



Proceeding Paper

Evaluating the Impact of Argan Oil, Sugar, and Peptone Concentrations on the Survival of *Escherichia coli*[†]

Youssef Ezzaky , Mariem Zanzan, Ahmed Elidrissi, Kaoutar Boussif and Fouad Achemchem *

Bioprocess and Environment Team, LASIME Laboratory, Agadir Superior School of Technology, Ibn Zohr University, Agadir 80150, Morocco; youssef.ezzaky@edu.uiz.ac.ma (Y.E.); mariem.zanzan@edu.uiz.ac.ma (M.Z.); ah.elidrissi@uiz.ac.ma (A.E.); kaoutarboussif@gmail.com (K.B.)

* Correspondence: f.achemchem@uiz.ac.ma; Tel.: +212-528-232-583

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Abstract: This study developed a predictive model for *Escherichia coli* survival in Amlou, a Moroccan almond–honey–argan oil spread, focusing on argan oil, sugar, and peptone concentrations. Based on the Doehlert matrix design, relationships were established between these ingredients and the death rate (DR) and survival period (SP). Sugar affected the DR, while argan oil and peptone influenced the SP. The DR model demonstrated an RMSE of 0.0095 and SEP of 22.22%, confirming its accuracy. This work offers valuable insights for food producers about *E. coli*'s behavior in Amlou-like foods.

Keywords: *Escherichia coli*; survival; response surface; argan oil; sugar; protein concentration

1. Introduction

Amlou, a popular product in Moroccan culinary traditions, is a blend of almonds, honey, and distinctive argan oil. Its rich texture and unique flavor profile have led to its widespread consumption across Moroccan households and its increasing global popularity [1]. However, the largely artisanal methods of its production, often relying on traditional practices passed down through generations, have raised concerns about its microbiological safety. Artisanal foods, though treasured for their authenticity and taste, often come with increased risks of microbial contamination due to the use of non-standardized production practices [2].

Escherichia coli, a well-known pathogenic bacterium, has been identified in various food sources, leading to foodborne illnesses. Its presence in food items such as Amlou can pose serious health risks, especially if consumed by vulnerable populations like the elderly, children, and those with compromised immune systems [3]. Moreover, the unique composition of Amlou, with varying concentrations of argan oil, sugar, and proteins, could influence the survival dynamics of this bacterium. As such, a comprehensive understanding of *E. coli*'s survival in response to these constituents becomes imperative, not only for ensuring the safety of Amlou, but also for preserving its cultural significance in Moroccan food.

This study aims to bridge this gap by developing a predictive model detailing the survival of *E. coli* in an environment similar to Amlou.

2. Materials and Methods

2.1. Bacterial Strain and Preparation

The bacterial strain used in this study was *E. coli* ATCC 25922. Fresh overnight cultures of *E. coli* were prepared by inoculating a single colony into 10 mL of tryptic soy broth (TSB, Biokar Diagnostics, Allonne, France) and incubating it at 37 °C for 18 h. The cultures were then centrifuged at 4000 rpm for 10 min, and the resultant bacterial pellet was resuspended in a sterile 0.9% physiological solution to reach ~8 log CFU/mL.



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2.2. Preparation of Amlou-like Medium

To simulate the matrix of Amlou, a medium was prepared using argan oil, sugar and peptone derived from digested soymeal papain (Merck Millipore, Germany). The concentrations, ranging from argan oil (10–70% *v/v*), sugar (0–30% *w/v*), to peptone (10–70% *w/v*), were selected based on preliminary studies and traditional Amlou. This ensured a suitable environment for bacterial survival, while also representing a real-world consumption scenario.

After the sterilization of mixed argan oil and peptone in the autoclave (121 °C/15 min), filtered sugar solutions were added to the medium to give the concentrations described in the experimental design (Table 1). Subsequently, 100 mL samples were transferred to sterile 500 mL bottles, and then each Amlou-like medium was inoculated with the prepared *E. coli* suspension to achieve an initial concentration of approximately ~6 log CFU/mL. The samples were incubated under ambient temperatures (i.e., 25 °C) during constant shaking at 180 rpm.

Table 1. Results from the Baranyi model for *E. coli* survival under varying argan oil, sugar, and peptone concentrations in Amlou-like medium.

Factors			Output	
Peptone %	Sugar %	Argan Oil %	IR (log CFU/g/h)	SP (h)
40	15	40	−0.0814	0
40	15	40	−0.0766	0
40	15	40	−0.0738	0
70	15	40	0.0292	140.20
10	15	40	0.0107	199.72
40	30	40	−0.0253	0
40	0	40	0.00487	0
40	15	70	−0.0977	156.69
40	15	10	−0.0607	0
40	30	70	−0.0602	0
70	15	70	−0.0608	0
10	15	10	−0.0451	0
70	30	40	−0.0763	0
10	0	40	0.0113	25.41

2.3. Experimental Design

The study employed the Doehlert matrix design, an efficient and systematic method for simultaneously optimizing and studying the impact of multiple variables. The design allowed the evaluation of three factors (argan oil, sugar, and peptone concentrations) at multiple levels, resulting in 14 experimental runs. Each run was performed in duplicate.

2.4. Microbial Enumeration

At appropriate time intervals, 1 mL of the sample was drawn aseptically and subjected to serial dilution. Aliquots from appropriate dilutions were plated onto Tryptone Bile Glucuronide Agar plates (TBX, Biokar) to selectively enumerate the *E. coli*. The plates were incubated at 37 °C for 24 h, after which the colonies were counted.

2.5. Statistical Analysis and Modeling

The collected data were subjected to a response surface methodology (RSM) using the software package Design Expert (Version 12.0.12, Stat-Ease Inc., Minneapolis, MN, USA). The Baranyi model, renowned for its accuracy in predicting microbial survival kinetic in food systems, was used to fit the data [4]. Kinetic parameters refer to the death rate (DR, log CFU/g/h), which indicates the logarithmic reduction of the microbial population over

the study period. The shoulder phase (SP, h) represents the initial microbial resistance. DMFit version 3.5 was used to perform curve fitting.

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_{ii} + \sum_{i=1}^k \beta_{ij} X_{ij} + \varepsilon \quad (1)$$

2.6. Model Performance Assessment

To critically evaluate the efficacy and predictive accuracy of the developed models, several statistical parameters were employed, namely, the adjusted coefficient of determination (Adjusted R^2), the root-mean-square error (RMSE), and the standard error of prediction percentage (% SEP). It is noteworthy that % SEP offers a distinct advantage as a relative error metric; it remains unaffected by the magnitude of the observed data, a characteristic that has been underscored in previous studies [5]. The respective mathematical formulations for RMSE and % SEP are delineated as

$$RMSE = \sqrt{\frac{\sum (obs - pred)^2}{n}} \quad (2)$$

$$\%MSE = \frac{100}{|\overline{obs}|} \sqrt{\frac{\sum (obs - pred)^2}{n}} \quad (3)$$

where \overline{obs} represents the observed values, $pred$ designates values predicted by the respective model, \overline{obs} is the mean of the observed values, and n denotes the total number of data points utilized in the analysis.

3. Results and Discussion

The fitted kinetic parameter (DR and SP) values obtained for the Baranyi model are reported in Table 1. The Doehlert matrix design enabled the comprehensive assessment of the impact of sugar concentration on *E. coli*'s DR. Notably, with an increasing sugar concentration from 0% to 30% *w/v*, a significant increase in the DR was evident ($p < 0.05$). At the highest sugar concentration, the DR was approximately five-fold higher than that at the lowest concentration. This suggests that osmotic stress induced by elevated sugar levels exerts a detrimental effect on bacterial cells, corroborating the findings of our previous study [3].

Argan oil evidently has an influence on the SP, particularly with a p value of 0.005 (Table 2). This suggests that argan oil's primary role is in modulating the duration of bacterial survival. In contrast, its effect on the instantaneous rate of bacterial death appears minimal. In contrast, peptone's influence was dual-faceted. While lower concentrations increased the SP, possibly as a result of nutrient provision, concentrations above this level appear to decrease the SP. The observed variations in survival outcomes at different peptone concentrations may be attributed to the complex relationship between nutrient availability and bacterial physiology. Peptone is a complex mixture of peptides and amino acids that serves as a crucial nutrient source for bacterial growth and survival [6]. *E. coli* survival in the presence of peptone can be explained through several mechanisms, including nutrient limitation and growth dynamics, toxicity and inhibitory effects, and substrate utilization dynamics [6–8]. However, more research is needed to fully understand the mechanism via which peptone affects *E. coli* survival in Amlou [3].

Furthermore, the quadratic effects were highly significant for both the IR and SP ($p = 0.000$), especially those associated with the protein concentration (Table 2 and Figure 1). This observation highlights the presence of a non-linear relationship with increasing concentrations, suggesting that the ingredient's effect may show significant changes beyond certain concentrations.

Table 2. Significance of factors affecting *E. coli* survival in Amlou-like medium.

Factors	Response Variable			
	IR (log cfu/g/h)		SP (h)	
	F	p	F	p
Model	15.48	0.002	16.35	0.004
A-Argan oil	4.29	0.084	22.39	0.005
B-Sugar	19.40	0.005	1.04	0.356
C-Peptone	0.02	0.894	11.79	0.019
AB	-	-	20.48	0.006
AC	27.34	0.002	75.46	0.000
BC	45.58	0.001	51.45	0.001
A ²			18.25	0.008
B ²	38.29	0.001	-	-
C ²	70.56	0.000	85.86	0.000
Adjusted R ²	0.89		0.90	

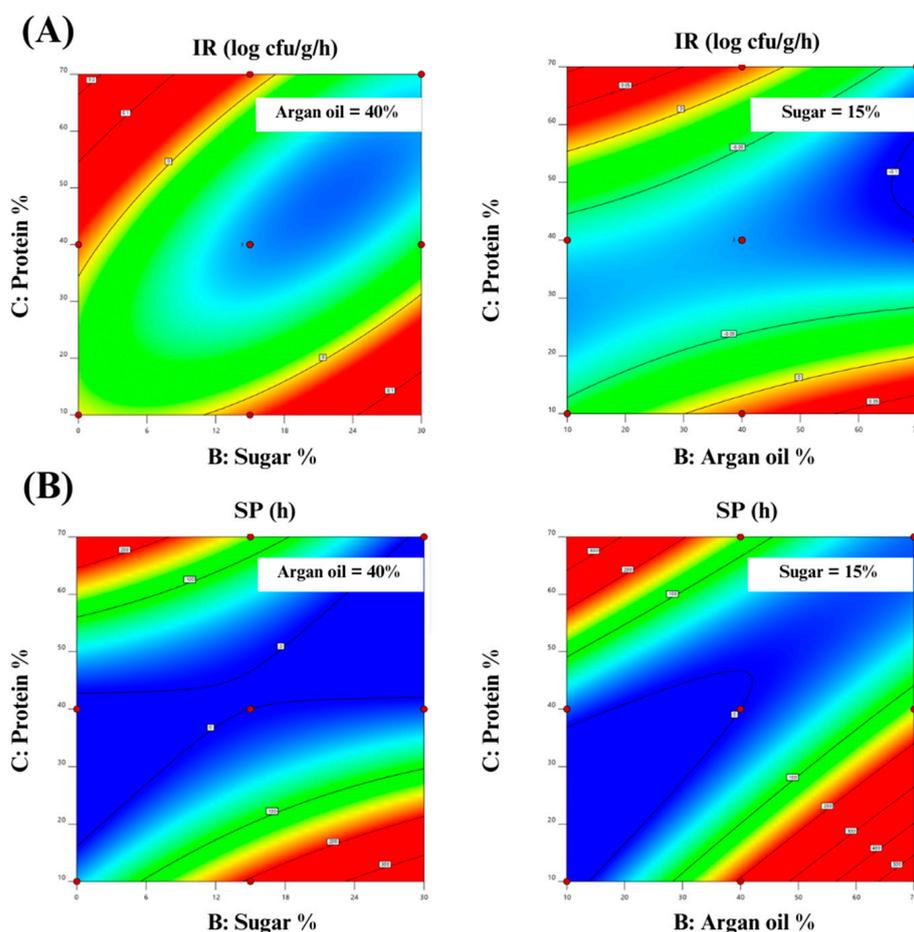


Figure 1. Quadratic response surfaces, predicting the IR (A) and SP (B) of *E. coli* in Amlou-like medium, as a function of argan oil, sugar, and peptone concentrations.

The adjusted R² values were 0.89 and 0.90 for the DR and SP, respectively, showing a good agreement between the predictions and observed inactivation data (Table 2). Furthermore, p values < 0.05 indicated that the models were adequate to describe the observed data. Additionally, the model values for the RMSE and SEP of 0.0095 and 22.22% were obtained for the DR, respectively. Based on these criteria, the models adequately described the experimental data and can be used to calculate DR and SP as functions of factors studied within the experiment’s limits.

Besides Amlou, the insights from this study have significance for a variety of artisanal food products that rely on a similar matrix. Sugar, oils, and proteins are ubiquitous in various culinary traditions, and understanding their microbial interplay can inform safer food production practices globally.

4. Conclusions

This study demonstrates the importance of maintaining a balance between traditional authenticity and microbial safety when producing artisanal Amlou. As well as enhancing the flavor, high sugar concentrations also inhibit *E. coli* growth. Additionally, argan oil provides a protective environment, which may extend the product's shelf life. In spite of this, careful calibration is recommended, especially in light of the interaction between the ingredients and the dynamics of microbes.

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