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# Optimization of Fermentation Factors for Vinegar Production from Prickly Pears using Response Surface Methodology (RSM)

Djamel Mamen[1], Dalila Abdessemed[1]

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## ABSTRACT

**Background:** Prickly pears are abundant in the Aurès region but are intended only for direct human consumption, due to the limited processing of this highly perishable fruit. To enhance the use of this precious resource, prickly pear vinegar [*Opuntia ficus indica* L. Miller], enriched with seed powder, can be produced by a double biological fermentation process, first alcoholic then acetic.

**Methods:** The Response Surface Methodology (RSM) was employed to optimize acetic fermentation process. This study investigated the influence of two independent variables, fermentation time and amount of added seed powder, on the chemical changes that affect five parameters (reducing sugar, ethanol, acetic acid, total phenolic and free radical scavenging activity) of vinegar obtained by the traditional method "Orléans".

**Result:** The fermentation time had a significant impact on all chemical properties examined, while the amount of added seed powder had a significant impact only on total phenolic contents and free radical scavenging activity. At the optimal point (fermentation time= 248.81 h and seed powder= 35.52%), the vinegar produced contained 1.73±0.31% reducing sugar, 0.42±0.02% ethanol, 5.55±0.5% acetic acid, 1670.14±2.9 mg GAE/L total phenolic and 61.33±0.45 mg AA/100 mL free radical scavenging activity. As a result, prickly pear vinegar enriched with seed powder was successfully made. It has a high level of bioactive components and a strong antioxidant potential, so it can be used as a functional food.


**Key words:** Fermentation, Optimization, Prickly pear, Response surface methodology, Seeds, Vinegar.

## INTRODUCTION

According to the Algerian Food Regulations (1997) (Official Journal of the Algerian Republic N°18) the name "vinegar" is reserved for liquid prepared solely from a suitable substrate that comprises starch and/or sugars which contains not less than 5 g of acetic acid per 100 ml that is generated through the process of alcoholic fermentation followed by acetic fermentation. The production of vinegar is a very old practice; it is mainly linked to the appearance of alcoholic fermentation from different vegetable raw materials with the beginning of agriculture in human history (Mazza and Murooka, 2009). Vinegar is generally produced using either traditional or rapid fermentation methods (Morales *et al.,* 2001). Vinegar is a functional food with several biological properties, including antibacterial and antioxidant activity (Budak *et al.,* 2014).

*Opuntia ficus-indica*, commonly known as the prickly pear, exhibits a wide distribution across various regions of the globe, including Africa. Its fruits are fleshy and elongated, with a set amount of tough seeds (Piga, 2004). Several scientific studies have recently proven the advantages gained from prickly pear fruit in treating various diseases (Ondarza, 2016).

During the production of prickly pear vinegar, partial fermentation at high temperatures, even up to 37°C, can result in a still-high polyphenol content, provided that thermotolerant bacteria are used. However, it may lead to a reduction in the content of volatile compounds (Es sbata *et al.*, 2022).

[1]LAPAPEZA Laboratory, Institute of Veterinary Sciences and Agricultural Sciences, Batna 1 University, Batna 05000, Algeria.

**Corresponding Author:** Djamel Mamen, LAPAPEZA Laboratory, Institute of Veterinary Sciences and Agricultural Sciences, Batna 1 University, Batna 05000, Algeria.
Email: mamen.djamel@univ-batna.dz

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The primary goal of this research was to explore optimal methods for promoting the utilization of this valuable fruit. The focus was on transforming it into vinegar enriched with seed powder, which serves as a functional food, adding positive value in terms of phenolic compounds and anti-radical activity. This approach contributes to the valorization of the bioeconomic resources available to producing countries.

## MATERIALS AND METHODS

### Plant material, collection and preparation

Ripe prickly pear fruits (*Opuntia ficus indica* L.), harvested in August 2021 in the Chechar region, about fifty kilometres from the city of Khenchela, in the northeast of Algeria and

were used as plant material. The alcoholic fermentation process employed *Saccharomyces cerevisiae* yeast as a starter culture, which was procured from Saf-Instant Yeast, a product of S.I. Lesaffre SA located in Marcq-en-Baroeul, France. The plant was also identified in the LAPAPEZA laboratory at Batna 1 University, situated in Algeria.

**Preparation of prickly pear juice**

Prickly pear fruits were manually cleaned with running water, peeled, cut and mechanically crushed using a standard household blender. Subsequently, the blend was passed through a filter with a pore size of 0.5 mm to extract the pure juice (Benattia, 2017). The prickly pear juice has a Brix value of 14%, which represents a very acceptable rate. It was pasteurized at 72°C for 5 min, rapidly cooled using cold water and then clarified by centrifugation at 10,000 rpm for 20 min (Cruz-Cansino *et al.*, 2016; De Wit *et al.*, 2020).

**Preparation of seed powder**

After the seeds were separated from their pulp, they were rinsed in distilled water and left to air-dry in the shade for a period ranging from one to three days. Once completely dry, an electric grinder was employed to crush them into a fine powder. This powder was subsequently stored in a sterile, hermetically sealed jar at a temperature of 4°C (Benattia, 2017).

**Alcoholic fermentation of prickly pear juice**

Alcoholic fermentation was carried out according to the protocol described by Kong *et al.* (2018), with slight modifications. 150 ml of pasteurized prickly pear juice was fermented in a 250 ml Erlenmeyer flask at a constant temperature of 25°C for seven days. The vial was sealed with cotton and parafilm to avoid exposure to oxygen and minimize evaporation of volatile components. To initiate the fermentation process, 0.20 g/L of active dry yeast *Saccharomyces cerevisiae*, previously activated at 35°C for 20 minutes, was also introduced (Es-Sbata *et al.* 2022). Throughout the alcoholic fermentation, samples were collected every other day on days 1, 3, 5 and 7. Finally, sediment yeast cells were separated from the wine (supernatant) through centrifugation at 10,000 rpm for 20 min.

**Optimization of acetic fermentation**

In this study, a specific quantity of dried prickly pear seed powder was introduced into a sterile 100 ml Erlenmeyer flask, which already contained a sample of prickly pear wine that had undergone 7 days of alcoholic fermentation. Acetic fermentation was conducted at 30°C, following the gradual "Orléans" process outlined by Dabija *et al.* (2014). Throughout this phase, the flasks were covered with cotton to maintain aerobic conditions. The acetic fermentation occurred spontaneously, slow and taking effect only at the surface of the liquid. When the population of acetic acid bacteria reached a significant level, a layer of mother vinegar formed on the surface. Diverse samples were collected using a food-grade plastic tube. The fermentation endpoint was determined based on physico-chemical measurements and subsequently the vinegar was filtered (Ameur and Heleili, 2022). The prickly pear juice, pasteurized juice, wine and vinegar were stored at 4°C before analysis.

We developed a set of approach models to optimize acetic fermentation, employing a Central Composite Rotable Design (CCRD) with Design Expert software, version 11.1.0.1. The optimization focused on fermentation time (Tf) and prickly pear seed powder concentration (Csp) concerning the quantity of seeds incorporated (the amount of prickly pear needed to produce 150 ml of net juice contains an average of 11.41±0.32 g of seeds, representing 100%), which were optimized for their effects on the final product. The software selected five centre points to probe the interaction and impact of each factor, with an alpha value of 1.414. The different analyses of variance shed light on the development of polynomial equations.

The levels of fermentation time factor are 112.72 h, 150 h, 240 h, 330 h and 367.28 h; and those of concentration seed powder are 9.64%, 20%, 45%, 70% and 80.35%. We introduced to the software some limits in order to determine the optimal point by minimizing the ethanol content while simultaneously maximizing the responses of the acetic acid content, the total phenol content and the scavenging activity of DPPH radicals. The reducing sugar content was determined within the range.

Finally, we compare the values obtained experimentally with the predicted values given by software.

**Chemical analysis**

This analysis includes the determination of total reducing sugar content (g/100 ml) using the DNS method, as outlined by Wood and Bhat (1988). Ethanol content (%) was determined using the sodium dichromate method described by Betiku and Taiwo (2015). Acetic acid content (%) was analyzed using the titratable acidity method as described by Mat Isham *et al.* (2019). Total phenolic content (mg GAE/L) was measured using the Folin-Ciocalteu method outlined by Chew *et al.* (2008). Radical scavenging activity (mg AA/100 ml) was assessed using the method described by Ho *et al.* (2016) and Chew *et al.* (2008).

The $IC_{50}$ (concentration of an inhibitor that causes a 50% decrease in activity) is as follows:

$IC_{50}$ (ascorbate) was (0.464±0.02) mg/ml.

$IC_{50}$ (BHA) was (0.162±0.03) mg/ml.

**Statistical analysis**

The statistical methods employed in this study involved the utilisation of analysis of variance (ANOVA) and Fisher's least significant difference test (LSD) with a significance level of $p<0.05$ in IBM SPSS Statistics, Version 25. The response surface method based on a CCRD design was adopted to optimize the acetic fermentation through Design Expert software version 11.1.0.1.

The values shown for each analysis are the average of three independent measurements.

## RESULTS AND DISCUSSION

### Pasteurization of prickly pear juice

Pasteurization played a crucial role in maintaining the cleanliness and usability of our samples both before and during fermentation. After pasteurization, there were notable changes in the prickly pear juice's characteristics. Specifically, the total phenol content and free radical scavenging activity were significantly decreased ($p<0.05$), while the reducing sugar concentration showed a significant increase ($p<0.05$). However, pasteurization had no discernible effect on the ethanol and acetic acid content.

Kumar *et al.* (2017) and Phanindrakumar *et al.* (2005) have highlighted that conventional pasteurization through natural juice heating results in an elevation of reducing sugars, potentially due to the conversion of non-reducing sugars to reducing sugars. There was a slight increase in reducing sugars since prickly pear juice intrinsically contains only a minimal amount of non-reducing sugars, with sucrose constituting a mere 0.19% in prickly pear pulp (Salim *et al.*, 2009).

On the other hand, the recorded drop in total phenol content and free radical scavenging activity can be attributed to the harmful impact of heat treatment on the juice. Chen *et al.* (2013), Cinquanta *et al.* (2010) and Tahar *et al.* (2019) have reported decreases of approximately 13% and 10% in carotenoids and polyphenols, respectively after traditional thermal pasteurization.

### Alcoholic fermentation

During alcoholic fermentation, the sugar content decreased significantly ($p<0.05$). Simultaneously, the ethanol content increased significantly ($p<0.05$), as illustrated in Fig 1. This decrease in sugar content is attributed to the transformation of fermentable sugars into ethanol by yeasts, a process that does not require oxygen (Fatima and Mishra, 2015; Yıkmış *et al.*, 2020).

The initial acetic acid content of 0.04% experienced a significant increase ($p<0.05$) during alcoholic fermentation, reaching a level of 0.27%. Erasmus *et al.* (2004) discovered that high sugar concentrations during alcoholic fermentation induced yeast to produce a modest quantity of extracellular organic acids, primarily acetic acid, as a by-product in response to hyperosmotic stress.

However, neither the total phenolic content nor the DPPH free-radical scavenging activity were significantly affected by alcoholic fermentation ($p>0.05$). Most phenols remained unchanged during the fermentation process (Nogueira *et al.*, 2008) and the sample's anti-oxidative activity did not change either (Pérez-Gregorio *et al.*, 2011).

### Optimization of acetic fermentation

(Tf) had a significant effect ($p<0.05$) on reducing sugar content, while (Csp) did not have a significant effect ($p>0.05$) on reducing sugar content. The content of residual reducing sugars from alcoholic fermentation decreased simultaneously with (Tf) during acetic fermentation. These reducing sugars serve as a substrate for potential microbial activity during acetic fermentation (EJEMNI and MEJRIS, 2006). Fig 2(a) shows that a longer Tf caused a lower content of reducing sugars in vinegar.

In terms of ethanol content, a significant impact ($P<0.05$) caused by higher (Tf) was noticed, while (Cps) showed no statistically significant impact ($p>0.05$) after acetic fermentation. During this fermentation, *Acetobacter aceti* bacteria convert alcohol molecules into acetic acid molecules (Tesfaye *et al.* 2002), leading to a decrease in ethanol content. Nevertheless, there are losses of ethanol due to evaporation during acetic fermentation (Romero and

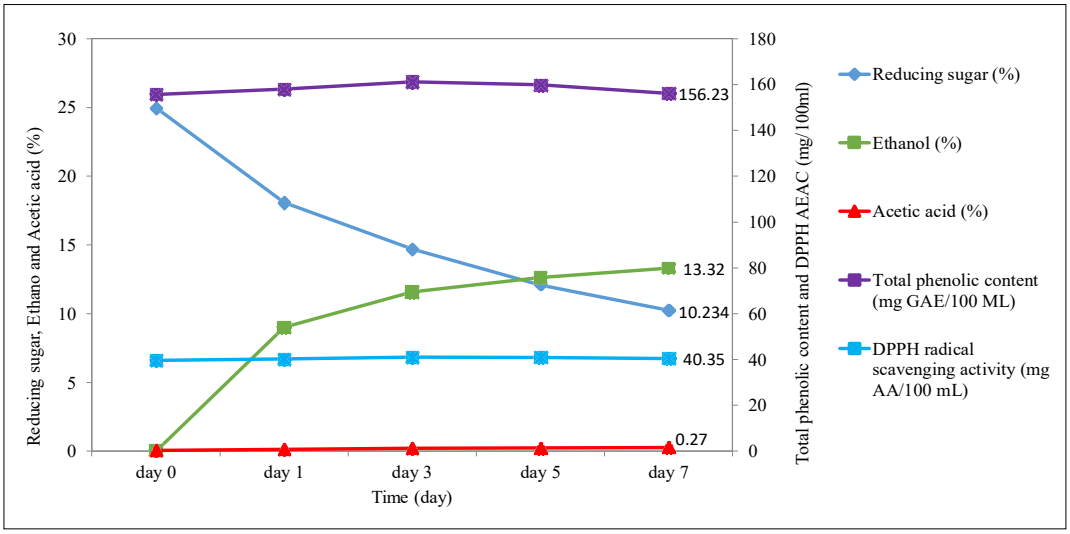



**Fig 1:** Changes of reducing sugar, ethanol, acetic acid, total phenolic content and DPPH AEAC during alcoholic fermentation.

Cantero, 1998). Therefore, as illustrated in Fig 2(b), a longer (Tf) resulted in a lower ethanol concentration in the sample.

Moreover, the findings in Table 1 reveal that (Tf) exerted a statistically significant impact ($p<0.05$) on acetic acid content during acetic fermentation, while (Cps) showed no significant impact ($p>0.05$). The acetic acid content initially increased, reaching a peak of more than 5%, but subsequently decreased gradually, as illustrated in Fig 2(c). It is noteworthy that acetic acid is a substance that evaporates rapidly during acetic fermentation (De Vuyst and Leroy, 2020).

Both (Tf) and (Cps) exhibited a significant effect ($p<0.05$) on total phenolic content and DPPH free radical scavenging activity, as indicated by Table 1 and Fig 2(d) and 2(e). According to studies by Andlauer *et al.* (2000) and Su and Chien (2007), acetification typically leads to a decrease in the total anthocyanin content, total phenolic content and antioxidant activities of vinegar during acetic fermentation. This decline is often attributed to the oxidation of bioactive compounds induced by continuous airflow. However, our results, as presented in Table 1 and Fig 2(d) and 2(e), contradict these findings. We observed a significant increase in the total phenolic content and antioxidant activity of vinegar, which can be attributed to the hydro-alcoholic extraction of bioactive compounds from the seed powder. Supporting this, Benattia (2017) reported that the hydro-ethanolic polar extract of prickly pear seeds is very rich in phenolic compounds and anti-radical activity.

**Verification of optimum point**

To assess the validity of the experimental design in optimizing the acetic fermentation process, response surface methodology was employed. Optimized conditions obtained from Design Expert software are (Tf)= 248.81 h and (Cps)= 35.52%, where the predicted values of the RSM model are compared to the experimental values of prickly pear vinegar samples produced in the verification process of acetic fermentation, as presented in Table 2.

The prickly pear vinegar produced 5.55% acetic acid content and 0.42% ethanol content, which meets the regulatory requirements for vinegar.

The prickly pear vinegar sample showed a significantly lower concentration of reducing sugars and total phenol content ($p<0.05$) compared to the predicted values, showing a very slight and acceptable deviation. Conversely, there were no statistically significant disparities ($p>0.05$) between the ethanol content, acetic acid content and free-radical scavenging activity of the prickly pear vinegar and the predicted values for these parameters. This proved that the RSM model is a good one.

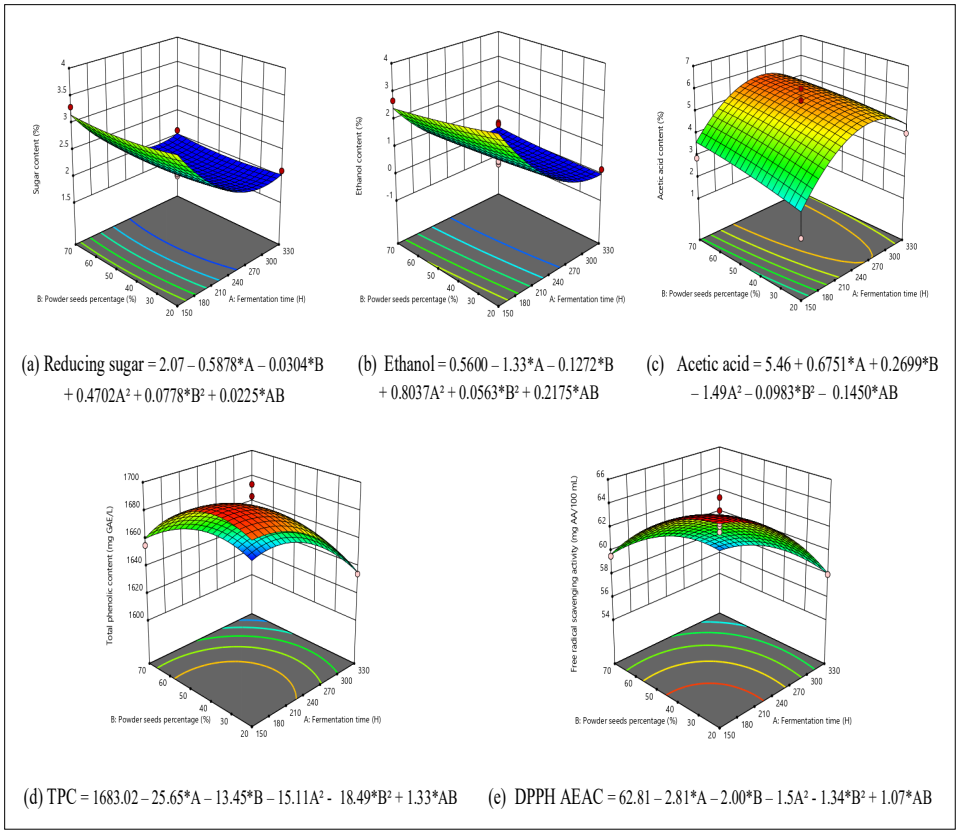



**Fig 2:** Response surfaces and models of fermentation time (factor A) and powder seeds concentration (factor B) towards (a) reducing sugar content, (b) ethanol content, (c) acetic acid content, (d) total phenolic content, (e) DPPH radical scavenging activity (AEAC) in acetic fermentation samples.

The prickly pear vinegar enriched with seed powder exhibited significantly ($p<0.05$) higher levels of reducing sugar content, total phenolics and free-radical scavenging activity compared to the non-enriched prickly pear vinegar prepared in the same laboratory and under identical conditions. Conversely, the content of acetic acid was significantly ($p<0.05$) lower in enriched prickly pear vinegar. No significant difference was recorded regarding the ethanol content.

A probable antimicrobial activity linked to the bioactive compounds extracted from prickly pear seed powder, causing a delay and a partial inhibition of the activity of *Acetobacter*, which explains the lower level of acetic acid in the enriched prickly pear vinegar. Moreover, this inhibition also affected the microbes responsible for the degradation of reducing sugars during acetic fermentation, causing a higher content of reducing sugars and the added seed powder causes a higher total phenolic content and a superior antioxidant power.

Table 2 displays the total phenolic content in enriched prickly pear vinegar samples, measured at (1670.18 mg GAE/L). This surpasses the levels found in unenriched prickly pear vinegar and exceeds the values reported for prickly pear vinegar by Hammouda *et al.* (2021) and traditional apple vinegar by Ozturk *et al.* (2015), which are (1631.44, 1636.75 and 434.88 mg GAE/L) respectively.

Notably, The IC50 of vinegar samples (concentration that inhibits 50% of the DPPH radical) was 0.16 for enriched prickly pear vinegar samples and (0.19; 0.19; and 3.43 mg/mL) for unenriched prickly pear vinegar samples, prickly pear vinegar reported by Hammouda *et al.* (2021) and traditional apple vinegar reported by Ozturk *et al.* (2015). It is well known that the antiradical activity is better when the IC50 value is lower.

**Chemical composition changes during the different stages of production of enriched prickly pear vinegar**

The changes in chemical composition from raw prickly pear juice through pasteurized juice and wine to vinegar aredepicted in Fig 3. Total phenolic content and free-radical scavenging activity of prickly pear juice dropped after pasteurization, while the reducing sugar concentration rose. Polysaccharide hydrolysis led to an increase in reducing sugar content. The significant reduction in total phenolic concentration and free radical scavenging

**Table 1:** Content of sugar, ethanol, acetic acid, total phenolic and free radical scavenging activity in 13 runs through CCRD design for acetic fermentation.

| Run | Factors | | | | | Responses | |
|---|---|---|---|---|---|---|---|
| | Fermentation Time (h) | Powder seed concentration (%) | Reducing sugar content (%) | Ethanol content (%) | Acetic acid content (%) | Total phenolic content (mg GAE/L) | Free radical scavenging activity (mg AA/100 ml) |
| 1 | 240 | 45 | 2.11 | 0.37 | 5.48 | 1675.24 | 63.43 |
| 2 | 367.279 | 45 | 2.13 | 0.18 | 3.52 | 1613.21 | 55.26 |
| 3 | 240 | 45 | 2.04 | 0.86 | 5.02 | 1673.39 | 61.63 |
| 4 | 330 | 70 | 2.09 | 0.28 | 4.64 | 1615.76 | 57.06 |
| 5 | 150 | 70 | 3.3 | 2.68 | 2.88 | 1655.39 | 59.58 |
| 6 | 240 | 45 | 2.14 | 0.48 | 6.02 | 1677.56 | 62.34 |
| 7 | 240 | 9.64466 | 2.16 | 0.58 | 5.72 | 1671.92 | 63.73 |
| 8 | 240 | 45 | 2.06 | 0.45 | 5.5 | 1698.64 | 62.12 |
| 9 | 240 | 45 | 2.01 | 0.64 | 5.3 | 1690.25 | 64.52 |
| 10 | 240 | 80.3553 | 2.08 | 0.32 | 5.96 | 1626.31 | 56.77 |
| 11 | 330 | 20 | 2.11 | 0.17 | 4.02 | 1634.65 | 58 |
| 12 | 150 | 20 | 3.41 | 3.44 | 1.68 | 1679.59 | 64.78 |
| 13 | 112.721 | 45 | 3.68 | 3.71 | 2.6 | 1698.53 | 64.58 |

**Table 2:** Chemical composition of improved prickly pear vinegar sample and predicted values from RSM model.

| Response | Predicted value | Experimental value (Prickly pear vinegar enriched with its seed powder) | Prickly pear vinegar |
|---|---|---|---|
| Reducing sugar (%) | 2.04 | 1.73±0.31 | 1.09±2.14 |
| Ethanol (%) | 0.48 | 0.42±0.02 | 0.39±0.08 |
| Acetic acid (%) | 5.40 | 5.55±0.50 | 6.32±0.15 |
| Total phenolic content (mg GAE/ L) | 1682.75 | 1670.14±2.90 | 1631.44±7.51 |
| Free-radical scavenging activity (mg AA/100 mL) | 63.04 | 61.33±0.45 | 59.54±0.36 |

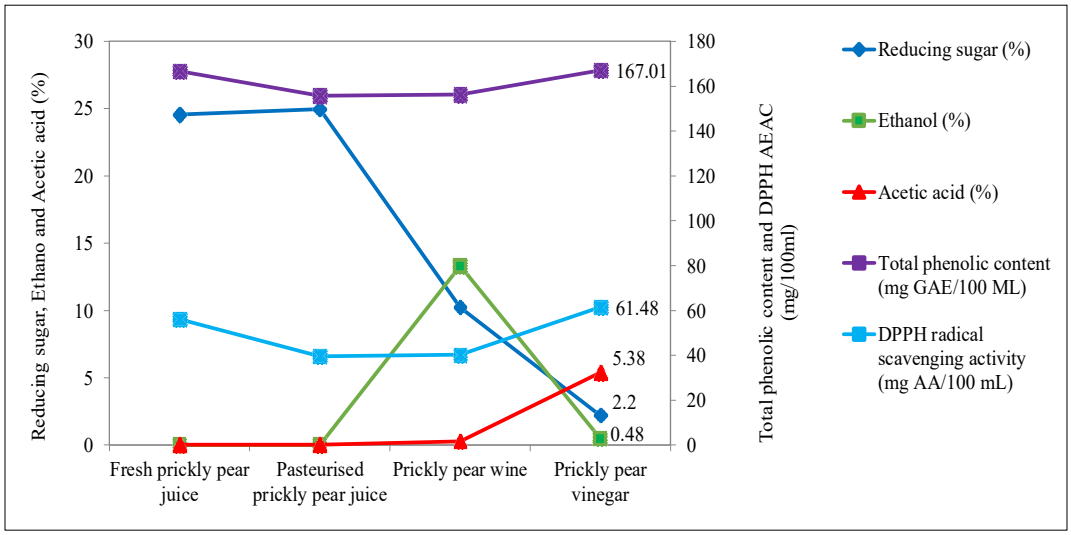



**Fig 3:** Chemical composition changes during the different stages of production of improved prickly pear vinegar.

activity is attributed to their destruction upon the application of heat treatment (Chen *et al.,* 2013). Heat processing had no effect on the ethanol and acetic acid contents.

During alcoholic fermentation, the increase in ethanol content and decrease in sugar content observed in pasteurised prickly pear juice is due to the transformation of sugar molecules into ethanol through the action of yeast (Fatima and Mishra, 2015; Yıkmış *et al.,* 2020). Acetic acid content in wine samples also increased. Total phenolic content and free radical scavenging activity did not differ significantly ($p>0.05$) between the juice and wine samples.

Acetic fermentation leads to an increase in acetic acid content and a decrease in ethanol content, as *Acetobacter* spp. convert ethanol into acetic acid. (Tesfaye *et al.,* 2002). A significant increase in the total phenolic content and antioxidant activity of vinegar compared to wine was recorded. This is simply because during the acetic fermentation, a hydro-alcoholic extraction of bioactive compounds from the seed powder occurred (Benattia and Arrar, 2018).

## CONCLUSION

Prickly pear vinegar enriched with seed powder was successfully produced using the slow Orléans process, meeting both national and global standards with an acetic acid rate of 5.55% and ethanol content of 0.42%. The optimization of acetic fermentation was also achieved, with the optimal fermentation time and seed powder concentration determined as 248.81 h and 35.52%, respectively. The results show no statistically significant difference ($p>0.05$) for the following parameters (ethanol content, acetic acid and free radical trapping activity) between the enriched prickly pear vinegar and the values predicted by the software. This proved that the RSM model is a good one. Additionally, prickly pear enriched vinegar exhibits superior quality in terms of total polyphenol bioactive compounds and antioxidant activity when compared to unenriched vinegar. In a global way, our research has demonstrated promising results in the production of prickly pear vinegar enriched with seed powder. This innovation has the potential to reduce waste by transforming this perishable fruit into a functional food. Currently, in-depth studies on the various biological activities attributable to our enriched vinegar are underway.

### Conflicts of interest

The authors assert that they have no conflicts of interest.

## REFERENCES

Algerian Food Regulations (Journal of the Republic of Algeria N 18.) (1997). Arrêté inter ministétriel du 24 Rajab 1418 correspondant on 25 November 1997 Relatif auxiliary techniques and auxiliary modalities and auxiliary conditions used in the consumption of vinegars. Accessed November 11, 2022, from https://www.commerce.gov.dz/fr/reglementation/arrete-interministeriel-du-25-novembre-1997.

Ameur, D. and Heleili, N. (2022). Comparative study on physico-chemical and sensory properties of vinegar produced from apple varieties. Agricultural Science Digest: A Research Journal. 42(5): 534-540. doi: 10.18805/ag.DF-401.

Andlauer, W., Stumpf, C. and Fürst, P. (2000). Influence of the acetification process on phenolic compounds. Journal of Agricultural and Food Chemistry: 48(8): 3533-3536.

Benattia, K. (2017). Analyse et application des extraits de pépins de Figues de Bararie. Doctoral dissertation. Université de Tlemcen-Abou Bekr Belkaid.

Betiku, E. and Taiwo, A.E. (2015). Modeling and optimization of bioethanol production from breadfruit starch hydrolyzate vis-à-vis response surface methodology and artificial neural network. Renewable Energy. 74: 87-94.

Budak, N.H., Aykin, E., Seydim, A.C., Greene, A.K., Guzel Seydim, Z.B. (2014). Functional properties of vinegar. Journal of Food Science. 79(5): R757-R764.

Chen, Y., Yu, L.J., Rupasinghe, H.V. (2013). Effect of thermal and non thermal pasteurisation on the microbial inactivation and phenolic degradation in fruit juice: A mini review. Journal of the Science of Food and Agriculture. 93(5): 981-986.

Chew, Y.L., Lim, Y.Y., Omar, M., Khoo, K.S. (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Sci Technol. 41(6): 1067-1072.

Cinquanta, L., Albanese, D., Cuccurullo, G., Di Matteo, M. (2010). Effect on orange juice of batch pasteurization in an improved pilot scale microwave oven. Journal of Food Science. 75(1): E46-E50.

Cruz Cansino, N.D.S., Montiel Columna, N.I., Bautista Velueta, P.G., Pérez Tinoco, M.R., Alanís García, E., Ramírez Moreno, E. (2016). Optimization of Thermoultrasound conditions for the processing of a prickly pear juice blend (*Opuntia ficus indica*) using response surface methodology. Journal of Food Quality. 39(6): 780-791.

Dabija, A., Hatnean, C.A. (2014). Study concerning the quality of apple vinegar obtained through classical method. Journal of Agroalimentary Processes and Technologies. 20(4): 304-310.

De Vuyst, L. and Leroy, F. (2020). Functional role of yeasts, lactic acid bacteria and acetic acid bacteria in cocoa fermentation processes. FEMS Microbiology Reviews. 44(4): 432-453.

De Wit, M., Du Toit, A., Osthoff, G. and Hugo, A. (2020). Antioxidant content, capacity and retention in fresh and processed cactus pear (*Opuntia ficus-indica* and *O. robusta*) fruit peels from different fruit-colored cultivars. Frontiers in Sustainable Food Systems. 4: 133.

Ejemni, M. and Mejri, S. (2006). Valorisation des écarts de triage de dattes. cas d'application: Production biologique du vinaigre. Actes des 13émes Journées Scientifiques sur les Résultats de la Recherche Agricoles. Hammamet: 861-870.

Erasmus, D.J., Cliff, M. and Van Vuuren, H.J. (2004). Impact of yeast strain on the production of acetic acid, glycerol and the sensory attributes of icewine. American Journal of Enology and Viticulture. 55(4): 371-378.

Es-sbata, I., Castro, R., Carmona-Jiménez, Y., Zouhair, R. and Durán-Guerrero, E. (2022). Influence of different bacteria Inocula and temperature levels on the chemical composition and antioxidant activity of prickly pear vinegar produced by surface culture. Foods. 11(3): 303.

Fatima, B. and Mishra, A.A. (2015). Optimization of Process Parameter for the Production of Vinegar from Banana Peel and Coconut Water. International Journal of Science, Engineering and Technology. 3(3): 817-823.

Hammouda, M.B., Mahfoudhi, A., Gharsallah, H., El Hatmi, H., Attia, H. and Azabou, S. (2021). Traditional homemade Tunisian vinegars: Phytochemical profile, biological, physicochemical and microbiological properties. LWT. 152: 112293.

Ho, C. W., Lazim, A.M., Fazry, S., Zaki, U.K.H.H. and Lim, S.J. (2017). Varieties, production, composition and health benefits of vinegars: A review. Food Chemistry. 221: 1621-1630.

Isham, N.K.M., Mokhtar, N., Fazry, S., Lim, S.J. (2019). The development of an alternative fermentation model system for vinegar production. LWT. 100: 322-327.

Kong, C.T., Ho, C.W., Ling, J.W.A., Lazim, A., Fazry, S., Lim, S.J. (2018). Chemical changes and optimisation of acetous fermentation time and mother of vinegar concentration in the production of vinegar-like fermented papaya beverage. Sains Malaysiana. 47(9): 2017-2026.

Kumar, S., Khadka, M., Mishra, R., Kohli, D. and Upadhaya, S. (2017). Effects of conventional and microwave heating pasteurization on physiochemical properties of pomelo (*Citrus maxima*) juice. Journal of Food Processing and Technology. 8(07): 8-11.

Mazza, S. and Murooka, Y. (2009). Vinegars through the ages. Vinegars of the World. pp. 17-39.

Morales, L.M., González, G.A., Casas, J.A., Troncoso, A.M. (2001). Multivariate analysis of commercial and laboratory produced Sherry wine vinegars: Influence of acetification and aging. European Food Research and Technology. 212: 676-682.

Nogueira, A., Guyot, S., Marnet, N., Lequéré, J.M., Drilleau, J.F., Wosiacki, G. (2008). Effect of Alcoholic Fermentation in the Content of Phenolic Compounds in Cider Processing. Brazilian Archives of Biology and Technology. 51: 1025-1032.

Ondarza, M. A. (2016). Cactus mucilages: nutritional, health benefits and clinical trials. Journal of Medical and Biological Science Research. 2(6): 87-103.

Ozturk, I., Caliskan, O.Z.N.U.R., Tornuk, F., Ozcan, N., Yalcin, H., Baslar, M. and Sagdic, O. (2015). Antioxidant, antimicrobial, mineral, volatile, physicochemical and microbiological characteristics of traditional home-made Turkish vinegars. LWT-Food Science and Technology. 63(1): 144-151.

Pérez-Gregorio, M. R., Regueiro, J., Alonso-González, E., Pastrana-Castro, L. M., Simal-Gándara, J. (2011). Influence of alcoholic fermentation process on antioxidant activity and phenolic levels from mulberries (*Morus nigra* L.). LWT-Food Science and Technology. 44(8): 1793-1801.

Phanindrakumar, H.S., Radhakrishna, K., Mahesh, S., Jagannath, J.H., Bawa, A.S. (2005). Effect of pretreatments and additives on the thermal behavior and hygroscopicity of freeze dried pineapple juice powder. Journal of Food Processing and Preservation. 29(5 6): 307-318.

Piga, A. (2004). Cactus pear: a fruit of nutraceutical and functional importance. Journal of the Professional Association for Cactus Development. 6: 9-22.

Romero, L.E. and Cantero, D. (1998). Evaluation of ethanol evaporation losses in acetic acid fermentations. Bioprocess Engineering. 18(4): 289-291.

Salim, N., Abdelwaheb, C., Rabah, C., Ahcene, B. (2009). Chemical composition of *Opuntia ficus-indica* (L.) fruit. African Journal of Biotechnology. 8(8): 1623-1624.

Su, M.S. and Chien, P.J. (2007). Antioxidant activity, anthocyanins and phenolics of rabbiteye blueberry (*Vaccinium ashei*) fluid products as affected by fermentation. Food Chemistry. 104(1): 182-187.

Tahar, H.S., Hamid, G., Rachid, H.B., Souad, H. (2019). Evolution of bioactive components of Prickly Pear Juice (*Opuntia ficus indica*) and cocktails with orange juice. Nature and Technology. (20): 45-53.

Tesfaye, W., Morales, M.L., Garcıa-Parrilla, M.C., Troncoso, A.M. (2002). Wine vinegar: Technology, authenticity and quality evaluation. Trends in Food Science and Technology. 13(1): 12-21.

Wood, T.M. and Bhat, K.M. (1988). Methods for Measuring Cellulase Activities. In: Methods in enzymology. Vol. 160. Academic Press: 87-112.

Yıkmış, S., Bozgeyik, E., Şimşek, M.A. (2020). Ultrasound processing of verjuice (unripe grape juice) vinegar: Effect on bioactive compounds, sensory properties, microbiological quality and anticarcinogenic activity. Journal of Food Science and Technology. 57: 3445-3456.