biology and life sciences forum

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Proceeding Paper

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

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† Presented at the 4th International Electronic Conference on Foods, 15–30 October 2023; Available online: https://foods2023.sciforum.net/.

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 death rate (DR) and survival period (SP). Sugar affected DR, while argan oil and peptone influenced SP. The DR model demonstrated a 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

Citation: Ezzaky, Y.; Zanzan, M.; Elidrissi, A.; Boussif, K.; Achemchem, F. Evaluating the impact of argan oil, sugar, and peptone concentrations on the survival of *Escherichia coli*. *Biol. Life Sci. Forum* **2023**, *3*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Name

Published: date

1. Introduction

Amlou, a popular product in Moroccan culinary traditions, offers a blend of almonds, honey, and the 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 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, France) and incubating 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.

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 soymeal papain-digested (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 real-world consumption scenarios.

After sterilisation 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, 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) with constant shaking at 180 rpm.

2.3. Experimental Design

The study employed the Doehlert matrix design, an efficient and systematic method for optimizing and studying the impact of multiple variables simultaneously. The design allowed for 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 *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 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 in 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 the curve fitting.

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

2.7. 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 squared error (RMSE), and the standard error of prediction percentage (% SEP). It's 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 mathematical formulations for RMSE and % SEP are delineated as:

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

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

where *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 a comprehensive assessment of the impact of sugar concentration on *E. coli* DR. Notably, with 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 5-fold higher than 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].

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

Argan oil became evident as its influence on the SP was observed, 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 [6,8]. *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 [6,7]. However, more research is needed to fully understand the mechanism by which peptone affects *E. coli* survival in Amlou [3].

Furthermore, the quadratic effects were highly significant for both IR and SP (p = 0.000), especially those associated with 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	

The adjusted R^2 values were 0.89 and 0.90 for DR and SP, respectively, showing good agreement between predictions and observed inactivation data (Table 2). Furthermore, *p* values < 0.05 indicated that the models were adequate to describe the observed data. Additionally, model values for RMSE and SEP of 0.0095 and 22.22% were obtained for DR. 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 experiments' limits.

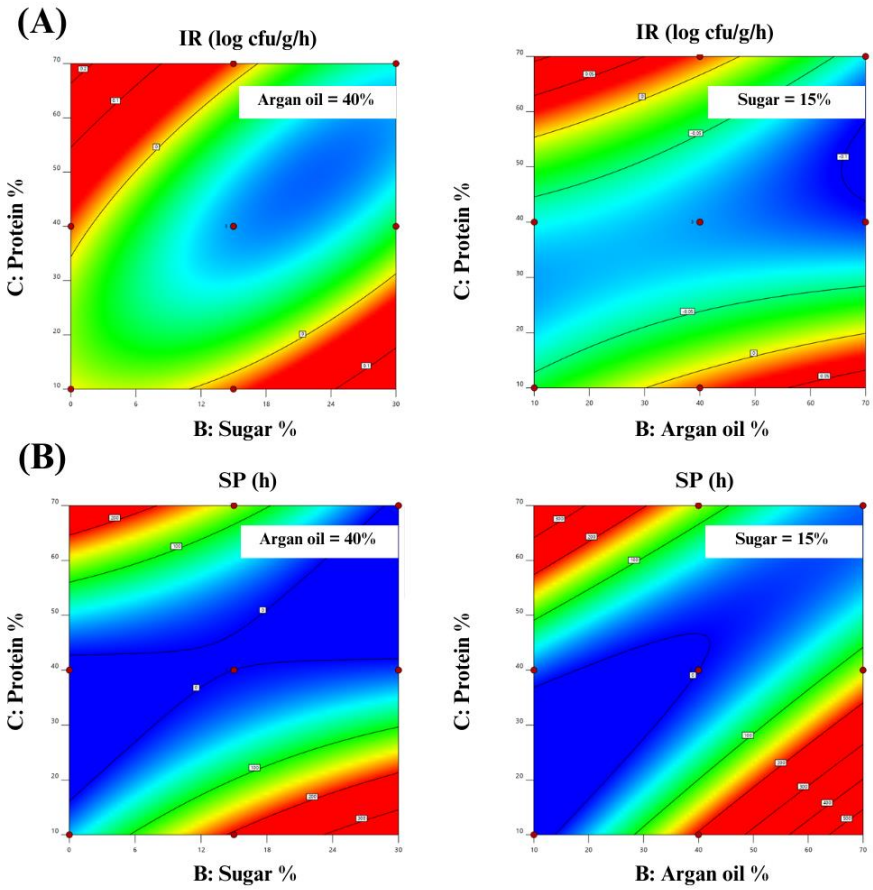

Figure 1. Quadratic response surfaces, predicting the IR (**a**) and SP (**b**) of *E. coli* in Amlou-like Medium, as a function of argan oil, sugar and peptone concentration.

Beyond 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 flavor, high sugar concentrations also inhibit E. coli growth. Additionally, argan oil provides a protective environment, which may extend product shelf life. In spite of this, careful calibration is recommended, especially in light of the interaction between ingredients and the dynamics of microbes.

Author Contributions: Conceptualization, Y.E.; methodology, Y.E. and M.Z.; validation, A.E., K.B.; formal analysis, Y.E.; investigation, Y.E. and M.Z.; writing—original draft preparation, Y.E.; writing—review and editing, F.A. and M.Z.; visualization, A.E.; supervision, F.A. All authors have read and agreed to the published version of the manuscript.

Funding:

Institutional Review Board Statement:

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Charrouf, Z.; Guillaume, D. Should the amazigh diet (regular and moderate argan-oil consumption) have a beneficial impact on human health? *Crit. Rev. Food Sci. Nutr.* **2010**, *50*, 473–477.
2. Capozzi, V.; Fragasso, M.; Russo, P. Microbiological Safety and the Management of Microbial Resources in Artisanal Foods and Beverages: The Need for a Transdisciplinary Assessment to Conciliate Actual Trends and Risks Avoidance. *Microorganisms* **2020**, *8*, 306.
3. Ezzaky, E.; Zanzan, M.; Achemchem, F.; Valero, A.; Hamadi, F. Microbiological profile and modeling the survival of *Escherichia coli* ATCC 25922 in Amlou: A traditional Moroccan spread food. *Microb. Risk Anal.* **2022**, *21*, 100216.
4. Baranyi, J.; Roberts, T.A. Mathematics of predictive food microbiology. *Int. J. Food Microbiol*. **1995**, *26*, 199–218.
5. Dong, Q.; Tu, K.; Guo, L.; Li, H.; Zhao, Y. Response surface model for prediction of growth parameters from spores of *Clostridium sporogenes* under different experimental conditions. *Food microbiol.* **2007**, *24*, 624–632.
6. Davami, F.; Eghbalpour, F.; Nematollahi, L.; Barkhordari, F.; Mahboudi, F. Effects of Peptone Supplementation in Different Culture Media on Growth, Metabolic Pathway and Productivity of CHO DG44 Cells a New Insight into Amino Acid Profiles. *Iran. Biomed. J.* **2015**, *19*, 194–205.
7. McQuestin, O.J.; Shadbolt, C.T.; Ross, T. Quantification of the relative effects of temperature, pH, and water activity on inactivation of *Escherichia coli* in fermented meat by meta-analysis. *Appl. Environ. Microbiol*. **2009**, *75*, 6963–6972.
8. Amezaga, M.R.; Booth, I.R. Osmoprotection of *Escherichia coli* by peptone is mediated by the uptake and accumulation of free proline but not of proline-containing peptides. *Appl. Environ. Microbiol*. **1999**, *65*, 5272–5278.