DOI: 10.29050/harranziraat.499249
Harran TarımveGıdaBilimleriDerg. 2019,23(3):324-334

Research Article/AraştırmaMakalesi

# A preliminary study of probiotic apple snack production with assisting food technologies

## *Yardımcı gıda teknolojileri ile elmadan probiyotik atıştırmalık üretiminin ön çalışması*

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***To cite this article:***
Dursun Saydam, D., Dakak, R. & Dalgıç, A.C. (2019). A preliminary study of probiotic apple snack production with assisting food technologies. Harran Tarım ve GıdaBilimleriDergisi, 23(3): 324-334.
DOI: 10.29050/harranziraat.499249

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***Received Date:***
19.12.2018
***Accepted Date:***
21.06.2019



**ABSTRACT**

A probiotic snack model food was formed with apple and *Lactobacillus rhamnosus* GG (LGG). The effects of dehydration process conditions with design of experiment (DoE) and dryer types used in the production were evaluated through optimization and probiotic qualification of the final product. Experiments on ultrasound assisted osmotic dehydration (US-OD) of apple cubes designed by Box-Behnken were conducted to obtain the maximum water reduction. Optimum values of sucrose solution concentration (40, 45, 50%), apple and solution ratio (1:4, 1:6, 1:8 w $w^{-1}$) and ultrasound application time (10, 20, 30 min) variables of the design were determined. The DoE methodology introduced the results that maximum water loss was reached at the conditions of 50% sucrose concentration, 1:4 apple and solution ratio and 10.05 min; sucrose concentration was the most effective variable; quadratic model submitted a good fitting ($R^2$=0.929) with the experimental results. Apple samples produced under the optimized conditions were dried with convectional and conventional dryers at specific temperature, 37 °C during 5 hours. The results showed that the remaining number of viable LGG cells ($10^6$-$10^7$cfu $g^{-1}$) was sufficient to qualify dried products as probiotic.

**Key Words:** Optimization, Osmotic dehydration, Probiotic dnack, Ultrasound assisting

**ÖZ**

Probiyotik atıştırmalık model yiyeceği elma ve *Lactobacillus rhamnosus GG* (LGG) ile oluşturulmuştur. Üretimde kullanılan deney tasarımı (DoE) ve kurutucu tipleri ile dehidrasyon işlemi koşullarının etkileri optimizasyon ve son ürünün probiyotik yeterliliği ile değerlendirilmiştir. Elma küplerinin ultrason destekli ozmotik dehidrasyonu (US-OD) ile maksimum düzeyde su içeriğini azaltmak için Box-Behnken tarafından tasarlanan deneysel tasarım çerçevesinde deneyler yürütülmüştür. Deneysel tasarımın sükroz çözelti konsantrasyonu (% 40, 45, 50), elma ve çözelti oranı (1: 4, 1: 6, 1: 8 w $w^{-1}$) ve ultrason uygulama süresi (10, 20, 30 dak) değişkenlerinin optimum değerleri belirlenmiştir. DoE metodolojisi, % 50 sükroz konsantrasyonu, 1:4 elma ve çözelti oranı ile 10,05 dakika koşullarında maksimum su kaybına ulaşıldığını ortaya koymuştur; sükroz konsantrasyonunu en etkili değişken olarak belirlemiştir; ve kuadratik modelin deneysel sonuçlar ile iyi bir uyum sağladığını ($R^2$=0.929) ortaya çıkarmıştır. Optimize edilen koşullar altında üretilen elma örnekleri, 5 saat boyunca 37 °C sıcaklıkta konveksiyonel ve konvensiyonel kurutucularla kurutulmuştur. Sonuçlar, canlı kalan LGG hücre sayısının ($10^6$-$10^7$kob $g^{-1}$) kurutulmuş ürünleri probiyotik olarak nitelendirmek için yeterli olduğunu göstermiştir.

**Anahtar Kelimeler:** Optimizasyon, Ozmotik dehidrasyon, Probiyotik atıştırmalık, Ultrason destekli

## Introduction

Functional foods used for the purpose of regulating health and developing diet were defined with the concept of "food as medicine" many years ago (Hasler, 2002). The Japanese brought forward the functional food term for

saving both human health and high health costs in 1980s (Plaza et al., 2008). Today simply, foods or food ingredients have the ability of improving human health that described as functional. Basic beneficial effects of functional foods are characterized by reducing high blood pressure, cholesterol and blood sugar, providing nutritional impacts, and reducing risks of heart diseases, cancer and osteoporosis (Charalampopoulos et al., 2002; Hasler, 2002).

Functional foods have introduced probiotics (Ps) that are viable microorganisms contributing to human health by affecting gastrointestinal system (GIS). They improve intestinal microbial balance and provide detoxification by enhancing the composition and activities of GIS, thereby they strengthen the immune system and reduce the risks of diseases. Ps are predominantly lactic acid bacteria (LAB), particularly *Lactobacillus* species such as *L. casei, L. plantarum, L. rhamnosus and L. acidophilus* (Charalampopoulos et al., 2002; Grajek et al., 2005; Farnworth, 2007; Plaza et al., 2008). *Bifidobacterium* species (*B. longum, B. animali and B. lactis*) are the other most known probiotic microorganisms (Charalampopoulos et al., 2002).

Foods with probiotics are among the most important and frequently consumed functional foods (Farnworth, 2007). Foods and beverages including probiotics can be animal or vegetable derived. Animal derived Ps are generally fermented dairy products like cheese, kefir and yoghurt whereas vegetable derived ones are fermented fruits and vegetables like pickles (Farnworth, 2007). The Japanese foundation, FOSHU (foods for specific health uses) started to produce probiotic products and sold them to the public in 1990s. Dietary fibre, oligosaccarides and LAB are three categories among the eleven ones referred functional components, which the FOSHU foundation has particularly designed for intestinal functions (Farnworth, 2007). Fruits comprise the two categories since they contain high, physically suitable and adequate amount of dietary fibre and have various oligosaccarides (Demirci et al., 2017). Dietary fibre and oligosaccaride in the fruits also provide prebiotic effect. The third category is corresponded by the probiotic added fruits. Probiotic-food consumers, vegans and the consumers of non-dairy and healthy foods are high interest in probiotic fruit products. (Pimentel et al., 2015; Bellary and Rastogi, 2016; Rodrigues et al. 2018). Li et al. (2018) informed that with the becoming aware of the relationship of fruits with health, the interest in healthier, good taste and probiotic fruit snacks has increased.

For snack food production, drying technology is highly used in addition to frying, coating, extruding, etc. Osmotic dehydration, vacuum drying, hot air drying, ultrasound application, emulsifying,microwave technology, fermentation, freezing, high pressure processing, and pulsed electric field treatment are common technologies for basic drying of fruit products. In addition, they are used as assisting applications in the drying (Garcia-Noguera et al., 2010; Chen et al 2016; Mierzwa et al., 2017; Nowacka et al., 2017; Zielinska and Markowski, 2017). Some of them are possible technologies for the production of probiotic foods (Tripathi and Gri, 2014; Ramya and Jain, 2017; Ambros et al., 2018). There are important points such a production in terms of food engineering approach: (*i*) The infusion of functional ingredients such as probiotics into the matrix of fruits as solid food products is different from liquid food ones due to the organoleptic properties and compositions of the solids. The techniques applied according to the structure, properties of such foods should be controlled, and the infusion should be improved (Bellary and Rastogi, 2016); (*ii*) Process optimization should be concerned for economics and product quality (Ambros et al., 2018). Drying time and kinetics are other important points where drying is the main technology for the production of dried probiotic fruit snack foods (Betoret et al., 2003).

Apple, which is commonly cultivated worldwide and consumed in various forms (juice, fresh, jam, etc.) (Rodrigues et. al., 2018) has 2% dietary fibre and rich oligosaccaride content (Erdoğan and Demirci, 2014). Impregnation of

probiotic lactic acid bacterium(a) in apple fruit and applying drying technology may create an opportunity for a functional snack. *Lactobacillus rhamnosus* GG (LGG) selected in this study for the targeted product is a proved and one of the most studied probiotic bacterial strains (Valík et al., 2008). It is also used in food industry as a probiotic and protective culture (Flores-Andrade et al., 2017). We aimed to produce probiotic apple snack with regard to the statements that literature knowledge of probiotic fruit products is limited (Puente et al., 2009); well-designed strategies and novel approaches based on keeping probiotic cells alive are necessary for the drying technology (Broeck et al., 2016; Flores-Andrade et al., 2017); optimization of air drying periods helps to reduce energy and costs (Garcia-Noguera et al., 2010); co- and/or pre- treatments are needed for avoiding organoleptic changes while drying by air (Mierzwa et al., 2017), and improving the final product quality (Garcia-Noguera et al., 2010). As the preliminary stage of the production, the conditions of the pretreatments, penetration time and drying technology were optimized. The process parameters of ultrasound assisting osmotic dehydration were modeled and optimized in the scope of Box-Behnken experimental design. ProbioticLGG penetration was then employed at different periods. Conventional and convectional drying procedures were putted in practice to discuss which one is the effective in terms of viable cell number and moisture content of the product.

## Materials and Methods

Operations and goals of this study were visualized in Figure 1.

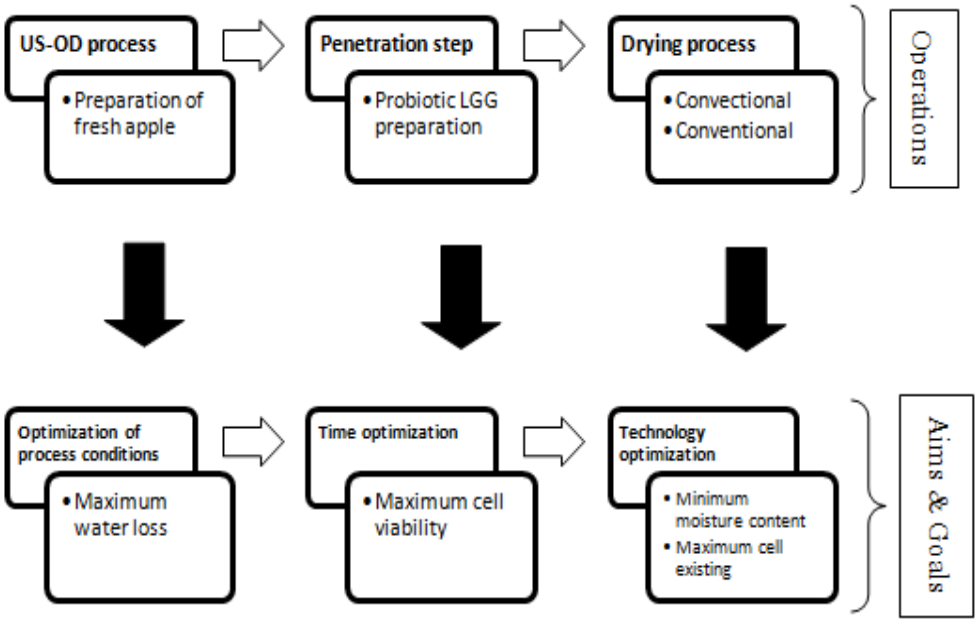


Figure 1. Diagram of the study
*Şekil 1. Çalışmanın diagramı*

*Preparation of apple cubes and microorganism inoculum*

Apples (*Malus domesticastarking delicious*) were purchased from a local market (Gaziantep, Turkey). They were washed with water, peeled and cut into cubes (1 cm) with a cutting device.

*Lactobacillus rhamnosus* GG was selected in this study since it is a proved and one of the most studied probiotic bacterial strains (Valík et al., 2008), and also used in food industry as a probiotic and protective culture (Flores-Andrade et al., 2017). *Lactobacillus rhamnosus* GG (ATCC 53103) was supplied from Bayburt University and used as probiotic bacteria in this study. The microorganism was maintained in Man, Rogosa and Sharp (MRS) broth/agar (Merck, Germany) as a selective medium for the *Lactobacillus* species. The inoculum was prepared using a 24-26 h fresh culture cultivated in 100 mL MRS at 37 °C to obtain an initial cell concentration of $7.2\times10^9$ cfu

$mL^{-1}$. After cultivation, the cells were removed from the medium and washed with physiological salt solution (PSS, containing 0.85% (w $v^{-1}$) NaCl) once to avoid the taste and smell of MRS medium. The procedures of cell transfer and inoculum were followed as described by Rodrigues et al. (2018) with minor modifications.

Viable cell count of the samples was determined placing 5 g sample in 45 ml sterile PSS and preparing a serial decimal dilution. Dilution series were obtained by mixing 1 mL previous dilution and 9 mL sterile PSS. Aliquots of 0.1 mL of dilution were plated on MRS agar. Plate counting was carried out after 2-3 days incubation at 37 °C and cell number was calculated by the equation below (Halkman, 2005):

$$N=C/[V^{x}d^{x}(n_1+(0.1^{x}n_2))] \quad (1)$$

*N*: colony number in 1 g or 1 mL sample (cfu $g^{-1}$)
*C*: total colony number at all plates
*V*: inoculation volume (mL)
*d*: dilution ratio of more concentrated dilution series
$n_1$: plate number counted of first dilution
$n_2$: plate number counted of second dilution

*Brix measurement*

The solute gain was observed by measuring Brix (°Bx) values of the US-OD treated and un-treated apple samples. The samples were pressed using a porcelain mortar and squeezed with a clean white cloth. The Brix value of the juice was measured with a refractometer (PTR 46 X, Index Instruments, USA) (AOAC, 1990).

*Ultrasound assisted osmotic dehydration process*

The design of experiments (DoE) approach was utilized in the US-OD pretreatment, which is consisting of experimental design, modeling and optimization transactions. The independent variables, levels and environmental conditions were decided considering the literature knowledge. Sharma et al. (2009) reported that rate of water loss in OD system depends on solution concentration, employment time and temperature, solid liquid ratio, and surface area of solid. Ramya and Jain (2017) informed that the OD operation of apple samples generally conducted at the conditions of 1:20 solid liquid ratio, 30 °C environment temperature, little pieces of apple, and 60% sucrose solution. In addition, the total OD period was discussed as 3 hours according to the knowledge that long OD periods may cause degradation of compounds in fruits and deformation of texture (Chottanom et al., 2016), and maximum water loss occurs within the first hour of OD application (Sharma et al., 2009).

*Experimental design*

Box-Behnken design (BBD) was generated using three independent variables; osmotic solution concentration (%), S:L ratio (w $w^{-1}$) and US time (min) at three levels by Design of Expert Version 7.1.6 program (Stat-Ease, Inc., Minneapolis, USA). High, middle and low codes of the variables were presented in Table 1. There were 15 runs with 3 centre points conducted through the design (Table 2). The depended variable -response- was moisture content expressing water loss.

Table 1. Levels of the independent variables for US-OD pretreatment
*Çizelge 1. US-OD ön işlem için bağımsız değişkenlerin seviyeleri*

| Independent variables<br>*Bağımsız değişkenler* | Symbol<br>*Sembol* | Low code<br>*Düşük kod* | Middle code<br>*Orta kod* | High code<br>*Yüksek kod* |
|---|---|---|---|---|
| Solution concentration (%),<br>*Çözelti konsantrasyonu (%)* | $x_1$ | 40 | 45 | 50 |
| S:L ratio (w $w^{-1}$),<br>*S:L oranı (w $w^{-1}$)* | $x_2$ | 1:4 | 1:6 | 1:8 |
| US time (min),<br>*US zamanı (dak)* | $x_3$ | 10 | 20 | 30 |

*US-OD pretreatment:*The edible apple cubes (20 g for each experiment) were immediately placed in 250 mL Erlenmeyer flasks containing sucrose solution with regard to the levels of osmotic solution concentration and S:L ratio. Firstly, ultrasound treatment was applied to the samples using an ultrasonic bath (Intersonic, Min 4 Model, Transducer Pzt=4, 350 W, Turkey) at constant frequency, 25 kHz and temperature, 30 °C according to the US time levels of the BBD design. Secondly, the rest of total osmotic dehydration time (3 h) was carried out in a rotary shaker (Innova 40R New Brunswick Scientific, USA) at 180 rpm and 30 °C temperature. The constant experiment conditions were applied with regard to the review study of Ramya and Jain (2017). When the US-OD pretreatment was completed in the scope of the experimental design, the samples were immediately utilized for moisture content analysis (AOAC, 1990).

*Modeling:*Quadratic model using second order polynomial equation (Montgomery, 2001) was selected for the modeling of experimental and predicted data of the moisture content response. The equation of the model is:

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j x_j + \sum_{j=1}^{k} \beta_{jj} x^2{}_j + \sum_{i=1}^{j-1} \sum_{j=2}^{k} \beta_{ij} x_i x_j \qquad (2)$$

Where $y$ is the predicted response, $\beta_0$ is model constant, $\beta_j$, $\beta_{ij}$ and $\beta_{jj}$ are the regression coefficients (linear, interaction, quadratic) and $x_j$ and $x_i$are the levels of the independent variables.

*Optimization*

Optimal conditions were determined according to the minimum moisture content value reached after the US-OD pretreatment. The experimental data were evaluated by regression and variance analyses (ANOVA) in Response Surface Methodology (RSM) at a significance level of 5% ($p<0.05$).

*LGG penetration*

After the optimized conditions were determined for the US-OD pretreatment, the apple sample was penetrated with LGG microorganism by agitating at 180 rpm and 30 °C in the rotary shaker during 5 and 10 minutes. The penetration periods were discussed considering the study of Krasaekoopt and Suthanwong (2008). When the penetration periods were ended, moisture content and viable cell analyses were carried out.

*Drying process*

After LGG penetration, drying process was performed using a hot air drier (UOP 8 Tray Drier, Armfield, UK) at constant velocity (2.0±0.05 m $s^{-1}$) and a vacuum dryer (VD 23 Binder, Germany) at constant pressure (1 atm) to create stationary heat source as in conventional or cabinet dryers. The drying processes were continued until reaching a constant weight reduction at 37 °C temperature degree for both drier. The viable cell number and moisture content of the dried samples was analysed when the drying period for both driers were ended.

*Statistical analysis*

One-way ANOVA with Duncan[a] test in SPSS program (Version 22.0, IBM SPSS Software, USA) at a significance level of 5% was performed to investigate the mean differences of the moisture content and refractive analyses' results.

**Results and Discussion**

Ultrasound assisting or combining with osmotic dehydration is more efficient for reducing of water in fruit and vegetables (Goula et al., 2017; Nowacka et al., 2017). Thus, US application was used to assist the OD process as a pretreatment in this study. The results of the target response and Brix values of the samples obtained from the US-OD pretreatment with BBD matrix are presented in Table 2. The Brix values showed that the apple gained sucrose according to the un-pretreated apple (16.17 °Bx). When we evaluate the maximum (33.31) and minimum (26.22) Brix values, it is seen that the sucrose up-take increases with the increasing levels of each

variable. It is known that ultrasound helps mass transfer of OD system for fruits by increasing the cell permeability and transfer of solutes (Azarpazhooh and Ramaswamy, 2010; Bellary and Rastogi, 2016; Nowacka et al., 2017). Also, it is evaluated from the results that the higher S:L ratio helps this transfer at higher sucrose concentrations.

In Table 3, ANOVA results showed that statistical significance of the fitted second order quadratic model equation at 0.05 probability value. Further evidence of goodness of fit was provided by $R^2$ value (92.9%) determining the fitting between the experimental and predicted response values. Lower probability value (0.0209<0.05) of the model suggested showed that the quadratic model is appropriate. The validation of the model was introduced by not significant lack of fit value (0.7308>0.05). Regression analysis indicated that a significant effect ($p<0.05$) of the osmotic solution concentration variable ($x_1$) and quadratic terms ($x_1$^2, $x_2$^2, $x_3$^2) of the moisture removal. Coded coefficients of the model terms denoted that the direction in which the target is affected. Negative coefficient value of the solution concentration variable (-2.2938) exhibit that the moisture content in the product decreased as the level of the variable increased.

Visual results of DoE approach are represented as 3D plots in Figure 2 demonstrating the interaction effects of the independent variables on the moisture content left in the samples while the third one was kept constant. All the figures point that the minimum water content values in the samples reached with the pretreatment, while US time has the minimum level (10 min), and S:L ratio and solution concentration variables have the maximum levels (1:8 w $w^{-1}$ and 50%) in Figure 2a, 2b and 2c, respectively.

The three parameters of the US-OD pretreatment were optimized in ranges depicted in Figure 3. According to the results, the lowest moisture content (69.63%) was reached at the conditions of 50% sugar concentration, 1:4 solid liquid ratio and 10.05 min US time. The dehydration effect of higher concentration of sucrose is already known (Barman and Barwaik, 2017; Ramya and Jain, 2017), which is a parallel consequent with the highest concentration result of the study. The lowest US time is interpreted as a score for the further study since there are results indicating long US periods that causes deformations in texture of fruits (Nowacka et al., 2017). Contour plot in Figure 4 shows how the solid-liquid ratio and solution concentration relationship affects the moisture level in the sample at a constant US time and indicating the maximum water loss prediction. The lines in the blue area depict the approach to the target. Consequently, the amount of water in fresh apple (84.43%) was reduced by 9.7-16.46% by employing the US-OD pretreatment for 3 hours. The DoE approach showed that a water reduction in the ratio of 17.87% could be reached.

Amami et al. (2017) studied the kinetics of the US-OD process of strawberry in the scope of BBD with the variables of US-OD time (10, 20, 30 min), Brix of solution (0, 32.5, 65 °Bx) and temperature (20, 30, 40 °C). BBD and RSM provided an effective approach for modeling with quadratic equation and optimizing the US-OD process conditions as found out in our study. Quadratic model was adequate at $p<0.05$ level and showed a good fit for loss of water with 0.989 R-squared value. Ultrasound application time and solution Brix were the significant terms. When the both of them increased, the water loss was increased. Ultrasound application revealed a great reduction of drying time by increasing the water loss. Optimal conditions of the US-OD process are 20.5 min US time, 47.5% solution concentration, and 31 °C temperature. The optimum solution concentration found and temperature value implemented in the presented study is parallel with the results of Amami et al. (2017).

Garcia-Noguera et al. (2010) dried strawberry with a US-OD pretreatment. Constant sucrose solution temperature (30 °C), S:L ratio (1:2) and air drying conditions (60 °C, 0.5 m $s^{-1}$) were used for OD alone and US-OD processes. At different sucrose concentrations (0, 25, 50%) and US

application periods (10, 20, 30, 45 min), they determined that the ultrasound helps to reduce total drying time, the US-OD pretreatment reduces the drying time, the maximum sucrose concentration provides maximum water loss, and optimum US time is 30 min at 25 kHz. Reducing the water content with US assisted OD pretreatment with the highest solution concentration is an effective method for decreasing drying time of strawberry. A similar approach was exhibited at constant US application frequency and OD temperature level as the selected constant values in our study. They revealed that the effectiveness of US-OD process for the removal of moisture in fruits as we did for apple.

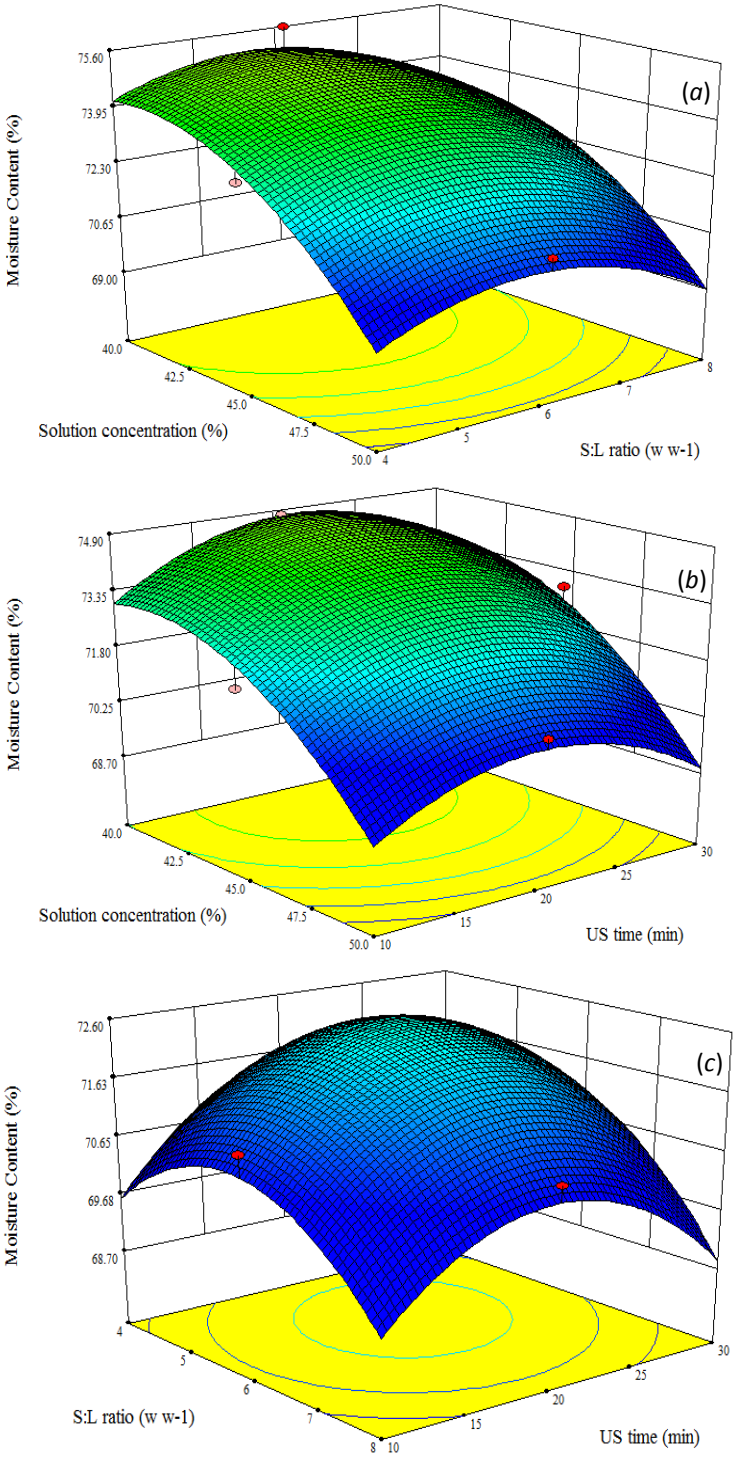


Figure 2. 3D plots demonstrating the interaction effects of the independent variables on the response; *a*: solid and liquid ratio and solution concentration, *b*: ultrasound application time and solution concentration, *c*: solid and liquid ratio and ultrasound application time

*Şekil 2. Yanıt üzerindebağımsız değişkenlerin interaksiyon etkilerini gösteren 3 boyutlu grafikler; a: katı ve sıvı oranı ile ozmotik çözelti konsantrasyonu, b: ultrason uygulama süresi ile ozmotik çözelti konsantrasyonu, c: katı ve sıvı oranı ile ultrason uygulama süresi*

Table 2. Box-Behnken design matrix with responses of the independent variables
*Çizelge 2. Bağımsız değişkenlerin yanıtlarıyla Box-Behnken dizayn matriksi*

| Run<br>*Deney* | $x_1$ (%) | $x_2$ (w $w^{-1}$) | $x_3$ (min) | Moisture content (%)<br>*Nem miktarı* | Fruit Brix<br>*Meyve Briksi* |
|---|---|---|---|---|---|
| 1 | 50 | 1:8 | 20 | 70.70±0.20$^{a}$ | 33.31±0.08$^{j}$ |
| 2 | 40 | 1:8 | 20 | 74.75±0.51$^{c}$ | 29.37±0.38$^{g}$ |
| 3 | 50 | 1:6 | 30 | 70.53±0.23$^{a}$ | 32.15±0.11$^{i}$ |
| 4 | 45 | 1:6 | 20 | 75.39±0.54$^{c}$ | 27.09±0.01$^{bc}$ |
| 5 | 40 | 1:6 | 30 | 75.70±0.52$^{c}$ | 27.02±0.09$^{bc}$ |
| 6 | 50 | 1:4 | 20 | 71.66±0.58$^{ab}$ | 30.62±0.06$^{h}$ |
| 7 | 45 | 1:8 | 10 | 71.78±0.23$^{ab}$ | 29.43±0.36$^{g}$ |
| 8 | 50 | 1:6 | 10 | 71.04±0.46$^{ab}$ | 32.60±0.03$^{i}$ |
| 9 | 45 | 1:6 | 20 | 75.41±0.33$^{c}$ | 28.09±0.01$^{de}$ |
| 10 | 45 | 1:4 | 30 | 75.26±0.84$^{c}$ | 26.86±0.13$^{b}$ |
| 11 | 40 | 1:4 | 20 | 76.24±0.16$^{cd}$ | 30.45±0.56$^{h}$ |
| 12 | 40 | 1:6 | 10 | 75.59±0.03$^{c}$ | 26.22±0.02$^{a}$ |
| 13 | 45 | 1:6 | 20 | 77.53±1.57$^{d}$ | 27.67±0.02$^{cd}$ |
| 14 | 45 | 1:4 | 10 | 72.90±0.32$^{b}$ | 28.89±0.14$^{fg}$ |
| 15 | 45 | 1:8 | 30 | 72.90±0.34$^{b}$ | 28.53±0.05$^{ef}$ |
| | | Un-pretreated apple<br>*Ön işlem yapılmayan elma* | | 84.43±0.08 | 16.17±0.02 |

$^{a-j}$: expresses the mean differences in the column (p<0.05).
$^{a-j}$*: sütundaki ortalamadan farklılıkları açıklamaktadır (p<0.05).*
±: standard deviation.
*±: standart sapma.*

Table 3. ANOVA results of the modeling based on moisture content measurements
*Çizelge 3. Nem içeriği ölçümlerine dayanan modellemenin ANOVA sonuçları*

| Source<br>*Kaynak* | Sum of squares<br>*Kareler toplamı* | Prob>F | Coded coefficients<br>*Kodlu katsayılar* |
|---|---|---|---|
| Model, *Model* | 67.8380 | 0.0209* | |
| Intercept ($\beta_0$), *İntersept* | | | 76.11 |
| Linear terms, *Lineer terimler* | | | |
| $x_1$ (%) ($\beta_1$) | 42.0903 | 0.0014 | -2.2938 |
| $x_2$ (w/w) ($\beta_2$) | 4.3956 | 0.0946 | -0.7413 |
| $x_3$ (min) ($\beta_3$) | 1.1858 | 0.3339 | 0.3850 |
| Interaction terms, *İnteraksiyon terimleri* | | | |
| $x_1x_2$ ($\beta_1\beta_2$) | 0.0702 | 0.8051 | 0.1325 |
| $x_1x_3$ ($\beta_1\beta_3$) | 0.0961 | 0.7731 | -0.1550 |
| $x_2x_3$ ($\beta_2\beta_3$) | 0.3844 | 0.5693 | -0.3100 |
| Quadratic terms, *Kuadratik terimler* | | | |
| $x_1$^2 ($\beta_{11}$) | 7.0699 | 0.0476 | -1.3838 |
| $x_2$^2 ($\beta_{22}$) | 7.1211 | 0.0471 | -1.3888 |
| $x_3$^2 ($\beta_{33}$) | 8.4328 | 0.0358 | -1.5113 |
| Residual | 5.1877 | | |
| Lack of Fit | 2.1629 | 0.7308** | |
| Pure Error | 3.0248 | | |
| Cor Total | 73.0258 | | |
| Statistic results, *İstatistik sonuçları* | | | |
| Standard deviation, *Standart sapma* | 1.0186 | R-Squared | 0.9290 |
| Mean, *Ortalama* | 3.82537 | Adj. R-Squared | 0.8011 |
| C.V. % | 1.3797 | Pred. R-Squared | 0.4329 |
| PRESS | 41.4126 | Adeq. Precision | 7.2985 |

*: significant and **: not significant (p<0.05).
**: anlamlı **: anlamlı değil (p<0,05).*

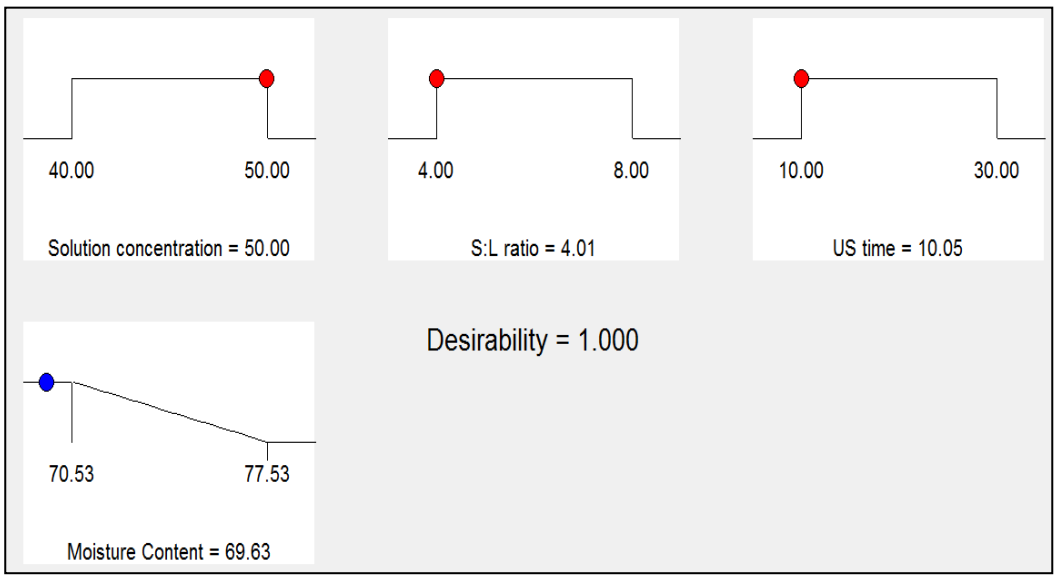


Figure 3. The optimum conditions of the US-OD pretreatment in terms of coded values. The circular signs on the interval lines of the parameters indicate the optimum results

*Şekil 3. Kodlu değerler üzerinden US-OD ön işleminin optimum koşulları. Parametrelerin aralık çizgileri üzerindeki dairesel işaretler optimum sonuçları belirtir*

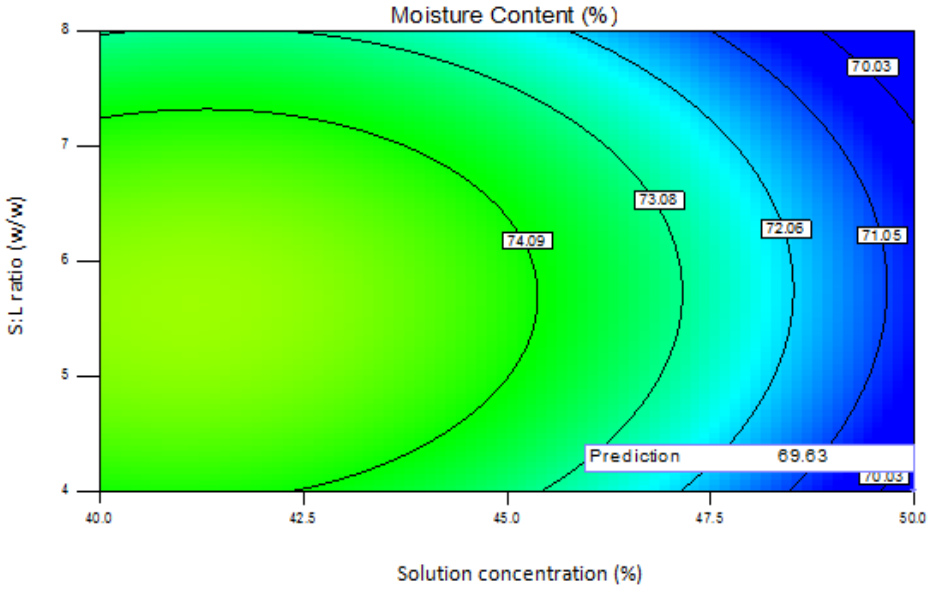


Figure 4. The predicted minimum moisture content value depicted by contour plot

*Şekil 4. Tahmin edilen minimum nem miktarı değerini gösteren kontür çizimi*

Table 4. Moisture content and viable cell number results

*Çizelge 4. Nem miktarı ve canlı hücre sayısı sonuçları*

| Apple samples<br>*Elma numuneleri* | Moisture content (%)<br>*Nem miktarı (%)* | Viable cell number (cfu ($mL^{-1}$) $g^{-1}$)<br>*Canlı hücre sayısı (kob ($mL^{-1}$) $g^{-1}$)* |
|---|---|---|
| Fresh, *Taze* | 85.78±0.52 | |
| US-OD pretreated,<br>*US-OD ön işlemi uygulanmış* | 73.34±0 | |
| 5 min penetrated,<br>*5 dak penetre edilmiş* | 80.48±0 | $6.3\times10^{9}$ |
| 10 min penetrated,<br>*10 dak penetre edilmiş* | 80.94±0 | $6.7\times10^{9}$ |
| Convectional dried,<br>*Konveksiyonel kurutulmuş* | 8.79±1.40 | $0.1\times10^{7}$ |
| Conventional dried,<br>*Konvensiyonel kurutulmuş* | 23.02±3.65 | $0.5\times10^{6}$ |

LGG penetration was followed for the sample dehydrated at the optimized conditions. It was observed that there was no difference between the number of viable cells and moisture content in the five and ten minute penetrated apple samples as presented in Table 4. Meanwhile, it was seen that the apple cubes received some water (≈10%). Krasaekoopt and Suthanwong (2008) penetrated guava and papaya fruits with *L. casei* during 5, 10 and 15 min. Suitable penetration conditions for guava and papaya were found as 5 and 10 min respectively.

After penetration time optimization, the apple samples were penetrated for 10 min and the process was continued with drying processes. The amount of water in the apple samples was reduced at the ratios of 89% and 72% with convectional and with conventional dryers respectively in 5 h. The results of the convectional drying process showed more desirable results for a probiotic apple snack with higher viable cell and lower moisture content. Rodrigues et al. (2018) studied such a product at 10, 40 and 60 °C temperatures and 1 m $s^{-1}$ velocity of convectional dryer. They obtained a probiotic product for all temperatures as a result of drying until 80% of the sample weight was reduced. They reported that the number of living cells decreased as the temperature increased. Additionally, they revealed the result that 100 g of probiotic apple snack intake provides 100 million probiotic cell intake. In the presented study, same probiotic cell in take result with same hot source system was obtained at 37 °C.The conventional dried apple cubes had higher moisture content. It is thought that more drying time is needed for vacuum dryer. Nevertheless, probiotic apple cubes could be produced. Li et al. (2018) tried to produce probiotic-enriched apple snack using a cabinet air dryer at 40 °C for 12 h. They achieved to produce probiotic qualified snack.

## Conclusions

Approximately 17% water loss could be obtained by the US-OD pretreatment in the scope of experimental design, which is a good result for the effectiveness of the drying process. Kinetic study will be performed to increase the productivity of the pretreatment and understand mass diffusivity phenomena of osmotic dehydration.

It is revealed that the probiotic apple snack could be produced by the study performed. However, a detailed drying process and shelf-life will be studied under the optimized conditions, and organoleptic properties (flavour, colour, texture, etc.) and nutritional content will be investigated in the further studies.

## Acknowledgement

We thank to Assoc. Prof. Dr. Enes Dertli from Bayburt University for providing probiotic microorganism.

There is no conflict of interest.

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