Research Article

Investigating the Shelf Life of Mango Blends Enhanced with Olive Leaves Extract

Waseef Ullah Khan*, Yasser Durrani and Muhammad Ayub

Department of Food Science and Technology, Faculty of Nutrition Sciences, The University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan.

Abstract | The mango fruit is widely recognized as one of the most delectable, popular, and vital fruit crops in tropical and subtropical regions. Its renowned taste is celebrated worldwide, whether enjoyed fresh or in processed forms. This research was undertaken to assess the efficacy of employing both natural olive leaves extract (OLE) and minimal chemical preservatives (sodium benzoate and potassium sorbate) for preserving mango OLE blend over a 90 days period. The findings demonstrated that the inclusion of OLE effectively preserved the chemical quality and sensory characteristics of mango OLE blend with added chemical preservatives. Results showed that all samples retained total soluble solids between (17.89-20.80°Brix), pH (4.06- 4.91), color (6.94 to 8.01), flavor (5.34 to 6.34) and taste (5.59-6.99). The results concluded that OLE can serve as a dual-purpose solution, acting as a natural antioxidant and antimicrobial preservative for refrigerated mango OLE blend.

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***Correspondence** | Waseef Ullah Khan, Department of Food Science and Technology, Faculty of Nutrition Sciences, The University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan; **Email:** yasser@aup.edu.pk, ffresh_4u@yahoo.com
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Introduction

Mango (*Mangifera indica* L.) stands as a symbol of tropical indulgence, with its sweet, succulent flesh tantalizing taste buds across the globe. This luscious fruit is not only cherished in its fresh form but also finds its way into numerous processed products, including the ever-popular mango pulp products. The increasing demand for convenience and the desire to retain exotic savors of mangoes year-round has spurred the need for effective preservation techniques.

Preservation is paramount in ensuring the availability of high-quality mango pulp beyond its short harvesting season. While chemical preservatives have traditionally played a role in extending shelf life, the modern consumer is increasingly seeking natural and healthier alternatives. Consumer preferences, informed by a growing awareness of the impact of dietary choices on health and well-being, have catalyzed a shift towards functional and health-enhancing food and beverage options (Caponio *et al*., 2019). Olive Leaves Extract (OLE), a promising natural preservative derived from the leaves of the olive tree (*Olea europaea*). OLE has garnered attention for its potential to enhance the storage stability of various food products while aligning with the growing trend towards clean-label and sustainable food preservation (Khalifa *et al*., 2017).

This research work delves into the innovative approach of blending mango pulp with OLE and judiciously applying a minimum amount of chemical preservatives. By synergizing the antioxidant properties of olive leaves with carefully selected chemical preservatives. Our study aims to investigate the effectiveness of OLE with lesser amount of chemical preservatives in inhibiting microbial growth, preserving the chemical quality, and retaining the sensory attributes of mango OLE blend over an extended storage period, specifically 90 days. By doing so, we intend to contribute to the evolving landscape of food preservation, offering an eco-friendly and health-conscious alternative to traditional chemical preservatives for mango fruit products. As the global food industry pivots towards more sustainable practices, the utilization of OLE in mango pulp preservation holds the promise of enhancing both product quality and consumer satisfaction.

Materials and Methods

Fresh variety of mango Chaunsa was selected in order to prepare the pulp. Olive leaves were obtained from Agriculture Research Institute Tarnab, Peshawar for obtaining olive leaves extract (OLE). In the present study 4% OLE, and different concentrations of Potassium Sorbate and Sodium benzoate were used for the preservation of mango OLE blend. The grades used during experimentation with following abbreviations mango pulp blend with olive leaves extract ($M_{POE0)}$, mango pulp (MP), sodium benzoate (SB) and potassium sorbate (PS). Treatments for experimentation were planned as T0= MP+0.025% SB+0.025% PS, T1= MPOE+0.025% SB, T2= MPOE+0.025% PS, T3= MPOE+0.05% SB, T4=MPOE4+0.05% PS, T5= MPOE+0.1% SB, T6= MPOE+0.1% PS. These grades were stored under refrigerated temperature for the period of three months and their physicochemical as well as sensory attributes were evaluated.

Preparation of juice and squash of mango blend

Mango juice preparation of all the treatments was tailored to match the sensory preferences of 50 assessors (students of the department of Food Science and Technology) with slight modifications in the method initially outlined by (Carbonell *et al*., 2013). Subsequently, we created ready to serve juice (RTS) and squash variants by carefully adjusting the total soluble solids content to reach 17°Brix and 55°Brix, respectively. This adjustment was achieved through the addition of water and sugar, following the methodology described by (Awan and Salim.1999).

Mango OLE blends were analyzed for different characteristics including protein, fat, ash content, total soluble solids TSS, pH, acidity, using the standard methods proposed by (AOAC, 2012).

Crude protein

The crude protein was determined using micro Kjeldahl method as describe in (AOAC, 2012). 5g of mango OLE blend samples were taken in a Kjeldahl flask and 30 ml concentrated sulfuric acid (H_2SO_4) was added followed by the addition of 10 g potassium sulphate and 1 g copper sulphate. The mixture was heated first gently and then strongly once the frothing had ceased. When the solutions became colorless, samples were heated for another hour, allowed to cool, diluted with distilled water (washing the digestion flask) and transferred to 800 ml Kjeldahl flask. Three or four pieces of granulated zinc and 100 ml of 40% caustic soda were added and the flasks were connected with the splash heads of the distillation apparatus. Next 25 ml of 0.1 N sulphuric acid was taken in the receiving flask and distilled. When two-thirds of the liquid had been distilled, samples were tested for completion of reaction. The flasks were removed and titrated against 0.1 N caustic soda solution using methyl red indicator for determination of Kjeldahl nitrogen, which in turn gave the protein content. The nitrogen percent was calculated by the following formula.

$$N\% = 1.4(V2 - V1) \times \text{Normality of HCl} X\, 250\,(\text{dilution}) / \text{Weight of sample}$$

Whereas, protein content was estimated by conversion of nitrogen percentage to protein (James, 1995).

$$\text{Protein } \% = N\% \times \text{Conversion factor } (6.25)$$

Where conversion factor = 100/N (N% in fruit products)

Crude fat

Crude fat was determined by Mojonnier method

(James, 1995). The fat content was determined gravimetrically after extraction with diethyle ether (ethoxyethane) and petroleum ether from an ammonia alcoholic solution of all the treatments. About 10 g of sample was taken into a Mojonnier tube one by one. Added 1 ml of 0.880 with 10 ml ethanol mixed well and cooled. Added 25 ml diethyl ether, stopper the tube, shacked vigorously and then added 25 ml petroleum ether and left the tube to be stand for 1 h. The extraction was repeated thrice using a mixture of 5 ml ethanol, 25 ml diethyl ether and 25 ml petroleum ether and adding the extraction to the distillation flask. Distilled off the solvents, dried the flask for 1 h at 100°C and reweighed. The percentage fat content of the sample was calculated by the following formula which gave the difference in the weights or the original flask and the flask plus extracted fat represent the weight of fat present in the original samples.

$$\%Fat= W_2-W_1/W_3$$

Where; W = Weight of empty flask (g), W = Weight offlask + fat (g) and W = Weight of sample taken (g).

Ash content

Ash content of mango OLE blend was determined according to the standard method of (AOAC, 2012). About 5g of each sample was taken in crucibles and was partially closed with lids. The partially closed crucibles were placed in muffled furnace for 16 hours at 550 °C until the samples turned into grey ash. The crucibles were removed from the furnace and placed in desiccator until cooled. The final weight of the samples was determined and using the below given formula ash content was determined.

$$Ash\ content\ (\%) = \frac{Initial\ wight\ of\ pulp - Final\ weight\ of\ pulp}{Initial\ weight\ of\ pulp} \times 100$$

Total soluble solids

The total soluble solids (TSS) present in the mango OLE blend was evaluated using standard methods of (AOAC, 2012). Small amount of the tested sample was placed on to the prism of digital refractometer and the samples TSS was determined on refractometer screen.

Titratable acidity

The (AOAC, 2012) method was followed for the determination of titratable acidity of mango OLE blend. The quantity of 10g sample was poured in beaker which was then diluted with 50mL of distilled water and phenolphthalein (2 to 3 drops) were added and titrated against NaOH (0.1N).

pH

The pH of all blends samples was measured following the standard procedure of (AOAC, 2012) pH meter (Mettler-Toledo (S) Pte Ltd, Singapore) was used after standardizing it with the buffer solution. The electrodes were rinsed, marked, and then immersed into a beaker containing sample and the value shown in the screen was noted.

Sensory evaluation

The organoleptic assessment of the juice samples was conducted at the (Sensory Evaluation Laboratory), Department of Food Science and Technology, the University of Agriculture Peshawar. For this experiment (200) assessors, consisting of both university students and employees, participated in the sensory analysis. The juice samples were presented to each assessor in triplicate. The judges systematically evaluated the samples based on its color, flavor, taste, overall acceptability, by utilizing a 9-point hedonic scale as described by (Larmond, 1977). Each judge consistently assessed the same sample under uniform conditions, and the results were recorded as an average rating.

Statistical analysis

All the research parameters results were analyzed and compiled using SPSS in conjunction with MiniTab software. The various variables and their impacts were determined through a completely randomized design, employing two-factorial analyses. The least significant difference (LSD) method, as outlined by (Steel and Torrie, 1960), was utilized to assess the probability at $P \leq 0.05$ for the applied treatments.

Results and Discussion

Physico-chemical analyses of the mango olive leaves extract blend

The effect of minimal amounts of sodium benzoate and potassium sorbate in presence of olive leaves extract (OLE) in mango blend on its physico-chemical characteristics was investigated and is presented in Table 1. The data highlights the changes in protein, fat, ash contents, total soluble solids (TSS), acidity, and pH in response to different treatments (T0 to T6).

The protein content in the mango OLE blend samples showed variations across the treatments. Treatment

T0, which served as the control, exhibited the highest protein content (0.68±0.021%), while treatments T1, T2, T3, T4, T5 and T6 showed lower protein content, with T1 having the lowest (0.62±0.042%). This reduction in protein content with the addition of OLE and chemical preservatives may be attributed to enzymatic activities and degradation during the preservation process. Similarly, the fat content displayed variations among the treatments. Treatment T0 had the highest fat content (0.59±0.031%) while T5 had the lowest (0.49±0.091%). The changes in fat content were relatively subtle, indicating that the addition of OLE and chemical preservatives had a limited impact on fat levels in mango OLE blend. The ash content of mango OLE blend also exhibited variation. Treatment T1 displayed the highest ash content (0.58±0.004%), while T4 had the lowest (0.49±0.009%). The differences in ash content may reflect the mineral composition of the added preservatives and the initial composition of the mango OLE blend. Total soluble solids (TSS) are an important parameter reflecting the sweetness of the mango OLE blend. Treatment T2 exhibited the highest TSS (20.80±0.75°Brix), while T0 had the lowest (19.05±0.04°Brix). The increase in TSS in some treatments could be attributed to changes in sugar concentrations during storage and may influence the sensory perception of sweetness in the preserved mango OLE blend. It is noted during research that during storage the sugar concentration of mango juice increases, it can enhance the perceived sweetness of the juice. This is because the taste receptors on our tongues are sensitive to sugar molecules, and higher sugar concentrations trigger a stronger perception of sweetness (Arvanitoyannis and Ladas, 2008). The acidity of mango OLE blend showed variability among the treatments. Treatment T6 exhibited the highest acidity (0.59±0.013%), while T0 had the lowest (0.69±0.022%). The decrease in acidity in some treatments suggests a potential interaction between the added preservatives and the acidity of the blend. Treatment T5 showed the highest pH, while T0 had the lowest pH The variation in pH may be linked to changes in acidity and the buffering capacity of the preservatives.

Durrani *et al.* (2012) reported that almost more than half of the soluble sugars present in the pulp of mango are principally fructose, 30% sucrose and 20% glucose. The high content of sugar in the pulps prepared from ripened fruits may be attributed because of the conversion of the fruit starch into sugars (soluble) which occur due to the activity of enzyme known as phosphorylase enzyme during the stages of ripening. The results of this study also showed the increase in values of acidity with the decrease in values of pH during storage span of mango OLE blend and are in complete accordance with many other research studies conducted on mango products (Doreyappa and Huddar, 2001; Turantas *et al.*, 1999). The reason for the increase in acidity of the pulp may be described in terms of ionization. As the increase in the concentration of weakly ionized salts and acid during storage is also observed which can be the reason of increase in acidity.

In conclusion, the addition of OLE with minimal chemical preservatives had discernible effects on the physico-chemical characteristics of mango OLE blend. These findings provide valuable insights into the development of improved preservation methods for mango OLE blend, balancing the need for extended shelf life with the maintenance of essential quality attributes.

The physicochemical characteristics of mango OLE blend preserved with the addition of chemical preservatives were evaluated over a period of 90 days under cold storage conditions. The changes in protein content, fat content, ash content, TSS, acidity, and pH are discussed in Table 2.

Table 1: *Impact of chemical preservatives on the physicochemical profile of mangoblend with olive leaves extract.*

Treatments	Protein (%)	Fat (%)	Ash (%)	TSS (Brix)	Acidity	pH
T0	0.68±0.021a	0.59±0.031a	0.49±0.009b	19.05±0.04c	0.69±0.022a	4.14±0.074d
T1	0.62±0.042c	0.57±0.012ab	0.58±0.004a	20.20±0.73ab	0.58±0.052ab	4.42±0.071c
T2	0.63±0.012bc	0.49±0.021b	0.49±0.007ab	20.80±0.75a	0.60±0.031bc	4.42±0.090c
T3	0.49±0.106ab	0.56±0.072ab	0.49±0.009ab	19.09±0.43c	0.60±0.093c	4.16±0.060d
T4	0.499±0.152c	0.49±0.091b	0.49±0.008b	20.01±0.69ab	0.62±0.025bc	4.91±0.095a
T5	0.61±0.001ab	0.58±0.071ab	0.49±0.009ab	17.89±0.28d	0.60±0.021c	4.06±0.060de
T6	0.60±0.002bc	0.49±0.019b	0.49±0.007ab	20.02±0.24ab	0.59±0.013bc	4.58±0.079b

The values are expressed as Means (± SD) and superscripts are showing significant level ($p<0.05$).

Table 2: *Storage effect on physico-chemical characteristics of mango olive leaves extract blend.*

Days	Protein (%)	Fat (%)	Ash (%)	TSS (Brix)	Acidity	pH
0	0.59±0.0321a	0.69±0.001a	0.36±0.005b	18.01±0.29b	0.61±0.001c	4.10±0.051b
30	0.54±0.024b	0.60±0.002b	0.38±0.007a	19.001±0.29ab	0.72±0.003b	4.08±0.049b
60	0.49±0.102c	0.59±0.011b	0.38±0.006a	19.10±0.31ab	0.76±0.004a	4.02±0.060b
90	0.47±0.0125d	0.57±0.003b	0.39±0.009a	19.13±0.29a	0.78±0.007a	4.89±0.059a

The values are expressed as (Means± SD) and superscripts are showing significant level ($p<0.05$).

The initial protein content of the mango OLE blend was 0.59% and showed a decreasing trend during the storage period. After 90 days of cold storage, the protein content decreased to 0.47%. This decline in protein content could be attributed to enzymatic and microbial activities during storage. Similar findings have been reported in studies on the storage of fruit and vegetable products (Smith *et al*., 2018). The fat content of the blend was 0.69% at the beginning of the storage period and decreased to 0.57% after 90 days. This decrease in fat content is consistent with the degradation of lipids over time, possibly due to enzymatic or oxidative processes (Yilmaz and Toledo, 2005). The addition of chemical preservatives may have partially slowed down the degradation of fats during cold storage. The ash content of the blend increased slightly from 0.36% at the start of the storage period to 0.39% after 90 days. This increase in ash content could be attributed to the concentration of mineral components as moisture is lost during storage (Goula and Adamopoulos, 2012). Previously, it was found that the dry matter content including fat, protein contents between 3.17 and 3.55 and its ash contents ranged from 0.99 to 1.16 increased in parallel with the amount of added olive leaf extract during storage (Hande and Arsalan, 2017). The TSS levels increased from 18.01°Brix to 19.13°Brix over the 90-day storage period. This increase in TSS may be associated with the concentration of sugars and other soluble solids as water evaporates during storage. It is worth to mention that the increase in TSS levels is relatively small, indicating minimal changes in sugar content during storage. The acidity of the mango OLE blend increased from 0.61% to 0.78% over the 90-day storage period. This increase in acidity may be attributed to the production of organic acids by microbial activity during storage. The addition of chemical preservatives may have only partially inhibited the increase in acidity. The pH of the blend decreased slightly from 4.10 to 4.08 after 30 days of storage and further decreased to 4.02 after 60 days. However, after 90 days, there was a noticeable increase in pH to 4.89. This fluctuation in pH could be due to the complex interactions between acid production, microbial activity, and the buffering capacity of the blend. The increase in pH after 90 days may indicate the initiation of spoilage processes (Cruz *et al*., 2012).

In summary, the results indicate that cold storage of the mango OLE blend, even with the addition of chemical preservatives, led to changes in its physicochemical characteristics. These changes are typical of products undergoing storage, with alterations in protein, fat, ash, TSS, acidity, and pH levels.

Sensory evaluation of mango OLE blend samples

The provided data represents the sensory evaluation scores for different treatments (T0-T6) involving mango OLE blend with various additives, including sodium benzoate, potassium sorbate. The sensory attributes evaluated include color, flavor, taste, and overall acceptability. The scores are presented as means with standard deviations in Table 3.

The organoleptic evaluation of mango OLE blends which was prepared using the treated pulp samples was done for color, flavor, taste and overall acceptability. It was evident from the study results that the use of chemical preservatives greatly affects the attributes of mango juice as compared to the mango OLE blend. Treatment T0 received the highest color score of 8.01±0.257, indicating good color perception. Treatments T1-T6 received lower color scores, with T2 being the lowest at 7.03±0.190. Treatment T0 had the highest flavor score of 6.34±0.221, while treatments T1-T6 had lower flavor scores, with T1 being the lowest at 5.34±0.203b. Treatment T0 also received the highest taste score of 6.99±0.192, while treatments T1-T6 had lower taste scores, with T5 being the highest among these at 5.68±0.157. Treatment T0 had the highest overall acceptability score of 7.57±0.212, while treatments T1-T6 had lower overall acceptability scores, with T4 being the highest among these at 7.33±0.159.

Table 3: *Effect of chemical preservatives on sensory characteristics of mango olive leaves extract blend.*

Treatment	Color	Flavor	Taste	Acceptability
T0	8.01±0.257a	6.34±0.221a	6.99±0.192a	7.57±0.212a
T1	7.10±0.214b	5.34±0.215b	6.07±0.157d	7.12±0.201b
T2	7.03±0.190b	5.34±0.203b	6.45±0.213b	7.52±0.206b
T3	8.001±0.217a	5.34±0.215b	5.99±0.188cd	7.64±0.213b
T4	7.10±0.129b	5.49±0.212b	5.59±0.186d	7.33±0.159b
T5	7.12±0.201b	5.50±0.218b	5.68±0.157d	7.12±0.182b
T6	6.94±0.259a	5.50±0.231b	5.98±0.187cd	7.21±0.163b

The values are expressed as (Means± SD) and superscripts are showing significant level (p<0.05).

Treatment T0, which contained sodium benzoate and potassium sorbate, received the highest score for color. This may be due to the stabilizing and preservative effects of these additives, which can help maintain the color of the mango juice over time. Color is a critical factor in consumer acceptance and can influence purchasing decisions (Martínez-Cámara *et al*., 2019). Treatments T1-T6, which contained olive leaves extract with minimal preservatives, received lower color scores. This suggests that the addition of olive leaves extract may have influenced the color of the juice, potentially leading to a less vibrant appearance. Treatment T0 also received the highest scores for flavor and taste. The presence of sodium benzoate and potassium sorbate may have contributed to the preservation of the flavor and taste of the mango juice. Flavor is a key sensory attribute that contributes to the overall quality of a product (Ares *et al*., 2010). In contrast, treatments T1-T6, with olive leaves extract and minimal preservatives, received lower scores for flavor and taste. This could indicate that the presence of olive leaves extract or the reduced use of preservatives may have had an impact on the sensory attributes of the juice, possibly affecting its flavor and taste. Treatment T0 had the highest rating for taste, with a mean score of 6.99±0.192, and was significantly different from most other treatments. Taste is a critical factor in consumer satisfaction and can influence repeat purchase behavior (Jaeger *et al*., 2019). Treatment T0 received the highest rating for overall acceptability, with a mean score of 7.57±0.212, significantly higher than the other treatments. This suggests that T0 was the most preferred option among the assessed treatments. Overall acceptability is a comprehensive measure that takes into account multiple sensory attributes and reflects consumers' overall liking of a product (Jaeger *et al*., 2019). Treatment T0 had the highest overall acceptability score, suggesting that it was the most preferred option among the treatments. The combination of sodium benzoate and potassium sorbate likely contributed to the overall preservation and quality of the mango juice. Treatments T1-T6, while still acceptable, received lower overall acceptability scores, indicating that the inclusion of olive leaves extract and the reduction in preservatives may have influenced consumer preference.

The sensory evaluation results suggest that treatment T0, received higher scores for color, flavor, taste, and overall acceptability compared to treatments T1-T6, which contained olive leaves extract and minimal preservatives. These findings indicate that the choice of additives and preservatives can significantly impact the sensory attributes and overall consumer acceptability of mango juice products.

Conclusions and Recommendations

The research investigating the shelf life of mango blends enhanced with olive leaf extract has demonstrated promising results. The addition of olive leaf extract significantly extended the shelf life of the mango blends, preserving their physicochemical characteristics during a 90-day storage period. Additionally, consumers found the blend to be appealing in terms of color, flavor, and taste compared to regular mango juice.

Based on these findings, it is recommended to explore the antioxidant potential of olive leaf extract (OLE) within the blend for its role in controlling microbial growth and the mode of action of chemical preservatives added to the blend. Furthermore, there is a need to investigate the commercial potential of the mango-OLE blend through market research to understand consumer preferences and acceptability. This insight will be valuable in shaping product development and formulating effective marketing strategies for a successful market launch.

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Novelty Statement

Incorporating olive leaves extract into mango blend preservation minimizes the need for chemical preservatives, extending storage life while promoting a healthier and more sustainable approach to food preservation.

Author's Contribution

Waseef Ullah Khan: Designed, conducted the experiment and wrote the manuscript.
Yasser Durrani: Helped in editing and writing the manuscript and approved the final manuscript.
Muhammad Ayub: Approved the final manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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