195     -2,46 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
Lentil 30%     3   7,0  A
```

Table 5.24 (Continued)

```
Control-PE     3   6,9  A
Lentil 20%     3   6,7  A B
Lentil 25%     3   6,7  A B
Control-Mg     3   6,1    B

Means that do not share a letter are significantly different.
```

Table 5.25 One way ANOVA and Tukey's Comparison Test for weight loss of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% pea flour concentrations

General Linear Model: WL versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  Control-Mg; Control-PE; Pea 20%; Pea 25%;
Pea 30%


Analysis of Variance for WL, using Adjusted SS for Tests

Source         DF   Seq SS   Adj SS   Adj MS     F      P
Concentration   4  1,01549  1,01549  0,25387  4,24  0,029
Error          10  0,59908  0,59908  0,05991
Total          14  1,61457


S = 0,244761   R-Sq = 62,90%   R-Sq(adj) = 48,05%


Unusual Observations for WL

Obs       WL      Fit   SE Fit  Residual  St Resid
 24  6,99301  6,49351  0,14131   0,49950      2,50 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
Control-PE     3   6,9  A
Pea 25%        3   6,6  A B
Pea 30%        3   6,5  A B
Pea 20%        3   6,4  A B
Control-Mg     3   6,1    B

Means that do not share a letter are significantly different.
```

Table 5.26 Two way ANOVA and Tukey's Comparison Test for color of cakes prepared with double emulsions containing 20%, 25% and 30% lentil and pea flour concentrations

General Linear Model: Color versus Flour; Concentration

```
Factor         Type   Levels  Values
Flour          fixed       2  Lentil; Pea
Concentration  fixed       3  20; 25; 30


Analysis of Variance for Color, using Adjusted SS for Tests

Source               DF  Seq SS  Adj SS  Adj MS     F      P
Flour                 1   5,138   5,138   5,138  3,24  0,122
Concentration         2   4,531   4,531   2,266  1,43  0,311
Flour*Concentration   2   0,607   0,607   0,304  0,19  0,831
Error                 6   9,517   9,517   1,586
Total                11  19,794


S = 1,25945   R-Sq = 51,92%   R-Sq(adj) = 11,85%


Unusual Observations for Color

Obs    Color      Fit  SE Fit  Residual  St Resid
  7  31,6944  33,7256  0,8906   -2,0312     -2,28 R
  8  35,7568  33,7256  0,8906    2,0312      2,28 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   N  Mean  Grouping
Lentil  6  33,1  A
Pea     6  31,8  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
30             4  33,0  A
25             4  32,9  A
20             4  31,6  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Concentration  N  Mean  Grouping
Lentil  25             2  33,7  A
Lentil  30             2  33,3  A
Pea     30             2  32,7  A
Lentil  20             2  32,4  A
```

Table 5.26 (Continued)

```
Pea     25            2  32,0  A
Pea     20            2  30,9  A

Means that do not share a letter are significantly different.
```

Table 5.27 One way ANOVA and Tukey's Comparison Test for color of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% lentil flour concentrations

General Linear Model: Color versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  Control-Mg; Control-PE; Lentil 20%; Lentil
25%;
                              Lentil 30%


Analysis of Variance for Color, using Adjusted SS for Tests

Source         DF  Seq SS  Adj SS  Adj MS     F      P
Concentration   4  21,643  21,643   5,411  2,85  0,140
Error           5   9,483   9,483   1,897
Total           9  31,125


S = 1,37716   R-Sq = 69,53%   R-Sq(adj) = 45,16%


Unusual Observations for Color

Obs    Color      Fit  SE Fit  Residual  St Resid
  7  31,6944  33,7256  0,9738   -2,0312     -2,09 R
  8  35,7568  33,7256  0,9738    2,0312      2,09 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
Lentil 25%     2  33,7  A
Lentil 30%     2  33,3  A
Lentil 20%     2  32,4  A
Control-PE     2  30,4  A
Control-Mg     2  30,1  A

Means that do not share a letter are significantly different.
```

Table 5.28 One way ANOVA and Tukey's Comparison Test for color of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% pea flour concentrations

General Linear Model: Color versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  Control-Mg; Control-PE; Pea 20%; Pea 25%;
Pea 30%


Analysis of Variance for Color, using Adjusted SS for Tests

Source         DF   Seq SS  Adj SS  Adj MS     F      P
Concentration   4   9,1039  9,1039  2,2760  5,41  0,046
Error           5   2,1037  2,1037  0,4207
Total           9  11,2076


S = 0,648648   R-Sq = 81,23%   R-Sq(adj) = 66,21%


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
Pea 30%        2  32,7  A
Pea 25%        2  32,0  A
Pea 20%        2  30,9  A
Control-PE     2  30,4  A
Control-Mg     2  30,1  A

Means that do not share a letter are significantly different.
```

Table 5.29 One way Anova and Tukey's Comparison Test for taste (from sensory analysis) of cakes without magnesium, with direct magnesium addition and with magnesium encapsulated in double emulsions containing 20% lentil and pea flour

General Linear Model: taste versus sample

```
Factor  Type   Levels  Values
sample  fixed       4  1; 2; 3; 4


Analysis of Variance for taste, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS  Adj MS      F      P
sample   3  133,857  133,857  44,619  40,30  0,000
Error   24   26,571   26,571   1,107
Total   27  160,429


S = 1,05221   R-Sq = 83,44%   R-Sq(adj) = 81,37%
```

Table 5.29 (Continued)

```
Unusual Observations for taste

Obs    taste      Fit   SE Fit  Residual  St Resid
  1  5,00000  7,57143  0,39770  -2,57143     -2,64 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

sample  N  Mean  Grouping
3       7   7,7  A
1       7   7,6  A
4       7   7,6  A
2       7   2,6    B

Means that do not share a letter are significantly different.
```

Table 5.30 One way Anova and Tukey's Comparison Test for hardness (from sensory analysis) of cakes without magnesium, with direct magnesium addition and with magnesium encapsulated in double emulsions containing 20% lentil and pea flour

General Linear Model: hardness versus sample

```
Factor  Type   Levels  Values
sample  fixed       4  1; 2; 3; 4


Analysis of Variance for hardness, using Adjusted SS for Tests

Source  DF  Seq SS  Adj SS  Adj MS     F      P
sample   3  31,250  31,250  10,417  7,48  0,001
Error   24  33,429  33,429   1,393
Total   27  64,679


S = 1,18019   R-Sq = 48,32%   R-Sq(adj) = 41,86%


Unusual Observations for hardness

Obs  hardness      Fit   SE Fit  Residual  St Resid
  1   4,00000  7,71429  0,44607  -3,71429     -3,40 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

sample  N  Mean  Grouping
3       7   7,9  A
1       7   7,7  A
4       7   7,6  A
```

Table 5.30 (Continued)

```
2       7   5,3    B

Means that do not share a letter are significantly different.
```

Table 5.31 One way Anova and Tukey's Comparison Test for color (from sensory analysis) of cakes without magnesium, with direct magnesium addition and with magnesium encapsulated in double emulsions containing 20% lentil and pea flour

General Linear Model: color versus sample

```
Factor  Type   Levels  Values
sample  fixed       4  1; 2; 3; 4


Analysis of Variance for color, using Adjusted SS for Tests

Source  DF   Seq SS   Adj SS  Adj MS     F      P
sample   3   0,7143   0,7143  0,2381  0,51  0,677
Error   24  11,1429  11,1429  0,4643
Total   27  11,8571


S = 0,681385   R-Sq = 6,02%   R-Sq(adj) = 0,00%


Unusual Observations for color

Obs    color      Fit   SE Fit  Residual  St Resid
 17  7,00000  8,28571  0,25754  -1,28571     -2,04 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

sample  N  Mean  Grouping
3       7   8,3  A
1       7   8,1  A
4       7   8,0  A
2       7   7,9  A

Means that do not share a letter are significantly different.
```

Table 5.32 Two way ANOVA and Tukey's Comparison Test for baking stability of cakes prepared with double emulsions containing 20%, 25% and 30% pea flour concentrations

General Linear Model: Baking Stability versus Flour; Concentration

```
Factor         Type   Levels  Values
Flour          fixed       2  Lentil; Pea
Concentration  fixed       3  20; 25; 30


Analysis of Variance for Baking Stability, using Adjusted SS for Tests

Source               DF   Seq SS   Adj SS   Adj MS       F      P
Flour                 1  197,435  197,435  197,435  120,16  0,000
Concentration         2   68,378   68,378   34,189   20,81  0,002
Flour*Concentration   2    7,155    7,155    3,578    2,18  0,195
Error                 6    9,859    9,859    1,643
Total                11  282,828


S = 1,28185   R-Sq = 96,51%   R-Sq(adj) = 93,61%


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   N  Mean  Grouping
Pea     6  88,7  A
Lentil  6  80,6    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
20             4  88,0  A
25             4  83,5    B
30             4  82,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Flour   Concentration  N  Mean  Grouping
Pea     20             2  93,2  A
Pea     25             2  87,0    B
Pea     30             2  86,0    B
Lentil  20             2  82,9    B C
Lentil  25             2  79,9      C
Lentil  30             2  79,1      C

Means that do not share a letter are significantly different.
```

Table 5.33 One way ANOVA and Tukey's Comparison Test for baking stability of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% lentil flour concentrations

General Linear Model: Baking Stability versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  Control-Mg; Control-PE; Lentil 20%; Lentil
25%;
                              Lentil 30%


Analysis of Variance for Baking Stability, using Adjusted SS for Tests

Source         DF  Seq SS  Adj SS  Adj MS        F      P
Concentration   4  9762,0  9762,0  2440,5  1670,36  0,000
Error           5     7,3     7,3     1,5
Total           9  9769,3


S = 1,20874   R-Sq = 99,93%   R-Sq(adj) = 99,87%


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
Lentil 20%     2  82,9  A
Lentil 25%     2  79,9  A
Lentil 30%     2  79,1  A
Control-PE     2  71,7    B
Control-Mg     2   0,8      C

Means that do not share a letter are significantly different.
```

Table 5.34 One way ANOVA and Tukey's Comparison Test for baking stability of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% pea flour concentrations

General Linear Model: Baking Stability versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       5  Control-Mg; Control-PE; Pea 20%; Pea 25%;
Pea 30%


Analysis of Variance for Baking Stability, using Adjusted SS for Tests

Source         DF   Seq SS   Adj SS  Adj MS        F      P
Concentration   4  11691,7  11691,7  2922,9  2391,49  0,000
Error           5      6,1      6,1     1,2
Total           9  11697,9
```

Table 5.34 (Continued)

```
S = 1,10554   R-Sq = 99,95%   R-Sq(adj) = 99,91%


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
Pea 20%        2  93,2  A
Pea 25%        2  87,0    B
Pea 30%        2  86,0    B
Control-PE     2  71,7      C
Control-Mg     2   0,8        D

Means that do not share a letter are significantly different.
```

Table 5.35 One way ANOVA and Tukey's Comparison Test for baking stability of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, 25% and 30% lentil and pea flour concentrations

General Linear Model: Baking Stability versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       8  Control-Mg; Control-PE; Lentil 20%; Lentil
25%;
                              Lentil 30%; Pea 20%; Pea 25%; Pea 30%


Analysis of Variance for Baking Stability, using Adjusted SS for Tests

Source         DF   Seq SS   Adj SS  Adj MS        F      P
Concentration   7  12329,9  12329,9  1761,4  1210,85  0,000
Error           8     11,6     11,6     1,5
Total          15  12341,5


S = 1,20611   R-Sq = 99,91%   R-Sq(adj) = 99,82%


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
Pea 20%        2  93,2  A
Pea 25%        2  87,0    B
Pea 30%        2  86,0    B
Lentil 20%     2  82,9    B C
Lentil 25%     2  79,9      C
Lentil 30%     2  79,1      C
Control-PE     2  71,7        D
Control-Mg     2   0,8          E

Means that do not share a letter are significantly different.
```

Table 5.36 Two way ANOVA and Tukey's Comparison Test for heat stability of double emulsions containing 20%, 25% and 30% lentil and pea flour

General Linear Model: baking stability versus flour type; concentration

Factor	Type	Levels	Values
flour type	fixed	2	Lentil; Pea
concentration	fixed	3	20; 25; 30

Analysis of Variance for baking stability, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
flour type	1	0,3688	0,3688	0,3688	3,09	0,129
concentration	2	8,5157	8,5157	4,2579	35,70	0,000
flour type*concentration	2	0,1937	0,1937	0,0968	0,81	0,487
Error	6	0,7157	0,7157	0,1193		
Total	11	9,7938				

S = 0,345365 R-Sq = 92,69% R-Sq(adj) = 86,60%

Grouping Information Using Tukey Method and 95,0% Confidence

flour type	N	Mean	Grouping
Lentil	6	93,4	A
Pea	6	93,1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

concentration	N	Mean	Grouping	
30	4	94,4	A	
20	4	92,8		B
25	4	92,5		B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

flour type	concentration	N	Mean	Grouping	
Lentil	30	2	94,4	A	
Pea	30	2	94,4	A	
Lentil	20	2	93,0		B
Lentil	25	2	92,8		B
Pea	20	2	92,7		B
Pea	25	2	92,1		B

Means that do not share a letter are significantly different.

Table 5.37 Two way ANOVA and Tukey's Comparison Test for heat stability of double emulsions containing 20%, 25% and 30% lentil flour concentrations and the cakes containing them

General Linear Model: baking stability versus medium; concentration

```
Factor         Type   Levels  Values
medium         fixed       2  Cake; DE
concentration  fixed       3  20; 25; 30


Analysis of Variance for baking stability, using Adjusted SS for Tests

Source                DF  Seq SS  Adj SS  Adj MS       F      P
medium                 1  490,94  490,94  490,94  493,97  0,000
concentration          2    5,26    5,26    2,63    2,64  0,150
medium*concentration   2   13,74   13,74    6,87    6,91  0,028
Error                  6    5,96    5,96    0,99
Total                 11  515,90


S = 0,996921   R-Sq = 98,84%   R-Sq(adj) = 97,88%


Grouping Information Using Tukey Method and 95,0% Confidence

medium  N  Mean  Grouping
DE      6  93,4  A
Cake    6  80,6    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

concentration  N  Mean  Grouping
20             4  87,9  A
30             4  86,7  A
25             4  86,4  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

medium  concentration  N  Mean  Grouping
DE      30             2  94,4  A
DE      20             2  93,0  A
DE      25             2  92,8  A
Cake    20             2  82,9    B
Cake    25             2  79,9    B
Cake    30             2  79,1    B

Means that do not share a letter are significantly different.
```

Table 5.38 Two way ANOVA and Tukey's Comparison Test for heat stability of double emulsions containing 20%, 25% and 30% lentil flour concentrations and the cakes containing them

General Linear Model: baking stability versus medium; concentration

```
Factor         Type   Levels  Values
medium         fixed       2  Cake; DE
concentration  fixed       3  20; 25; 30


Analysis of Variance for baking stability, using Adjusted SS for Tests

Source                DF   Seq SS  Adj SS  Adj MS      F      P
medium                 1   56,230  56,230  56,230  73,16  0,000
concentration          2   24,993  24,993  12,497  16,26  0,004
medium*concentration   2   40,250  40,250  20,125  26,19  0,001
Error                  6    4,611   4,611   0,769
Total                 11  126,084


S = 0,876674   R-Sq = 96,34%   R-Sq(adj) = 93,29%


Grouping Information Using Tukey Method and 95,0% Confidence

medium  N  Mean  Grouping
DE      6  93,1  A
Cake    6  88,7    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

concentration  N  Mean  Grouping
20             4  92,9  A
30             4  90,2    B
25             4  89,6    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

medium  concentration  N  Mean  Grouping
DE      30             2  94,4  A
Cake    20             2  93,2  A
DE      20             2  92,7  A
DE      25             2  92,1  A
Cake    25             2  87,0    B
Cake    30             2  86,0    B

Means that do not share a letter are significantly different.
```

Table 5.39 One way ANOVA and Tukey's Comparison Test for release of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, lentil and pea flour concentrations in simulated gastric juice

General Linear Model: Gastric Juice versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       4  Control-Mg; Control-PE; Lentil 20%; Pea 20%


Analysis of Variance for Gastric Juice, using Adjusted SS for Tests

Source         DF   Seq SS  Adj SS  Adj MS     F      P
Concentration   3   94,104  94,104  31,368  4,65  0,086
Error           4   26,991  26,991   6,748
Total           7  121,095


S = 2,59766   R-Sq = 77,71%   R-Sq(adj) = 60,99%


Grouping Information Using Tukey Method and 95,0% Confidence

Concentration  N  Mean  Grouping
Control-Mg     2  66,6  A
Control-PE     2  63,9  A
Lentil 20%     2  59,2  A
Pea 20%        2  58,1  A

Means that do not share a letter are significantly different.
```

Table 5.40 One way ANOVA and Tukey's Comparison Test for release of cakes prepared with non-capsulated magnesium, primary emulsion with double emulsions containing 20%, lentil and pea flour concentrations in simulated intestinal juice

General Linear Model: Intestinal Juice versus Concentration

```
Factor         Type   Levels  Values
Concentration  fixed       4  Control-Mg; Control-PE; Lentil 20%; Pea 20%


Analysis of Variance for Intestinal Juice, using Adjusted SS for Tests

Source         DF   Seq SS   Adj SS  Adj MS      F      P
Concentration   3  206,395  206,395  68,798  10,00  0,025
Error           4   27,526   27,526   6,881
Total           7  233,921


S = 2,62325   R-Sq = 88,23%   R-Sq(adj) = 79,41%


Grouping Information Using Tukey Method and 95,0% Confidence
```

Table 5.40 (Continued)

Concentration	N	Mean	Grouping
Control-PE	2	85,5	A
Control-Mg	2	85,1	A B
Pea 20%	2	75,8	A B
Lentil 20%	2	74,5	B

Means that do not share a letter are significantly different.

CURRICULUM VITAE

PERSONAL INFORMATION

Surname, Name : Kabakcı, Cansu

EDUCATION

Degree	Institution	Year of Graduation
BS	İstanbul University Child Development	-
BS Minor	METU Industrial Engineering	2014
BS	METU Food Engineering	2013
High School	Hüseyin Avni Sözen Anatolian High School, Ankara	2008

WORK EXPERIENCE

Year	Place	Enrollment
2013-Present	METU Department of Food Eng.	Research Assistant
2012 August	ETİ Biscuit Factory	Intern Engineering Student
2012 July	TÜBİTAK MAM Food Institute	Intern Engineering Student
2011 August	PINAR Milk Factory	Intern Engineering Student

FOREIGN LANGUAGES

Advanced English, Basic Spanish, Basic German

PUBLICATIONS

1. Kabakci, C., Sumnu, G., Sahin, S., Oztop, M. H. (2021). Encapsulation of Magnesium with Lentil Flour by Using Double Emulsion to Produce Magnesium Enriched Cakes. *Food and Bioprocess Technology, 0123456789.* https://doi.org/10.1007/s11947-021-02672-5

2. Kabakci, C., Sumnu, G., Sahin, S., (2022). Encapsulation of magnesium in

water-in-oil-in-water (W/O/W) double emulsions with pea flour: Physicochemical properties, stability, in vitro digestion and baking stability. (in progress)

3. Kabakci, C., Sumnu, G., Sahin, S., (2020, March). Encapsulation of Magnesium With Double Emulsion By Using Lentil Flour. Poster session presented at the 15th International Hydrocolloids Conference, Melbourne, Australia.

HOBBIES

Reading on psychology and child development, Doing puzzles, Skiing, Swimming