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ORIGINAL ARTICLE

Impact of Storage Duration on Kernel Quality of Offspring of 'Mamaei' and 'Marcona' Almond Hybrids

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K E Y W O R D S

Almond;
Biochemical traits;
Nut;
Oil content;
Storage times;
Vitamin E

A B S T R A C T

The influence of storing almond kernel genotypes derived from reciprocal crosses of 'Mamaei' and 'Marcona' cultivars (referred to as 'G1', 'G2', 'G3', 'G4', 'G5' and 'G6') on diverse quality parameters, encompassing moisture, ash, protein, oil, carbohydrates, fiber, and total vitamin E was investigated. The kernels were stored for 0, 6, and 12 months at room temperature. The results showed that the highest fresh kernel weight was observed in the 'Marcona' parent and two progenies, 'G5' and 'G3', at harvest time. The highest amounts of soluble carbohydrates were found in the 'G4' genotype, while the highest amounts of insoluble carbohydrates were observed in the 'Mamaei' parent and 'G5' genotype. The highest protein content was found in the 'Mamaei' parent and 'G4' genotype, while the maximum oil content was observed in the 'G5' genotype. The 'G6' genotype had the highest amount of total vitamin E. All studied traits showed a decreasing trend during the storage period, with the lowest amounts observed in all selected offspring after one year of storage. The results highlighted variations in traits such as fresh kernel weight, soluble and insoluble carbohydrates, protein, oil, and total vitamin E among different genotypes. Moreover, all traits exhibited a decline in values during storage, emphasizing the importance of selecting high-quality genotypes like 'G5' for almond breeding programs.

Introduction

Almond (*Prunus dulcis* L.) belongs to the genus *Prunus* and the subspecies Amygdalus within the Rosaceae family (Chen *et al*., 2005; Subhashinee *et al*., 2006). Almond is a nutritionally significant and economically valuable specialty nut crop that is grown in over 50 countries worldwide for both local

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consumption and export (Yada *et al*., 2011; Ansari and Gharaghani, 2019). Iran has been reported as the center of diversity and the origin of almonds, having nearly 20 wild almond species that is among the world's most significant almond producers, with a cultivated area of 79,392 ha. Its annual production of approximately 164,348 tons places it third globally, following the United States and Spain (Ansari and Gharaghani, 2019; FAO, 2021). Almond kernels are rich in protein, amino acids, fatty acids, carbohydrates, vitamins, minerals, and other beneficial compounds (Zahedi *et al*., 2020). The valuable properties of almond kernels can be preserved for an extended period through optimal storage after fruit harvest (Farooq *et al*., 2021).

Studying the appearance, phenotypic, genetic, and biochemical characteristics of plant materials is crucial and serves as the initial and fundamental step in identifying, preserving, and maintaining genetic resources (Akça *et al*., 2020). The phenotypic variation in these resources serve as the foundation for genetic research and breeding programs (Zokaee-Khosroshahi *et al*., 2014; Imani *et al*., 2021). The development and production of superior plants rely on the ability to accurately select between them, which are dependent on identifying their diversity and superior traits (Crossa *et al*., 2017). Almond is a hard-shelled fruit species that is highly valued for its positive impact on human health and abundant nutrient content. It is particularly notable for its various oils and rich nutritional composition (Ercik *et al*., 2023). Compared to other nuts, almond has a lower moisture content and higher antioxidant levels, which helps maintain its quality during storage to some extent. However, as storage time increases, there is a noticeable decline in the quality of its kernel. Research conducted on pistachio, walnut, macadamia, and hazelnut kernels has demonstrated that their quality and shelf life deteriorate over time and under inappropriate temperature conditions (Arena, 2013; Gama *et al*., 2018; Habibie *et al.*, 2019). The rate of quality reduction depends on the type of food. Almond fruit is typically harvested when dry, with a kernel moisture content of less than 7%. The moisture level of the fruit at the time of harvest and prior to handling and processing plays a crucial role in determining the mechanical response of the product during processing. To prevent mechanical damage during the pre-processing stage, it is desirable to maintain low fruit moisture levels (around 5.52%) (Gradziel, 2017).

Studies have shown that consumption of almond oil leads to a rapid and sustained decrease in low-density lipoprotein (LDL) cholesterol levels, without affecting high-density lipoprotein (HDL) cholesterol levels (Ercik *et al*., 2023). Furthermore, nutrient-rich cultivars can be utilized in almond breeding programs that aim to enhance the composition and nutrient content of almond oil (Gouta *et al*., 2021). If stored for extended periods and under improper conditions, the high levels of unsaturated fatty acids in almonds can lead to oxidation, unpleasant flavor, and a decrease in quality (Pleasance *et al*., 2018). Research indicates that almond kernels are more effective than almond powder in delaying lipid oxidation and enhancing oxidative stability (thus increasing shelf life) of almonds. This is attributed to the lower surface area of contact in whole kernels than in powdered form (Raisi *et al*., 2015). The quality of stored almonds is primarily influenced by factors such as kernel moisture content, fat content, storage temperature, relative humidity, oxygen levels, packaging type, form of the stored kernel (in-shell, peeled, roasted, etc.), peroxide value, light exposure, almond cultivars, and other conditions (Raisi *et al*., 2015; El Bernoussi *et al*., 2020). Agunbiade and Olanlokun (2006) conducted a study on the nutritional properties of Indian almonds. They reported that almonds contain 97.70% dry matter, 11.52± 0.10% crude protein, 6.76± 0.72% ash, 5.09± 0.84% crude fiber, 21.76± 1.20% fat, and 54.87± 2.80% carbohydrate. Their study showed that Indian almonds

have a significant amount of fat, protein, fiber, and minerals. Additionally, Ercik *et al*. (2023) reported that the F1 hybrids resulting from the crossbreeding of 'Gulcan 2' and 'Lauranne' almond cultivars ('Gulcan 2' x 'Lauranne') had higher levels of oleic acid and lower levels of linoleic acid compared to the F1 hybrids resulting from crosses between 'Guara' and 'Nurlu' almond cultivars and their respective parents ('Guara' x 'Nurlu'). The study also revealed a significant amount of variation in the fatty acid composition of the F1 populations, highlighting the potential for breeding new almond cultivars with desirable fatty acid profiles. The study suggests the importance of controlled crosses in almond breeding programs aimed at developing new cultivars with improved nutritional and sensory qualities. Barreca *et al*. (2020) found that the chemical compositions of different cultivated almond species vary due to factors such as genetics, ecology, and processing conditions. Despite extensive research by scientists (such as Lillian, 2017 and Tomishima *et al*., 2022) on the chemical compositions and properties of almonds, the observed variations in chemical composition among different almond species underline the importance of customized cultivation, processing, and breeding programs to produce almonds with specific desired nutritional and sensory characteristics.

Until now, limited scientific inquiry has been carried out regarding the impact of parent plants and the comparison of superior offspring resulting from controlled crossbreeding, along with an exploration of how storage durations affect the quality attributes of almond kernel fruits. Therefore, identifying or creating and introducing almond parents with superior physical and biochemical characteristics of nuts and kernels, while also maintaining kernel quality during storage, and conducting purposeful crosses between them to obtain offspring with desirable kernel traits and a long shelf life are important objectives of almond breeding programs. The purpose of this study is to investigate the impact of different storage times at normal temperature conditions on selected hybrid kernels, resulting from controlled superior crosses of 'Mamaei' and 'Marcona', in terms of various kernel traits.

Materials and Methods

This study was conducted between 2019-2021 and involved the examination of six selected almond hybrids, namely 'G1', 'G2', 'G3', 'G4', 'G5' and 'G6' (Fig. 1). These hybrids were selected due to their superior parentage, which involved the crossbreeding of 'M' and 'Mar' almonds. The study included a total of 62 offspring resulting from the crossbreeding of 'M' and 'Mar' almonds, which were available in the Seed and Plant Improvement Institute (SPII) orchards located in the Kamal Abad area, approximately 15 km west of Alborz province in Iran (Table 1).

Fig. 1. Some of the genotypes used in the current study.

Table1. The cultivars and progenies studied in this research.

No	Maternal parent	Pollinizers	Offspring	Code
1	'Marcona'	'Mamaei'	'MarM13-36'	'G1'
2	'Mamaei'	'Marcona'	'MMar13-40'	'G2'
3	'Mamaei'	'Marcona'	'MMar13-29'	'G3'
4	'Mamaei'	'Marcona'	'MMar13-39'	'G4'
5	'Mamaei'	'Marcona'	'MMar13-01'	'G5
6	'Mamaei'	'Marcona'	'MMar13-42'	'G6'
7	-	-	'Mamaei'	'M'
8	-	-	'Marcona'	'Mar'

'MarM': It stands for the maternal and paternal parents ('Marcona' and 'Mamaei', respectively).

Quantitative and biochemical traits

The study evaluated 10 qualitative and biochemical characteristics of almond nuts and kernels. To examine these traits, 100 fruits were randomly harvested from different directions of the tree. The traits evaluated are listed in Table 2. The storage times for the selected almond hybrids included three time points: at harvest, 6 months post-harvest, and 12 months post-harvest. It should be mentioned that the storage was carried out under regular conditions at a temperature of 24 °C, and the almond kernel samples were removed from their shells and they were stored inside plastic containers.

The storage conditions for all three examination times were maintained at a normal room temperature of 24 ℃. The fresh weight of the almond kernels was measured using a digital scale, which served as the initial weight. To measure the dry weight of the kernels, the samples were kept at 70 °C for 24h and weighed again, which served as the secondary weight (Zahedi *et al*., 2020). The percentage of kernel humidity was calculated through the following equation (Fernandes *et al*., 2022):

$$\text{Humidity percentage} = ((\text{initial weight} - \text{secondary weight}) / (\text{initial weight})) \times 100$$

Measurement of biochemical traits

The ash content was analyzed by burning the organic material at a temperature of 575°C, following the method described by Aktas et al. (2015). After conducting moisture analyses, 2.5 g of dry samples were heated to 250°C (at intervals of 10°C min^{-1}) in an electric furnace. The temperature was then maintained at 250 °C for 30 min before being raised to 575°C at a rate of 10°C $min^{-1}$and held there for 3 h. The samples were then cooled to ambient temperature and transferred directly to a desiccator before measurements. To prevent re-absorption of moisture, the samples were stored in airtight bags under desiccation after being weighed, as described by Aktas *et al*. (2015).

The total protein content was determined using the Kjeldahl technique (V50 model, Bakhshi Company, Iran), which followed the methods described by Okay (2002) and El Hawary *et al*. (2014). To generate ammonium sulfate by oxidizing the organic materials, the samples were heated with sulfuric acid. The ammonium was then converted into ammonia by distilling the solution with sodium hydroxide. The quantity of ammonia and the amount of nitrogen were calculated using back titration with sodium carbonate solution and boric acid, along with methyl orange as a pH indicator. Finally, considering the nitrogen-to-protein conversion coefficient of 5.6, the protein content was calculated.

The oil percentage was determined using the Soxhlet technique, which followed the methods described by Georges *et al*. 1992. After grinding, the almond powder

was extracted using the Soxhlet technique with pure methanol and chloroform in a 50:50 solvent ratio, at a temperature of 45 °C (SX100-G model, Bakhshi Company, Iran). The quantity of oil was determined after isolating the solvent in the extracted oil using an oven under a vacuum.

After separating the soluble sugars, the remaining pulp was recovered and dried in an oven (SHD96A, Iran) at a temperature of 50 °C for two hours to quantify the insoluble carbohydrates. The samples were mixed with 6 ml of 51% perchloric acid and 4.5 ml of distilled water, and then kept in the refrigerator at a temperature of 4 °C for 14 h. The measuring process was then conducted following the same procedure used for measuring the soluble sugars (Kochert, 1987).

Total vitamin E was analyzed using High-performance liquid chromatography (HPLC) (Unicam 200-Crystal model, England) with an array-photodiode detector, following the methods described by Nyandwi *et al*. (2019). The excitation and emission wavelengths of the fluorescence detector were set at 295 nm and 330 nm, respectively. The mobile phase consisted of a mixture of methanol and distilled water in a ratio of 97:3 (v/v), and the flow rate was set to 1.05 ml per min. Peaks were identified based on their retention times, which were compared with the four established standards (Sigma-Aldrich) (Nyandwi *et al.*, 2019). The fiber percentage was measured using the sulfuric acid digestion method, following the procedures outlined by Rico *et al*. (2016).

Statistical analysis

A completely randomized design (CRD) with three replicates per treatment was employed in this experiment. To evaluate the effects of the selected offspring (8 genotypes) and storage times (0, 6, and 12 months) on each dependent variable, a two-way analysis of variance was performed using SAS software (version 9.2). Mean values of the treatments were compared using the Least Significant Difference test (LSD, P = 0.05), and the graphs were created using Sigma Plot software version 12.3.

Table 2. List of evaluated traits in almond samples.

No.	Variable	Abbreviation	Unit	Measurement method
1	Kernel fresh weight	KWE	g	Electronic balance
2	Kernel dry weight	KDW	g	Electronic balance
3	Kernel humidity	HU	%	[(W-D)/(W)] ×100
4	Ash	Ash	%	Aven
5	Protein	Pr	%	Spectrophotometer
6	Oil	Oil	%	Soxhlet extractor
7	Soluble carbohydrate	Soca	%	Spectrophotometer
8	Insoluble carbohydrate	InSoca	%	Spectrophotometer
9	Total vitamin E	E vit	mg/100 g	HPLC
10	Fiber	Fib	%	Sulfuric acid digestion

*****W: fresh weight, D: Weight after drying

Results

The statistical analysis showed that the fresh and dry weight of kernel, humidity, ash, protein, oil, soluble carbohydrate, insoluble carbohydrate, fiber, and total vitamin E were all significantly affected by both genotype and storage times at the 1% level of significance. However, the humidity, ash, oil, soluble and insoluble carbohydrate, and fiber showed significant interaction effects between genotype and storage times (Table 3).

The analysis of average values obtained from the

offspring in comparison to the parental plants indicated that there was an increase observed in certain traits, while a decrease was noted in others. Furthermore, specific genotypes displayed intermediate averages lying between those of the parents. In particular, genotype ‘G5’ consistently demonstrated mean values higher than those of either parent or had intermediate mean values between the parents across all traits. Furthermore, regarding ash content, oil content, and also total vitamin E, the offspring displayed mean values higher than both parents or at the very least, one parent (Table 4).

Kernel fresh and dry weight

The results showed that, the highest value of kernel fresh weight was observed in ‘Mar’ and ‘M’ cultivars (1.19 and 1.13 g, respectively). Furthermore, ‘G3’ and ‘G5’ offspring exhibited the highest kernel fresh weight and were grouped statistically with the superior treatments. The lowest kernel fresh weight was obtained in offspring ‘G6’ (0.67 g). Offspring ‘G1’, ‘G2’, and ‘G4’ showed moderate kernel fresh weight among the genotypes (Table 4).

Based on the results of comparing all the offspring and parents, the 'Mar' genotype showed the highest kernel dry weight (1.15 g), although it did not exhibit a statistically significant difference compared to the ‘G4’ and ‘G5’ offspring and the 'M' cultivar. The genotype ‘G6’ exhibited the lowest kernel dry weight of 0.64 g, although it did not demonstrate a significant difference when compared to the ‘G1’ and ‘G2’ offspring. Notably, the kernel dry weight of the ‘G3’ offspring displayed a moderate value among the various genotypes. (Table 4). The maximum kernel dry weight was recorded during harvest, while the minimum value was noted after a 12-month storage period at room temperature. Despite that, the kernel dry weight of both parents and offspring exhibited a significant decrease as the storage duration progressed (Table 5).

Table 3. Variance analysis of the effect of different storage times on some quantitative and qualitative traits of the genotypes resulting from the crossing of 'Mamaei' and 'Marcona' almond cultivars

Treatment	df	Mean square (MS)									
		KWE	KDW	HU	Ash	Pr	Oil	Soca	InSoca	Fib	Total Vitamin E
Genotype (G)	7	0.32**	0.34**	419.76**	0.33**	53.18**	47.76**	3.26**	3.63**	9.10**	5543.20**
Time (T)	2	0.95**	0.99**	5346.69**	6.80**	118.00**	6366.67**	32.84**	4.15**	69.65**	21372.81**
G × T	14	0.10^{ns}	0.042^{ns}	414.06**	0.11**	8.68^{ns}	44.00**	1.11**	0.133**	3.40**	770.02^{ns}
Error	54	0.077	0.049	17.01	0.008	5.76	15.59	0.12	0.04	1.19	711.29
CV (%)	-	27.94	24.70	24.15	3.95	11.44	8.14	10.67	3.94	12.63	17.81

*, **, and ns represent significance at the 0.05 and 0.01 levels and non-significance, respectively.

Table 4. Comparison of mean (±SD) quantitative and qualitative traits in the genotypes obtained from the hybridization of 'Mamaei' and 'Marcona' almond cultivars

Genotypes	KWE (g)	KDW (g)	HU (%)	Ash (%)	Pr (%)	Oil (%)	Soca (%)	InSoca (%)	Fib (%)	Total Vitamin E (mg 100 g^{-1})
'G1'	0.81±0.35(-)	0.65±0.19(-)	21.3±27.8(+)	2.25±0.37(*)	18.32±3.38(-)	48.36±14.76(*)	3.65±0.46(+)	4.71±0.22(-)	9.59±1.51(*)	109.23±25.98(-)
'G2'	0.89±0.36(-)	0.75±0.26(-)	14.9±18.3(+)	2.37±0.18(*)	17.63±3.03(-)	43.57±14.72(-)	2.75±0.97(+)	4.93±0.44(-)	8.76±2.41(*)	139.57±24.11(*)
'G3'	1.15±0.71(*)	0.92±0.45(-)	19.5±26.1(+)	2.53±0.46(*)	18.73±1.87(-)	50.36±14.85(+)	3.38±1(+)	4.44±0.34(-)	7.74±2.31(-)	173.63±40.64(+)
'G4'	1.01±0.22(-)	0.96±0.21(-)	3.59±2.4(-)	2.37±0.61(*)	22.88±2.43(*)	50.26±15.38(+)	4.12±1.72(+)	4.68±0.34(-)	8.26±1.79(*)	173.29±47.63(+)
'G5	1.15±0.29(*)	1.1±0.26(*)	5.74±3.69(*)	2.28±0.51(*)	21.97±3.93(*)	50.53±15.4(+)	3.93±1.08(+)	5.88±0.65(*)	9.94±1.04(+)	145.31±28.57(*)
'G6'	0.67±0.3(-)	0.64±0.29(-)	12.4±12.1(*)	2.57±0.42(+)	22.55±2.25(*)	48.99±15.11(*)	3±0.71(+)	5.45±0.28(-)	7.42±2.04(-)	180.37±55.2(+)
'M'	1.13±0.42	1±0.19	14.4±21.3	2.55±0.63	24.24±2.59	47.22±13.77	2.68±1.56	6.19±0.43	9.81±1.09	127.77±22.9
'Mar'	1.19±0.29	1.15±0.29	4.49±3.54	1.99±0.6	21.55±4.69	49.01±14.68	2.53±1.29	5.6±0.61	7.82±2.75	149.02±37.34
LSD = 0.05	0.48	0.36	12.80	0.15	4.50	7.4	1.04	0.75	1.24	15.41

(+): Demonstrated an elevation relative to parental values, (-): Displayed a reduction in comparison to parents, (*): Carried an intermediary value between the parental traits

Table 5. The effect of different storage times on some quantitative and qualitative traits of the genotypes resulting from the crossing of 'Mamai' and 'Marcona' almond cultivars

Storage times	KDW (g)	HU (%)	Ash (%)	Pr (%)	Oil (%)	Soca (%)	InSoca (%)	Fib (%)	Total Vitamin E (mg 100 g^{-1})
At harvest	1.05±0.35	24.79±22.69	2.36±0.5	22.7±3.04	60.03±4.47	4.38±0.91	5.64±0.77	10.57±1.19	176.5±43.93
Month 6	0.91±0.34	10.17±13.55	2.17±0.51	21.78±3.31	55.68±7.13	3.34±0.59	5.27±0.61	8.15±1.65	155.25±34.01
Month 12	0.79±0.29	8.27±16.15	2.06±0.53	18.48±3.65	29.9±1.74	2.05±0.86	4.81±0.56	7.28±1.79	117.57±23.54
LSD = 0.05	0.13	2.58	0.13	1.39	7.50	0.23	0.14	0.13	15.48

Humidity percent

The highest humidity content was recorded in the kernels of the ‘G1’ offspring during harvest, while the lowest moisture content was observed in the kernels of the ‘G5’ offspring at the same time. However, no significant difference in kernel moisture content was observed among the ‘G4’, ‘G6’, and ‘Mar’ cultivar offspring during harvest. At 6 months post-harvest, there was a significant reduction in the humidity content in all offspring and parents. Furthermore, after 12 months post-harvest, the ‘G4’ genotype exhibited the lowest humidity content (1.12%) (Fig. 1). There was a close correlation between kernel weight and humidity percentage. These results showed that storage of almond nuts for one year significantly causes loss of kernel humidity, which can affect its edible quality (Fig. 2).

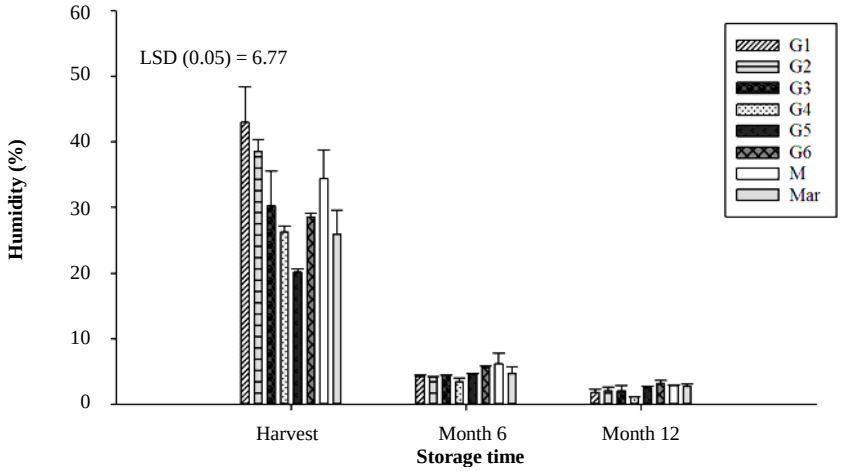

Fig. 2. Kernel humidity percent of the selected progeny from a cross between ‘Mamaei’ and ‘Marcona’ cultivars and its changes during 12 months storage under room temperature.

Ash content

Ash content signifies the level of mineral present in the sample. The ‘M’ cultivar exhibited the highest kernel ash percentage of 3.28% during harvest time. On the other hand, the lowest ash percentage at harvest time was observed in the offspring ‘G2’ (2.55%). After 6 months post-harvest, there was a decreasing trend in the percentage of ash in all offspring and their parents, although this decrease was not as significant as other traits, such as humidity content. The highest ash percentage was observed in offspring ‘G3’, reaching 2.64%. The ‘G3’ offspring did not exhibit a significant difference in the ash content compared to the highest value observed during fruit harvest (3.28%). However, the lowest ash content among all offspring, parents, and storage times (1.4%) was observed in the ‘Mar’ cultivar one year after harvest. These results suggest that some of the superior foreign almond cultivars, such as ‘Mar’, may not possess the desired quality in certain traits during storage under specific conditions, compared to superior domestic genotypes (Fig. 3).

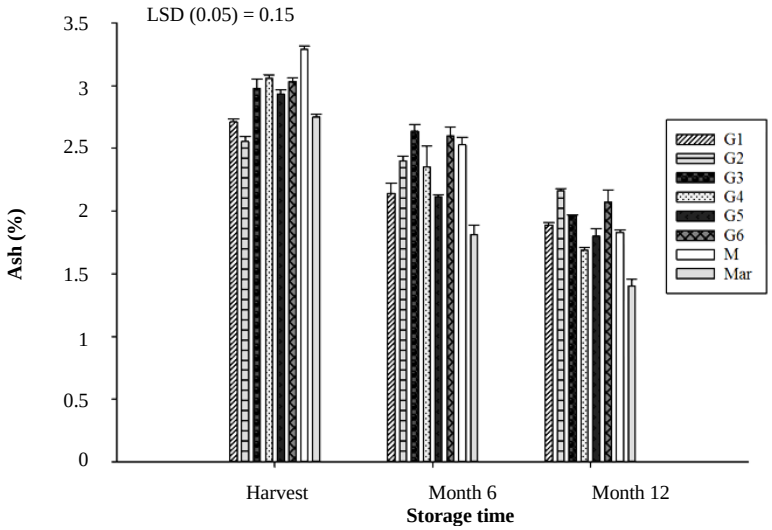

Fig. 3. Ash percent of the selected progeny from a cross between 'Mamaei' and 'Marcona' cultivars and its changes during 12 months storage under room temperature.

Protein content

Results illustrates that the protein content varied across the different offspring and cultivars, ranging from 17.63% in offspring 'G2' to 24.24% in the 'M' cultivar. The 'M' cultivar exhibited a higher protein content compared to the 'Mar' cultivar. Moreover, it and other selected offspring was superior in terms of some other qualitative traits, indicating that its selection as one of the parents in controlled crosses could influence the quality of the resulting kernel offspring (Table 4). The highest protein content was observed at harvest time, while the lowest protein content was measured 12 months after harvest (Table 5).

Oil content

According to the results at the harvest time, the kernel oil percentage varied from 43.57% in 'G2' offspring to 50.53% in the 'G5' offspring (Table 4). No significant difference was observed among genotypes in terms of oil content at the harvest level in the comparison of genotype interaction effects during storage. In comparison with harvest time, 6 months after harvest, although a decrease in kernel oil content was observed in the all offspring and parents, but this decrease was not significant, except to 'G2' offspring that its oil percent decrease significantly during the 6 months storage. The obtained results demonstrate that there was a significant reduction in the oil percentage of all genotypes after 12 months of storage compared to harvest time. Nonetheless, no notable variations were identified among different progeny and parental samples during this stage of storage (Fig. 4).

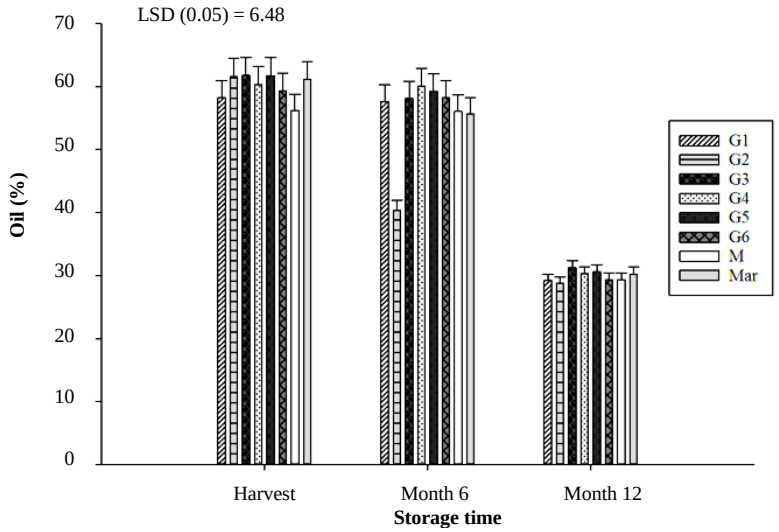

Fig. 4. Kernel oil content of the selected progeny from a cross between 'Mamaei' and 'Marcona' cultivars and its changes during 12 months storage under room temperature.

Soluble and insoluble carbohydrates

The 'G4' offspring exhibited the highest amount of soluble carbohydrates at harvest time (6.28%), while the lowest amount of soluble carbohydrates (3.40%) was observed in the 'G2' offspring. Following a 6-month period post-harvest, a reduction in soluble carbohydrate content was observed in both the offspring and their respective parents, ranging from 1.10% in the 'Mar' cultivar to 4.23% in the 'G5' offspring. At 12 months post-harvest, the 'M' cultivar exhibited the lowest amount of soluble carbohydrates, measuring at 0.62%, while the highest amount was observed in the 'G1' offspring (3.34%). Results indicate that the amount of soluble carbohydrates in all offspring and parents exhibited a decreasing trend from harvest time to one year of storage (Fig. 5).

At harvest time, the 'M' cultivar and 'G5' offspring exhibited the highest amount of insoluble carbohydrates, both measuring at 6.61%. In contrast, the lowest amount of insoluble carbohydrates at this stage (4.74%) was observed in the 'G3' offspring. After 6 months from the time of harvest, the amount of insoluble carbohydrates decreased in all offspring and their parents, similar to all the other measured traits. However, this decrease was not very significant, with the range varying from 4.52% to 6.19%. The amount of insoluble carbohydrates was most variable in the 'G3' offspring and the 'M' cultivar. The amount of insoluble carbohydrates measured in the 'G5' offspring 6 months after harvest (5.89%) was not significantly different from the 'M' cultivar. Nevertheless, the lowest content of insoluble carbohydrates in the 'G3' offspring's kernel after a year of storage reached 4.04%. Consistently, akin to the harvest time and the 6-month post-harvest period, the highest value for this characteristic, surpassing other progeny and the 'M' cultivar, was recorded at 5.76% (Fig. 6).

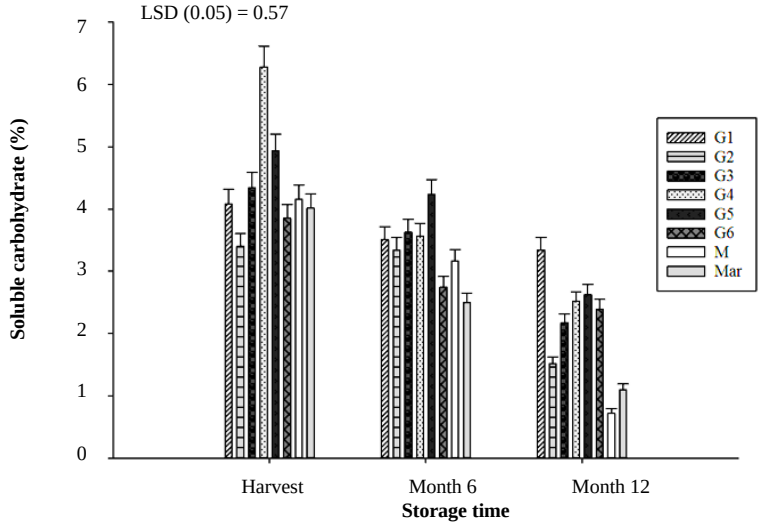

Fig. 5. Kernel soluble carbohydrate content of the selected progeny from a cross between 'Mamaei' and 'Marcona' cultivars and its changes during 12 months storage under room temperature.

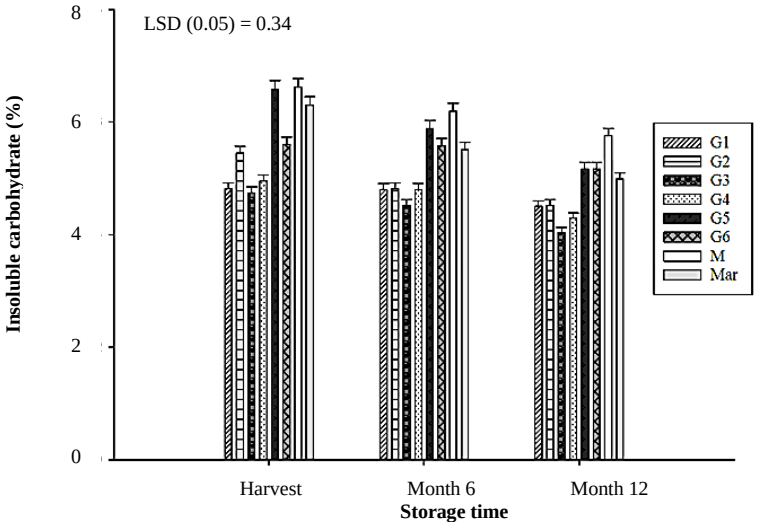

Fig. 6. Insoluble carbohydrate content of the selected progeny from a cross between 'Mamaei' and 'Marcona' cultivars and its changes during 12 months storage under room temperature.

Fiber content

The highest fiber content at harvest time were observed in the 'G2' offspring, the 'Mar' cultivar, and the 'G1' offspring (11.7%, 11.26%, and 11.1%, respectively). In contrast, the 'G4' offspring exhibited the lowest value with 9.30%. After 6- and 12-months post-harvest, the 'G5' offspring had the highest amount of fiber, while the 'G6' offspring had the lowest. Specifically, the 'G6' offspring had the lowest value of fiber among all treatments, measuring at 5.55% after 12 months of storage (Fig. 7).

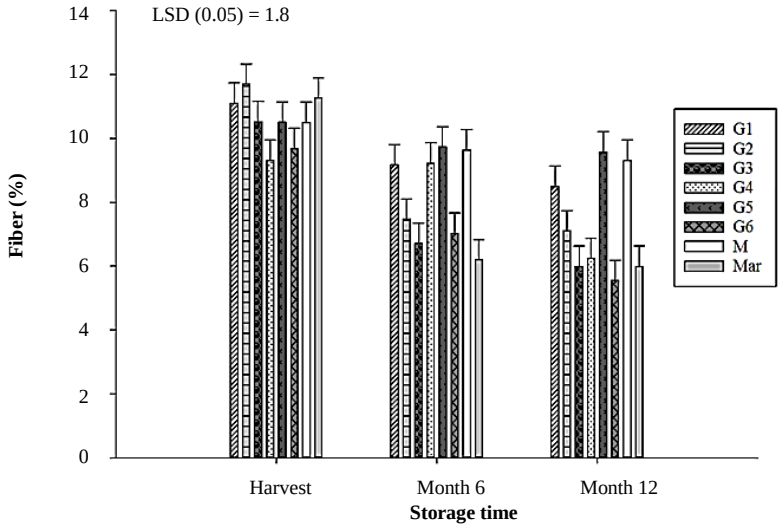

Fig. 7. Kernel fiber content of the selected progeny from a cross between 'Mamaei' and 'Marcona' cultivars and its changes during 12 months storage under room temperature.

Total vitamin E content

The outcomes suggest that the 'G6' progeny displayed a notable level of total vitamin E content. Among the offspring, the highest and lowest quantities of total vitamin E were found in the 'G6' and 'G1' offspring, recording 180.37 and 109.23 mg 100 g^{-1}, respectively (Table 4). The total vitamin E content within almond kernels exhibited a declining trend over the storage period. The peak total vitamin E content was observed at the time of harvest (176.5 mg 100 g^{-1}), while the lowest content was recorded 12 months post-harvest (117.57 mg 100 g^{-1}) (Table 5).

Discussion

The chemical and biochemical compositions of nuts play a crucial role in determining their nutritional value and quality during storage (Tasan *et al*., 2011;). Post-harvest storage time has a significant impact on the chemical composition of almonds. Factors such as cultivar, weather conditions during fruit growth, and storage conditions can affect kernel weight, which tends to decrease with the reduction of kernel humidity during post-harvest storage (Summo *et al*., 2018; Pakrah *et al.*, 2021). The kernel dry weight of all parents and offspring decreased significantly over the storage time (Table 5). The study results suggest that the kernel weight trait exhibits high heritability from the paternal 'Mar' parent, while the oil percentage trait exhibits high heritability from the maternal parent. Maintaining the appropriate humidity level of almonds is crucial for achieving the desired post-harvest operation and processing quality. This measure aids in preventing potential mechanical harm arising from processes such as peeling, shell removal, and handling. It ensures that the humidity level aligns with the kernel's mechanical attributes.

During the storage of fruits, a reduction in humidity percentage takes place, showing a substantial association with the mechanical properties of nuts and grains. This connection is vital in establishing the ideal moisture content level for processing and guaranteeing the quality of the ultimate product (Ekinci *et al*., 2010; Shahbazi, 2012; Shirmohammadi and Fielke, 2017; Shirmohammadi *et al*., 2018). To prevent almonds from being exposed to humidity and to extend their storage life, it is recommended to mechanically dry them. This method allows almonds to be stored for up to one year

(Luo *et al*., 2021).

The findings indicate that the kernel ash content ranged from 1.81% in the 'Mar' cultivar to 2.64% in the 'G3' offspring after 6 months of storage at room temperature conditions (Fig. 3). The results revealed that the ash content increased in all progenies when compared to the ‘Mar’ parent, whereas, with the exception of ‘G6’, the rest of the progenies exhibited a lower average than that of the ‘M’ parent. The variation in ash content between the parental almonds and their progeny can be attributed to factors encompassing genetic composition, cultivation and processing methodologies, growth conditions, and nutritional disparities between the parents (Suttle, 2022). In this particular investigation, given the consistency of three of these factors, the sole distinguishing element is the genetic composition. It seems that the elevated ash content in one of the parental almonds has contributed to the progeny derived from their cross displaying a higher average of this particular trait. Furthermore, the proximate composition of almonds has been reported to be affected by various factors, including soil type, climate, irrigation regime, fertilization, and geographical origin. These factors can cause changes in the compositional profile of the almond kernels (Levent Okan, 2022).

Protein is the second major chemical component of almond kernels after the oil fraction. The average protein content at harvest time was 22.70%. This result was consistent with the findings of Pérez-Sánchez *et al*. (2021), who reported protein content ranging from 15.7% to 23.39% of the kernel dry weight in Iberian almond cultivars. The respiration rate of the fruit plays a crucial role in causing a decline in protein content during storage. Protein is a necessary precursor in respiration, following sugar and fat. As the respiration rate increases during the storage period, sugar and fat are consumed, and protein precursors are used up as well. This can ultimately lead to a decrease in protein content over time. Protein is converted into energy and utilized in respiration, leading to a reduction in protein content during storage (Kader *et al*., 1982), which was consistent with the findings of the present study. Regarding protein content, the offspring also exhibited an average that ranged between that of the two parents, and in some cases, it was even lower. The ‘M’ parent displayed the highest average protein content. Research findings suggest that the ‘M’ cultivar possesses an elevated protein content in its kernel (Raisi *et al*., 2015).

The findings demonstrate that, with the exception of ‘G2’, all offspring resulting from the crossbreeding of ‘M’ and ‘Mar’ parental strains exhibit a substantial oil content derived from both parents or, at the very least, from one parent. The most notable mean value was observed in the ‘G5’. This could be due to a likely additive effect of oil-related genes from 'M' and 'Mar,' as oil content is influenced by multiple genes. ‘G5’ resulting from 'M' as the maternal parent and 'Mar' as the pollinizer, displayed the highest oil content, emphasizing the importance of maternal and paternal contributions (Zhang *et al*., 2006). Further research into the underlying genetic mechanisms can provide valuable insights for crop improvement. According to Kodad *et al*. (2010), although the oil content is influenced by genetics, environmental factors such as soil and climate play a substantial role in causing its fluctuations. The findings suggest that there was no significant alteration in the oil content of almond kernels following six months of post-harvest storage. Nevertheless, a noticeable decline in oil content was observed after one year of storage (Fig. 4). The oil content of the almond kernel plays a crucial role in determining its overall quality, and any factors that can affect the oil content are important considerations for commercial use. A lower lipid content, coupled with higher levels of phenolic compounds, can have a positive effect on the shelf life of nuts by limiting the oxidative process during storage (Zacheo *et al*., 2000; Chatrabnous *et al*.,

2018). The results mentioned earlier show notable differences in oil and protein concentrations among the studied sources. These variations can be attributed to environmental factors like climate conditions, soil properties, growth conditions, and agricultural practices. Differences among genotypes from the same sources may stem from genetic variations. These findings align with prior studies that have also noted differences in protein and oil content due to varietal diversity (Drogoudi *et al*., 2013).

The present study's results show that the amount of almond kernel oil decreased significantly after one year of harvest and storage, which can impact the overall quality of the kernels. Preserving the quality of almond oil during extended storage presents a challenge, necessitating suitable storage conditions to mitigate oxidative degradation of the oil. The high concentration of unsaturated fatty acids in almond lipids makes them particularly sensitive to spoilage changes. The prominent deterioration observed during storage is the lipid oxidation, which can result in the emergence of an undesirable odor linked to spoilage (Lee *et al*., 2014; Franklin and Mitchell, 2019).

Carbohydrates are present in almond kernels in the form of soluble sugars and polysaccharides, often associated with fiber. Although present in relatively low amounts, the soluble sugars are sufficient to make the kernels sweet-tasting (Gouta *et al*., 2020). The carbohydrate content of almond kernels has been reported to range from 3.3 (Vidal-Valverde *et al*., 1982) to 7.1 (Kodad, 2017) per 100 g of kernel weight. The reported variation in carbohydrate content can be attributed to the different cultivars that have been evaluated. The soluble sugars present in almond kernels are mostly non-reducing, with sucrose accounting for more than 90% of the total. Other sugars found in almond kernels include raffinose, glucose, fructose, sorbitol, and inositol (Barreira *et al*., 2010). The present study measured the amount of soluble carbohydrates in selected almond kernel nut offspring and their parents. The results showed that there was a decreasing trend in the amount of soluble carbohydrates from harvest to one year of storage in all the offspring and parents (Fig. 5). However, the reduction in the amount of non-soluble carbohydrates was not as rapid (Fig. 6). The study findings suggest that the reduction in the quantity of insoluble carbohydrates during the storage duration was comparatively smaller than the reduction observed in the amount of soluble carbohydrates. This difference in the rate of decrease could be due to the difference in the structure of the two types of carbohydrates. In all the offspring resulting from the crossbreeding of ‘M’ and ‘Mar’ cultivars, the soluble carbohydrate content was consistently higher compared to that of the parents, with the highest average observed in ‘G4’. Carbohydrates in almonds consist mainly of soluble sugars (mainly sucrose), starch and other polysaccharides such as cellulose and non-digestible hemicellulose (Ibourki *et al*., 2022). The variation of the carbohydrates content has already been linked to different factors such as cultivar, origin and harvest time (Roncero *et al*., 2020).

Except for ‘G1’, all the other offering exhibited elevated levels of total vitamin E content in comparison to either parents or at least one of them. Among the chosen almond kernel offspring, both 'G6' and 'G3' demonstrated elevated levels of total vitamin E content, offering not only significant benefits for human health due to their effective ROS scavenging capabilities but also for mitigating oxidative rancidity (Table 4). The higher accumulation of total vitamin E in ‘G6’ and ‘G3’ offspring makes them potential candidates for use in almond breeding programs, as they exhibit superior storability and higher health-promoting quality. Alpha-tocopherol, gamma-tocopherol, gamma tocopherol, alpha-tocotrienols, and total vitamin E content in 38 almond genotypes from Balıkesir province varied from undetectable levels to 1164.36 mg kg^{-1} oil, undetectable levels to 130.03 mg kg^{-1} oil, undetectable levels to 81.38

mg kg^{-1} oil, undetectable levels to 1252.24 mg kg^{-1} oil, respectively (Çelik *et al*., 2019). A research investigation into the tocopherol content of various *Prunus* species identified that the total vitamin E content in the examined almond genotypes ranged from 167.7 to 323.3 mg kg^{-1} (Matthaus and Ozcan, 2009). Vegetable oils are considered the main sources of vitamin E, which acts as a free radical scavenger in membranes and lipoproteins (Azzi, 2019). In almond oil, α-tocopherols play an essential role as a quality parameter by protecting the oil against lipid oxidation. Hence, the increased vitamin E content in almond kernels plays a crucial role in preserving the quality and extending the shelf life of almond oil. Research has indicated that the critical phase for the accumulation of almond tocopherols occurs between 74 and 95 days after flowering (Zhu *et al*., 2017). The content of tocopherol in almond genotypes and types of almond oils has been widely investigated, and its amount is mostly influenced by the genotype, as noted by Ouzir *et al*. (2021). Studies have shown that heating has a reducing effect on tocopherols in almond oil (Kodad and Alnso, 2018; Stuetz *et al*., 2017). In this study, almond kernels were stored at standard temperature conditions, and a reduction in vitamin E content was observed after one year. The decline in vitamin E content over time could be attributed to inherent degradation processes during storage, including oxidation, potentially compromising the nutritional value of almond kernels (Zaaboul and Liu, 2022). The assessment of vitamin E levels in the oil of diverse almond cultivars and genotypes revealed significant variability among the various tocopherol forms, with a substantial influence from environmental factors (Kodad, 2017).

Nonetheless, additional exploration is necessary to assess the robustness of these characteristics across various storage and processing scenarios, thereby ensuring the sustained excellence and nutritional integrity of almond products over extended periods.

Conclusions

According to the obtained results, the progeny resulting from the combination of ‘M’ and ‘Mar’ cultivars displayed notable variations in the examined characteristics. In certain attributes, such as ash content and soluble carbohydrates, the offspring had higher averages than at least one of the parents. Conversely, in some traits like kernel dry weight and insoluble carbohydrates (except for ‘G5’), the offspring exhibited lower averages. The current research proposes that certain well-performing almond kernel nut offspring, like ‘G5’ and ‘G6’, could be potential candidates for inclusion in almond breeding initiatives, with the potential to be promoted as superior cultivars. Further investigation into the stability of their traits over time would be essential in this regard. Furthermore, the findings from this study highlight a noticeable decline in the assessed characteristics over a year of storage in warehouse settings. It is crucial to take these aspects into account when assessing the nutritional value and longevity of almonds, and in devising effective storage and processing approaches to uphold their quality over time.

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Conflict of interests

The authors declare no conflicts of interest related to this research.

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