Journal of Environmental Biology
p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

JEB®

Original Research Paper
Volume 45 Issue 1 January 2024 pp. 106-116
DOI: http://doi.org/10.22438/jeb/45/1/MRN-5168

J. Environ. Biol., 2024

Journal website : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Fatty acid profiling of almond germpalsm grown in the Western Himalayan region of India

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Received: 18 August 2023 **Revised:** 31 September 2023 **Accepted:** 06 December 2023

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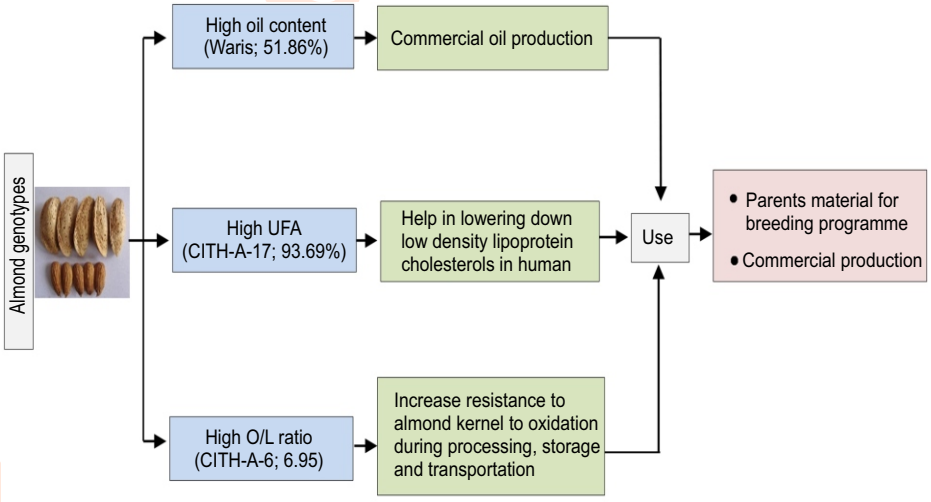
Abstract

Aim: Nutritional profiling of almond genetic resources for their utilization and further use in the breeding programme.

Methodology: The nuts of 32 almond genotypes were collected from the experimental farm of ICAR-CITH, Srinagar. Using a Soxhlet fat apparatus and 100ml of petroleum ether as a solvent, almond oil was extracted from 5 g of ground kernel. The fatty acid content of almond oil was analyzed by GC 30 (Perkin Elmer Autosystem XL) equipped with a CP-Wax 52 CB column (Varian Inc.) (50 x 0.25 mm, 0.2 µm).

Results: The total oil content in these genotypes ranged between 41.82% (CITH-A-21) and 51.86% (Waris). The major fatty acids found were oleic acid (53.70-80.81%), linoleic acid (11.63-36.88%), and palmitic acid (5.19-8.13%). Stearic acid (1.01-2.53%) was estimated at a lower proportion, while α-linolenic acid was found in trace amounts (0.05-0.18%). The oleic: linoleic acid ratio varied from 1.46 (CITH-A-21) to 6.95 (CITH-A-06), conversely the highest (115.42) and lowest (93.96) iodine values were recorded in CITH-A-21 and CITH-A-06, respectively. The lowest content of saturated fatty acids (6.32%) and the highest content of unsaturated fatty acids (93.69%) were found in CITH-A-17 whereas contrasting results were observed in Nonpareil. The highest monounsaturated fatty acids (80.81%) and lowest polyunsaturated fatty acids (11.71%) were found in CITH-A-6. Oleic acid content was negatively correlated with linoleic acid (r = -1.00), palmitic acid (r = -0.67) and stearic acid (r = -0.68). Principal component analysis indicated that among the fatty acids, the oleic acid, linoleic acid, and Oleic/Linoleic ratio were largely responsible for the separation on the PC 1. Among the different almond genotypes, CITH-A-17 and CITH-A-06 were found to be promising in terms of fatty acid composition.



Interpretation: The present study identified the genotypes with a high Oleic/Linoleic acid ratio, which is important for crop improvement programme and commercialization of these genotypes. Further, the present study also confirms that the Kashmir region is suitable for growing almonds with fatty acids composition at par with commercial almond cultivars grown in major almond-growing countries in the world.

Key words: Almond, Breeding programme, Fatty acid profile, Germplasm, Oil content

How to cite: Kumawat, K.L., M.K. Verma, D. Kumar, D.B. Singh, S. Lal, J.I. Mir, O.C. Sharma, W.H. Raja and L. Chand: Fatty acid profiling of almond germpalsm grown in the Western Himalayan region of India. *J. Environ. Biol.*, **45,** 106-116 (2024).

Introduction

Almond [*Prunus dulcis* (Mill.) D.A. Webb. Syn. *Prunus amygdalus* (L.) Batsch]; *Prunus communis* L.; *Amygdalus communis* L. is a species of genus *Prunus* and subgenus *Amygdalus* (family Rosaceae, subfamily Prunoidae). It is one of the oldest domesticated plants, native to Southwestern and central Asia (Zaurov *et al.,* 2015). In terms of commercial production, almond is the most significant temperate fruit crop, widely cultivated for dried kernel in many parts of the world. In India, almond cultivation is restricted to Jammu and Kashmir and Himachal Pradesh. India produced 11 thousand MT of almond from 10 thousand hectares of the irrigated and rainfed area during 2021-22 (Horticulture Statistics at a glance, 2022). *Prunus dulcis* is widely regarded as a drought-tolerant species (Houmy *et al.,* 2016; Prgomet *et al.,* 2020) and a large share of almonds produced in India comes from the rainfed area. The climatic conditions of Kashmir, include a long growing season from March to November with abundant snowfall from December to February, moderate rainfall during the early growing season (March to May), and an abundance of light throughout the season. Fertile soils with high water holding capacity, offer the opportunity to grow high-quality almonds under rainfed conditions in Kashmir.

Among the nuts, almonds are the richest source of fiber, protein, Vitamin E (alpha-tocopherol), riboflavin, and calcium (Kumawat *et al*., 2017). The medicinally rich phytonutrients available in almond improve heart, blood, skin, muscles, bone, oral and mental health. In addition, they improve digestive function, memory, and metabolism. They also boost the immune system, help in managing diabetes, and prevent different types of cancer and abnormalities in infants (Bravatti *et al.,* 2019; Dreher, 2021). Due to so many health benefits, almonds are known as an all-rounder or best nut for disease prevention. However, the health benefit of almond consumption varies with its nutritional composition. Amongst various factors influencing kernel composition of almond the genotype and climatic conditions during growth and fruit developmental stages are most important. Fatty acids are an important part of human diet and are essential for the normal functioning of the body. They function as energy storage molecules, plasma membrane components, and signalling molecules that control gene expression, cell development, and differentiation (Calder, 2015). Through these functions and actions, fatty acids can influence physiological function, overall health, and disease risk.

Fatty acids play a significant role in the prevention and management of chronic and metabolic diseases such as cardiovascular disease, type 2 diabetes, inflammatory diseases, and cancer (Calder, 2015; Marventano *et al.,* 2015). However, individual fatty acid have their own specific actions, consequently, each fatty acid or group have a different type of effect on human health. Different types of nuts contain different amounts of fat and different types of fatty acids. Almond is considered one of the healthiest nut as it contains lower saturated fatty acids (8-10%) and higher amounts of unsaturated fatty acids (90-92%) (Wojdylo *et al.,* 2022). The higher amounts of heart-healthy unsaturated fatty acids present in almonds are negatively associated with serum cholesterol levels (Nishi *et al.,* 2014) and a controlled trial showed that 73 g of almonds in the daily diet reduced LDL cholesterol upto 9.4% (Jenkins *et al.,* 2002), thus almond consumption reduces the risk of coronary heart disease (Chen *et al.,* 2006). The oil presents the major component in the almond kernel ranging from 25.19 (Askin *et al.,* 2007) to 79% (Ozcan *et al.,* 2020). This oil has a variety of uses in food, cosmetics, and supplementary medicine due to its anti-inflammatory, anti-hepatotoxic, immunity-boosting, and modulatory effects on inflammation properties (Lin *et al*., 2018; Moore *et al.,* 2020).

Nutritional improvement of almond crops through breeding programme is gaining importance, as the nutritional value of the almond kernel is one of the most important factors in determining the choice of the almond cultivar, and now-a-days many public health organizations recommend a daily intake of almonds as part of an overall healthy diet. Nutritional profiling of almond genetic resources is essential as this helps in the selection of almond genotypes for their utilization and preparation of value-added products. Moreover, it also helps to identify parents for breeding programme to improve kernel chemical composition. Almond kernels with high oil and unsaturated fatty acid contents are desirable. Although several studies have been reported on the oil content and fatty acids composition of almonds, however, the oil content and fatty acid compositions vary with genotype (Maestri *et al.,* 2015; Gouta *et al.,* 2020), weather condition on the crop site (Rabadan *et al.,* 2019), water supply (Zhu *et al.,* 2015), kernel weight (Askin *et al.,* 2007), soil composition and cultural practices. Therefore, this study was conducted to estimate the oil and fatty acid compositions of some commercial and locally collected almond genotypes grown under rainfed conditions in Kashmir for direct commercialization and utilization in future breeding programme to improve existing commercial cultivars.

Materials and Methods

The nuts of 32 almond genotypes (Fig. 1) were collected from the Experimental Farm of ICAR-Central Institute of Temperate Horticulture, Srinagar (34.05' N, 74°50', E and 1640 m a.s.l.), Jammu and Kashmir, India during 2018. The orchard was established during 1998-1999 on seedling rootstock through budding. The study location experience mild summer (maximum temperate up to 34ºC) and severe winter (minimum temperature about -7ºC) with an annual average temperature of about 13.5ºC. The precipitation (last decade) ranges from 274 to 658 mm per year and with an average of 357 mm per year and the majority of precipitation received during January to May. Fruits of each almond genotype were harvested at appropriate maturity time *i.e.* when mesocarp was dried and split along the suture, and their mesocarp (hull) were removed from endocarp (shell). Hull splitting began in different almond genotypes between 122 and 157 days after full bloom and attained harvestable maturity (90 % hull split) between 130 and 164 days after full bloom. Harvested

nuts were dried under natural conditions and three independent samples each containing 50 nuts were randomly selected for analysis. Using a Soxhlet fat apparatus and 100 ml of petroleum ether as a solvent, almond oil was extracted from 5 g of the ground kernel. A rotary evaporator was used to evaporate the solvent under a vacuum, and the oil was recovered (Bligh, Dyer 1959). The optimum period of oil extraction was approximately, 2 hr including the recovery period. The percentage of oil was represented as the difference in weight of the dried almond kernel samples before and after soxhlet extraction.

A method described by Slover and Lanza (1979) was used to prepare fatty acid methyl esters. The fatty acid content of almond oil was analyzed by GC 30 (Perkin Elmer Autosystem XL) equipped with a CP-Wax 52 CB column (Varian Inc.) (50 x 0.25 mm, 0.2 µm). One milliliter of Na-ethylate (0.5 g Na-methylate + 80 ml methanol + 20 ml isooctane) solution was added onto 40 µl of oil sample, and esterified. Before injecting into the GC, 0.25 ml of isooctane was added to the tube and thoroughly shaken. Thereafter with a microinjector, 0.5 ml was drawn from the upper phase, which had become clear, and injected into the GC apparatus (Perkin Elmer Autosystem XL). The were as follows: GC conditions: FID detector; injector and detector temperatures: He as a carrier gas at 0.069 MPa; pressure and later flow rate 30 ml min^{-1} at 250ºC. The oven temperature was set at 80º C for 4 min and later increased to 175ºC. After 25 min at this temperature, the temperature was increased to 215º C. After 2 min at 215º C, the temperature was steadily increased to 240º C and held for 10 min. Peaks were found by comparing them with the relative retention times of standards (Supelco 37 component FAME Mix), and the results were represented as percentages of peak areas. The following formula was used to compute iodine values (IV) from oleic, linoleic, and linolenic acid percentages (Martinez *et al.,* 2008):

IV = (% oleic acid x 0.899) + (% linoleic acid x 1.814) + (% linolenic acid x 2.737)

The statistical analysis was performed in triplicate from three different samples from each genotype. The compiled data were statistically analyzed by Analysis of Variance (ANOVA), Principal Component Analysis (PCA), and Correlation to ascertain the superlative genotypes exhibiting high-quality parameters with respect to fatty acids. All statistical tests were done using SAS (Version 9.3). The obtained experimental data were subjected to statistical analysis based on the ANOVA with the PROC GLM procedure. Duncan's Multiple Range Test was used to compare the means with a probability of 0.05 and 0.01. Pearson's test was used for correlation analysis (CORR procedure). PCA was performed on the correlation matrix using the PRINCOMP Procedure.

Results and Discussion

The results for the oil contents in the kernel of different almond genotypes are presented in Fig. 2. The genotypes showed significant variation (p<.0001) in oil content ranging from 41.82% (CITH-A-21) to 51.86% (Waris). Apart from Waris, the oil content of Pranyaj, California Paper Shell, and Merced was found to be more than 50 per cent. Whereas, ten genotypes recorded oil content between 48 to 50 per cent. A large variation in oil content in different almond genotypes has been previously reported by Askin *et al.,* 2007 (25.19 to 60.77% in 26 genotypes from Turkey); Colic *et al.,* 2017 (36.3 to 62.86% in 23 genotypes from Serbia) and Ozcan *et al.,* 2020 (36.7 to 79% in 31 genotypes from Turkey).

In the present investigation, the variation observed in oil content could be due to the difference in the genotypes, as genotype has a significant influence on oil content (Maestri *et al.,* 2015; Houmy *et al.,* 2016). Furthermore, the weather conditions also influence the oil content for instance in Nonpareil, the total oil content was reported as 56.78% in Isparta, Turkey (Yildirim *et al.,* 2016), 53% in San Juan Province, Argentina (Maestri *et al.,* 2015) and 47.88% in the present study (Kashmir, India). The effect of growing weather conditions on the oil content of almonds kernel was earlier reported by Rabadan *et al.* (2019). The results of the present investigation on the oil content in the almond genotypes are in good agreement with the studies of Piscopo *et al.* (2010) and Moayedi *et al.* (2011). However, in comparison to oil content observed in the present study, Yildirim *et al.* (2016) and Rapposelli *et al.* (2018) reported higher (52.03 to 64.47%) oil content in different almond genotypes. The heritability reported for almond oil content is high (0.57) indicating an additive gene action, being a trait less affected by environmental variables (Forcada *et al.,* 2011), thus genotypes with high oil content can be used in the breeding programmes to increase the oil content in existing commercial cultivars or directly in commercial oil production.

The fatty acids content is the most important feature used to characterise fats and oils in almond as it is associated with oxidative stability and some nutritional characteristics (Fernandes *et al.,* 2017). Fatty acid profiling was done in 32 almond genotypes to reveal the variability across the genotypes. Highly significant variation was observed across the studied almond genotypes with respect to fatty acids (p-<.0001). Data presented in Table 1 revealed that irrespective of genotypes major fatty acids found were oleic acid- C18:1 (71.99%), linoleic acid- C18:2 (19.88%), and palmitic acid- C16:0 (6.37%). The stearic acid (C18:0) was present at a lower proportion (1.69%), while α-linolenic acid (C18:3) was found at a very low level (0.08%) and was not detected in five almond genotypes.

The palmitic acid content varied from 5.19 to 8.13% and the lowest and highest content was estimated in CITH-A-17 and Nonpareil, respectively. However, CITH-A-20 remained statistically at par with CITH-A-17. Apart from CITH-A-17 and CITH-A-20, the palmitic acid content was also found to be less than 6 per cent in five almond genotypes. Palmitic acid provides more stability to oil but is harmful to the cardiovascular system (Kodad and Socias i Company, 2008). The range of palmitic acid in the current investigation is following previous findings by Zamany *et al.* (2017); Rapposelli *et al.* (2018); Ozcan *et al.* (2020)

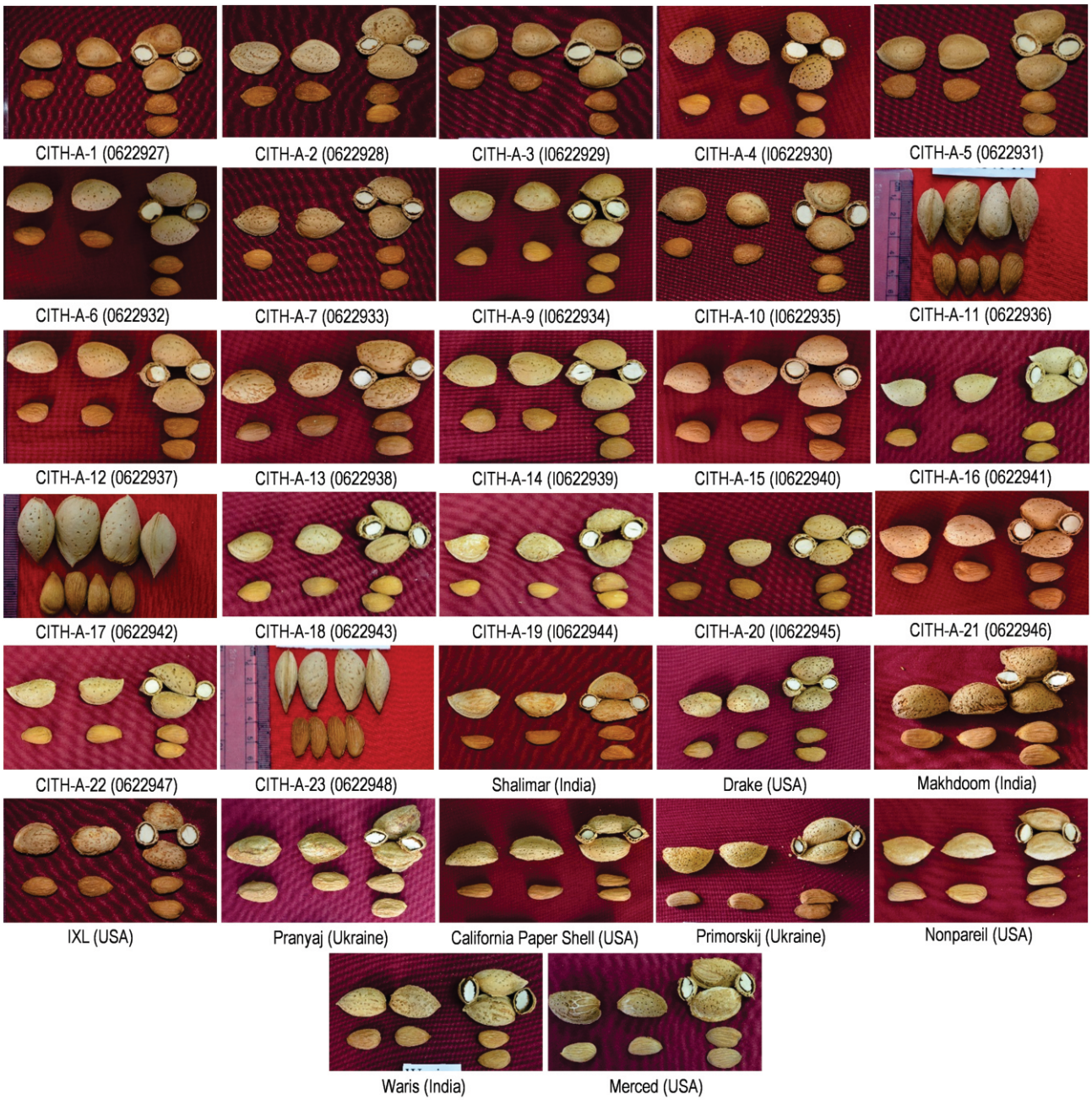


Fig.1: Nuts and kernels of almond genotypes included in the present study along with indigenous collection number/country of origin.

and Gouta *et al.* (2020). However, in some almond genotypes, Yildirim *et al.* (2016) and Csakvari *et al.* (2019) reported higher, on the other hand Karatay *et al.* (2014) and Colic *et al.* (2017) reported lower palmitic acid content compared with the results obtained in present study. Further, in present study stearic acid content varied from 1.01% (CITH-A-1) to 2.53% (CITH-A-21). Many researchers (Maestri *et al.,* 2015; Houmy *et al.,* 2016; Colic *et al.,* 2017, Rapposelli *et al.,* 2018) detected stearic acid content within the range obtained in the present study. However, in comparison to the present study, some researchers observed lower (Sathe *et al.,* 2008; Yildirim *et al.,* 2016) and higher (Kodad *et al.,* 2011; Csakvari *et al.,* 2019; Gouta *et al.,* 2020) stearic acid content in different almond genotypes.

Oleic acid was the predominant fatty acid, representing 53.70-80.81% of total fatty acid, followed by linoleic acid which represents 11.63-36.88% of total fatty acid and both together accounted for 89.91 to 93.59% of total fatty acid contents. The

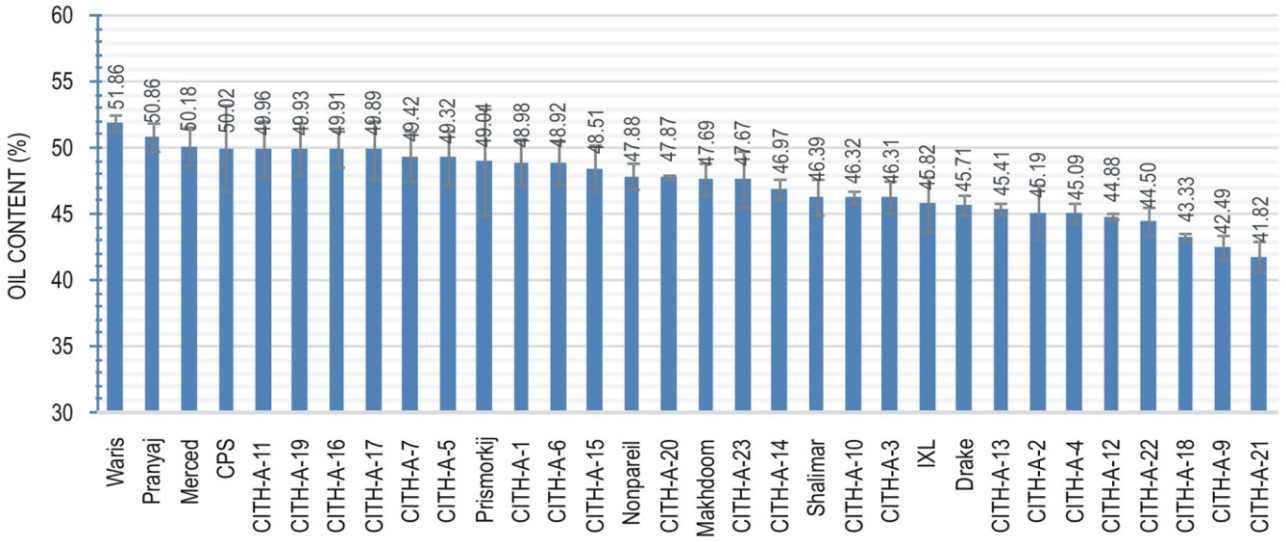


Fig. 2: Values of oil content of almond genotypes grown under rainfed conditions of the Western Himalayan region of India. The bars represent mean values ±S.D.

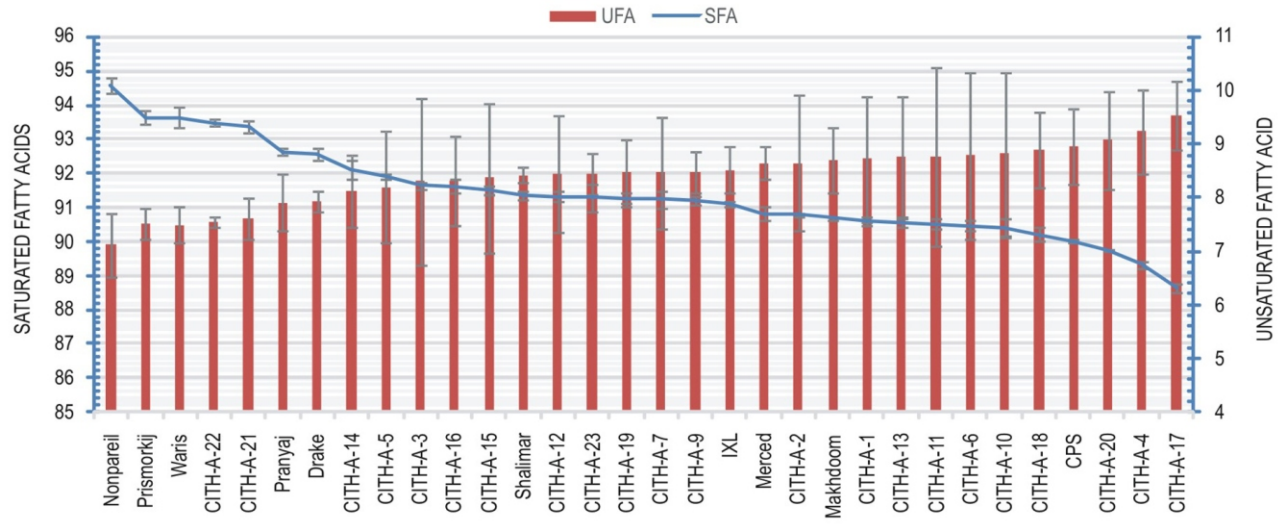


Fig. 3: Saturated and unsaturated fatty acid composition of almond genotypes grown under rainfed conditions of the Western Himalayan region of India. The bars represent mean values ±S.D.

genotype CITH-A-6 contained the highest amount of oleic acid and the lowest amount of linoleic acid. *Vice-versa* CITH-A-21 contained the lowest amount of oleic acid and the highest amount of linoleic acid. The oleic acid content in this study was in the range of 50.41 to 81.2% which corroborates with the study of Askin *et al.* (2007) for 26 almond genotypes at the eastern Anatolia region of Turkey. Furthermore, the present results are partly supported by the previous findings which reported that oleic acid content varied from 58.10 to 71.30 (Zhu *et al.,* 2015); 61 to 77% (Csakvari *et al.,* 2019); 62.43 to 76.34% (Ozcan *et al.,* 2020); 62.54 to 81.57 (Zamany *et al.,* 2017); 63.14 to 77.37% (Colic *et al.,* 2017); 65 to 76% (Gouta *et al.,* 2020); 68.64 to 78.29% (Rapposelli *et al.,* 2018) of total oil in the kernels of different almond genotypes. Oleic acid, a monounsaturated fatty acid helps to lower low-density lipoproteins (LDL) cholesterols in humans as effectively as polyunsaturated fatty acids (Mensik and Katan, 1989). Thus, it decreases the risk of cardiovascular diseases (Chen *et al.,* 2006) and 40 per cent of almond genotypes included in this investigation contained more than 75 per cent oleic acid of total fatty acids.

Table 1: Fatty acid compositions (%) in different almond genotypes grown in Western Himalayan region of India

Genotypes	Palmitic Acid (C16:0)	Stearic Acid (C18:0)	Oleic Acid (C18:1)	Linoleic Acid (C18:2)	Linolenic Acid (C18:3)	Oleic/ Linoleic ratio	Oleic+ Linoleic acid
CITH-A-1	6.56^{f} ± 0.06	1.01^{q} ± 0.07	75.68^{c-h} ± 2.62	16.66^{j-l} ± 0.83	0.09^{c-e} ± 0.01	4.55^{d-f} ± 0.37	92.34^{ab} ± 1.85
CITH-A-2	6.20^{ij} ± 0.06	1.49^{l-n} ± 0.05	78.69^{a-d} ± 2.13	13.56^{n} ± 0.54	0.06^{fg} ± 0.00	5.81^{b} ± 0.34	92.25^{ab} ± 1.97
CITH-A-3	6.75^{e} ± 0.03	1.48^{l-n} ± 0.05	74.53^{f-k} ± 1.59	17.16^{i-k} ± 0.87	0.08^{d-f} ± 0.02	4.35^{e-g} ± 0.12	91.69^{ab} ± 2.45
CITH-A-4	5.50^{o} ± 0.03	1.26^{o} ± 0.03	74.99^{f-l} ± 1.49	18.18^{h-j} ± 0.56	0.07^{e-g} ± 0.00	4.13^{f-h} ± 0.19	93.17^{a} ± 1.23
CITH-A-5	6.63^{f} ± 0.04	1.77^{de} ± 0.08	68.64^{m} ± 2.64	22.88^{e} ± 1.90	0.08^{d-f} ± 0.00	3.01^{kl} ± 0.24	91.52^{ab} ± 3.62
CITH-A-6	6.05^{kl} ± 0.08	1.43^{l-n} ± 0.02	80.81^{a} ± 2.09	11.63^{o} ± 0.41	0.08^{d-f} ± 0.03	6.95^{a} ± 0.11	92.44^{ab} ± 2.47
CITH-A-7	6.44^{g} ± 0.09	1.53^{i-m} ± 0.07	71.77^{j-l} ± 1.87	20.17^{fg} ± 1.77	0.09^{c-e} ± 0.01	3.57^{ij} ± 0.22	91.94^{ab} ± 3.63
CITH-A-9	6.30^{hi} ± 0.08	1.66^{e-l} ± 0.04	72.68^{h-l} ± 1.25	19.28^{gh} ± 1.22	0.08^{d-f} ± 0.02	3.78^{h-j} ± 0.31	91.96^{ab} ± 0.64
CITH-A-10	5.87^{m} ± 0.08	1.56^{h-l} ± 0.12	73.73^{g-k} ± 0.98	18.79^{g-l} ± 1.74	0.06^{fg} ± 0.01	3.95^{g-l} ± 0.36	92.52^{ab} ± 2.39
CITH-A-11	6.07^{k} ± 0.07	1.44^{k-n} ± 0.05	76.72^{b-g} ± 2.08	15.73^{k-m} ± 0.58	0.05^{g} ± 0.00	4.88^{c-e} ± 0.08	92.45^{ab} ± 2.62
CITH-A-12	6.38^{gh} ± 0.07	1.64^{e-l} ± 0.05	75.10^{e-h} ± 1.82	16.82^{i-l} ± 0.24	0.06^{fg} ± 0.00	4.47^{d-g} ± 0.15	91.92^{ab} ± 1.72
CITH-A-13	6.11^{jk} ± 0.07	1.43^{l-n} ± 0.03	77.02^{b-f} ± 1.95	15.38^{k-n} ± 0.26	0.07^{e-g} ± 0.01	5.01^{cd} ± 0.20	92.40^{ab} ± 1.75
CITH-A-14	6.04^{kl} ± 0.07	2.49^{a} ± 0.13	62.53^{n} ± 1.97	28.83^{c} ± 1.18	0.11^{bc} ± 0.02	2.17^{mn} ± 0.15	91.36^{ab} ± 1.07
CITH-A-15	6.57^{f} ± 0.12	1.56^{h-l} ± 0.06	74.84^{f-j} ± 1.71	16.97^{i-k} ± 0.59	0.06^{fg} ± 0.02	4.41^{e-g} ± 0.10	91.81^{ab} ± 2.20
CITH-A-16	6.21^{ij} ± 0.07	2.01^{c} ± 0.13	71.92^{j-l} ± 2.04	19.68^{gh} ± 0.83	0.18^{a} ± 0.03	3.66^{h-j} ± 0.26	91.60^{ab} ± 1.28
CITH-A-17	5.19^{p} ± 0.07	1.13^{p} ± 0.03	79.62^{ab} ± 2.04	13.97^{mn} ± 1.01	0.10^{cd} ± 0.02	5.73^{b} ± 0.57	93.59^{a} ± 1.02
CITH-A-18	5.62^{n} ± 0.07	1.70^{d-g} ± 0.10	76.72^{b-g} ± 1.96	15.89^{k-m} ± 1.00	0.07^{e-g} ± 0.00	4.85^{c-f} ± 0.43	92.61^{ab} ± 1.10
CITH-A-19	5.85^{m} ± 0.20	2.14^{b} ± 0.04	75.51^{d-h} ± 0.98	16.34^{j-l} ± 0.13	0.16^{a} ± 0.02	4.62^{d-f} ± 0.08	91.85^{ab} ± 0.96
CITH-A-20	5.23^{p} ± 0.07	1.80^{d} ± 0.08	78.24^{a-e} ± 1.64	14.67^{l-n} ± 0.82	0.06^{fg} ± 0.00	5.35^{bc} ± 0.36	92.91^{ab} ± 1.44
CITH-A-21	6.79^{e} ± 0.08	2.53^{a} ± 0.06	53.70^{p} ± 1.31	36.88^{a} ± 1.73	0.09^{c-e} ± 0.02	1.46^{o} ± 0.10	90.58^{ab} ± 0.61
CITH-A-22	7.46^{c} ± 0.07	1.94^{c} ± 0.03	64.53^{n} ± 0.50	26.01^{d} ± 0.58	0.06^{fg} ± 0.02	2.48^{m} ± 0.08	90.54^{ab} ± 0.13
CITH-A-23	6.01^{kl} ± 0.07	1.99^{c} ± 0.17	64.61^{n} ± 0.56	27.28^{cd} ± 1.04	0.10^{cd} ± 0.01	2.37^{mn} ± 0.11	91.89^{ab} ± 0.60
Shalimar	6.32^{g-l} ± 0.05	1.73^{d-f} ± 0.03	71.57^{kl} ± 1.30	20.32^{fg} ± 1.07	0.06^{fg} ± 0.00	3.53^{ij} ± 0.25	91.89^{ab} ± 0.23
Drake	6.59^{f} ± 0.07	2.22^{b} ± 0.04	74.45^{f-k} ± 1.49	16.74^{jk} ± 1.21	ND	4.47^{d-g} ± 0.42	91.19^{ab} ± 0.29
Makhdoom	6.22^{ij} ± 0.05	1.40^{mn} ± 0.06	70.57^{lm} ± 2.02	21.75^{ef} ± 1.42	0.07^{e-g} ± 0.02	3.26^{jk} ± 0.29	92.32^{ab} ± 0.95
IXL	6.32^{g-l} ± 0.06	1.57^{g-k} ± 0.06	75.87^{c-h} ± 0.52	16.24^{jkl} ± 1.06	ND	4.69^{d-f} ± 0.33	92.11^{ab} ± 0.66
Pranyaj	7.25^{d} ± 0.06	1.61^{f-j} ± 0.02	65.48^{n} ± 1.25	25.66^{d} ± 0.50	ND	2.55^{lm} ± 0.09	91.14^{ab} ± 0.83
CPS	5.93^{lm} ± 0.05	1.26^{o} ± 0.03	78.82^{a-c} ± 2.06	13.89^{mn} ± 1.06	0.09^{c-e} ± 0.02	5.70^{b} ± 0.58	92.71^{ab} ± 1.09
Prismorkij	7.25^{d} ± 0.07	2.25^{b} ± 0.08	63.62^{n} ± 1.29	26.84^{d} ± 1.19	0.05^{g} ± 0.00	2.37^{mn} ± 0.15	90.46^{ab} ± 0.44
Nonpareil	8.13^{a} ± 0.10	1.96^{c} ± 0.05	58.37^{o} ± 1.54	31.54^{b} ± 1.00	ND	1.85^{no} ± 0.10	89.91^{b} ± 0.92
Waris	7.81^{b} ± 0.10	1.68^{d-h} ± 0.10	63.57^{n} ± 1.27	26.80^{d} ± 1.45	0.13^{b} ± 0.02	2.38^{mn} ± 0.18	90.37^{ab} ± 0.54
Merced	6.32^{g-l} ± 0.09	1.38^{no} ± 0.04	78.71^{a-d} ± 1.49	13.59^{n} ± 1.30	ND	5.83^{b} ± 0.66	92.30^{ab} ± 0.47
Minimum	5.19	1.01	53.70	11.63	0.05	1.46	89.91
Maximum	8.13	2.53	80.81	36.88	0.18	6.95	93.59
Mean	6.37	1.69	71.99	19.88	0.08	4.01	91.87
Pr> F	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.5790

Fatty acids are expressed as per cent of total fatty acid. Mean values (± standard deviation) were the average of three independent measurements. Values followed by the same superscript letter within the columns are not significantly different (p=0.05) using Duncan's multiple range test.

Linoleic acid content obtained from different almond genotypes in the present study fall in the range reported by Askin *et al*. (2007) for 26 almond genotypes at the eastern Anatolia region of Turkey (6.21% to 37.13%). Furthermore, in different almond genotypes Rabadan *et al.* (2017); Colic *et al.* (2017); Rapposelli *et al.* (2018); Csakvari *et al.* (2019); Gouta *et al.* (2020) and Ozcan *et al.* (2020) reported linoleic acid content in accordance with the current investigation. Further, the highest linolenic acid (0.18%) was detected in CITH-A-16. which remained statistically at par with CITH-A-19. Linolenic acid content, on the other hand, was not detected in Drake, IXL, Pranyaj, Nonpareil and Merced. The range of linolenic acid in this investigation is in agreement with the previous reports (Sathe *et al.,* 2008; Colic *et al.,* 2017). Previous studies have shown that different genotypes can produce almonds with varied fatty acids profiles (Rabadan *et al.,* 2019; Levent, 2022). In the present study, the variation observed in the fatty acid profile was very high and the content of palmitic, stearic, oleic, and linoleic acid were significantly influenced by the almond genotype. Further, growing environmental conditions can also have a substantial influence on the fatty acids content of nuts. Table 2 shows the fatty acids content of four almond cultivars included in this study (Indian conditions) and grown in Argentina, Spain, Turkey, Australia and USA. This comparison can be useful for determining the effect of environmental conditions on the fatty acids composition of almonds between India and these countries. Differences in fatty

Table 2: Major fatty acids in four almond cultivar grown in India compared to Argentina, Spain, Turkey, Australia and USA

Major fatty acid (%)	Cultivars									
	Nonpareil					Drake		IXL		Primorskij
	Argentina[A]	Spain[B]	Turkey[C]	Australia[D]	USA[E]	Spain[B]	Turkey[F]	Argentina[A]	Spain[B]	Spain[B]
Palmitic Acid	6.13-6.87	6.31-6.56	8.15 8.13*	7.3	6.64	6.33-6.42 6.59*	7.04	6.28-6.83 6.32*	6.01-6.30	5.97-6.8 7.25*
Stearic Acid	1.32-1.49	1.41-1.52	2.18 1.96*	1.6	0.62	1.58-1.66 2.22*	2.25	1.17-1.20 1.57*	1.82-2.11	1.70-2.05 2.25*
Oleic Acid	69.9-71.9	65.31-69.93	61.22 58.37*	62.6	61.89	64.86-70.5 74.45*	59.84	68.8-73.0 75.87*	66.66-71.08	64.48 71.55 63.62*
Linoleic Acid	19.2-21.7	24.63-28.62	27.69 31.54*	24.7	30.31	20.43-26.13 16.74*	23.52	18.2-22.9 16.24*	19.87-23.67	19.76-24.31 26.84*
O/L ratio	3.22-3.75	2.10-2.67	2.21 1.85*	2.53	2.04	2.49-3.46 4.47*	2.54	3.00-4.03 4.69*	2.82-3.58	2.65-3.62 2.37*
Oleic+Linoleic	91.1-91.6	90.51-90.97	88.91 89.91*	87.3	92.19	90.93-90.99 91.19*	83.36	91.2-91.7 92.11*	90.33-90.95	88.79-91.31 90.46*

[A]Maestri *et al.,* 2015; [B]Kodad *et al.,* 2011; [C]Levent, 2022; [D]Zhu *et al.,* 2017; [E]Sathe *et al.,* 2008; [F]Beyhan *et al.,* 2011. * Value of fatty acids in India (present study).

acids content of Nonpariel, Drake, IXL, and Primorskij, confirm the effect of geographical areas on the composition of fatty acids. Variation between different studies conducted across different locations could be explained by temperature variation, rainfall pattern, and other plant performance factors such as water supply, soil type, soil fertility status, and interaction between these factors. Our results for Nonpareil are similar to those found in Turkey. Further, higher palmitic, stearic, and linoleic acid, were found in this study on comparing Nonpareil cultivar planted in India with USA (origin country), Argentina, Spain, and Australia. A similar pattern was also seen in the Primorskij variety planted in India and Spain. On the other hand, Drake and IXL produced higher oleic acid and O/L ratio in Indian conditions as compared to Spain, Argentina and Turkish conditions. The present study confirms that the Kashmir region is suitable for growing almonds even in rainfed conditions with fatty acids composition at par with commercial almond cultivars grown in major almond-growing countries in the world such as USA, Spain, Turkey and Australia. These encouraging results indicate the potential for successful almond cultivation in the Kashmir region and its adaptability to local conditions.

The oleic acid to linoleic acid ratio is regarded as a key indicator to assess the almond kernel quality because of its preventive effect on the oxidation of lipid (Zacheo *et al.,* 2000) and can also be used to distinguish almond genotype because it does not change over time (Kodad and Socias i Company, 2008). Due to the presence of carbon to carbon double bond linoleic acid is far more susceptible to oxidation than oleic acid. Thus, a high oleic acid and low linoleic acid content increases the resistance of almond kernel to oxidation during storage, processing, and transportation (Zacheo *et al.,* 2000; Kodad *et al.,* 2014; Zhu *et al.,* 2015). In the present study, the oleic: linoleic acid ratio varied from 1.46 (CITH-A-21) to 6.95 (CITH-A-06). In CITH-A-2, CITH-A-6, CITH-A-17, CITH-A-20, CPS, and Merced, the oleic: linoleic acid ratio was higher than five. The present investigation revealed large variability among the almond genotypes for oleic: linoleic acid ratio because of high variability in oleic and linoleic acid contents. The present findings are in agreement with earlier research findings by Kodad *et al.* (2011); Maestri *et al.* (2015); and Gouta *et al.* (2020), who reported 2.1 to 6.11; 2.49 to 6.01 and 2.92 to 5.67 oleic: linoleic acid ratio, respectively, in different almond genotypes.

The functional usefulness of almond kernels or oil depends on the fraction of saturated and unsaturated fatty acids. Hence, it is significant to compute the level of saturated (SFA), unsaturated (UFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids. In this study, saturated fatty acids were present at levels lesser than 10%. The lowest content of saturated (6.32%) and the highest content of unsaturated (93.69%) fatty acids were found in CITH-A-17. In contrast, the highest saturated (10.09%) and the lowest unsaturated (89.91%) fatty acids were found in Nonpareil (Fig. 3). CITH-A-6 had the highest content of monounsaturated (80.81%) and the lowest content of polyunsaturated (11.71%) fatty acids, while at the opposite extreme, CITH-A-21 had the lowest content of monounsaturated (53.7%) and the highest content of polyunsaturated (36.97%) fatty acid. The genotype CITH-A-17 had a higher UFA: SFA ratio (14.82) than other genotypes, while the lowest value of UFA: SFA ratio (8.91) was recorded in Nonpareil (Table 3). The predominance of oleic acid (MUFA) may aid in understanding why almonds have a longer shelf life than nuts with high levels of linoleic and linolenic acid (PUFA). The average ratio of PMS; PUFA: MUFA: SFA (1.99: 7.20: 0.81) in the present study indicated significant variation in relative proportion of fatty acids. The ratio reported in the present study was lower (4.12: 10.03: 1) than the ratio reported by Sathe *et al.* (2008) in almond.

Table 3: Fatty acid composition, derivatives, and respective Iodine values of almond genotypes grown in western Himalayan region of India

Genotypes	MUFA	PUFA	UFA/SFA	Iodine value
CITH-A-1	75.68^{c-h} ± 2.62	16.75^{jkl} ± 0.84	12.21^{ef} ± 0.18	98.50^{f-h} ± 1.01
CITH-A-2	78.69^{a-d} ± 2.13	13.62^{n} ± 0.54	12.00^{f-h} ± 0.20	95.50^{hi} ± 1.75
CITH-A-3	74.53^{f-k} ± 1.59	17.24^{i-k} ± 0.85	11.15^{jkl} ± 0.33	98.35^{f-h} ± 2.96
CITH-A-4	74.99^{f-l} ± 1.49	18.25^{h-j} ± 0.56	13.79^{b} ± 0.27	100.59^{e-g} ± 1.08
CITH-A-5	68.64^{m} ± 2.64	22.96^{e} ± 1.90	10.90^{kl} ± 0.50	103.43^{de} ± 4.66
CITH-A-6	80.81^{a} ± 2.09	11.71^{o} ± 0.39	12.37^{ef} ± 0.33	93.96^{i} ± 2.53
CITH-A-7	71.77^{jkl} ± 1.87	20.26^{fg} ± 1.77	11.55^{b-j} ± 0.45	101.36^{ef} ± 4.89
CITH-A-9	72.68^{h-l} ± 1.25	19.36^{gh} ± 1.23	11.56^{h-j} ± 0.12	100.53^{e-g} ± 1.36
CITH-A-10	73.73^{g-k} ± 0.98	18.85^{g-j} ± 1.73	12.46^{ef} ± 0.13	100.53^{e-g} ± 3.66
CITH-A-11	76.72^{b-g} ± 2.08	15.78^{k-m} ± 0.58	12.32^{ef} ± 0.19	97.64^{f-i} ± 2.85
CITH-A-12	75.10^{e-h} ± 1.82	16.88^{i-k} ± 0.24	11.47^{ij} ± 0.28	98.19^{f-h} ± 1.49
CITH-A-13	77.02^{b-f} ± 1.95	15.45^{k-n} ± 0.25	12.26^{ef} ± 0.37	97.33^{g-i} ± 1.42
CITH-A-14	62.53^{n} ± 1.97	28.94^{c} ± 1.19	10.72^{lm} ± 0.36	108.81^{bc} ± 0.97
CITH-A-15	74.84^{f-j} ± 1.71	17.03^{i-k} ± 0.61	11.30^{i-k} ± 0.38	98.23^{f-h} ± 2.47
CITH-A-16	71.92^{kl} ± 2.04	19.86^{gh} ± 0.80	11.17^{jkl} ± 0.32	100.85^{e-g} ± 0.66
CITH-A-17	79.62^{ab} ± 2.04	14.07^{mn} ± 1.03	14.82^{a} ± 0.36	97.19^{g-i} ± 0.06
CITH-A-18	76.72^{b-g} ± 1.96	15.96^{k-m} ± 1.00	12.66^{de} ± 0.30	97.99^{f-h} ± 0.68
CITH-A-19	75.51^{d-h} ± 0.98	16.50^{jkl} ± 0.11	11.52^{ij} ± 0.27	97.96^{f-h} ± 0.86
CITH-A-20	78.24^{a-e} ± 1.64	14.73^{l-n} ± 0.82	13.22^{c} ± 0.17	97.11^{g-i} ± 1.51
CITH-A-21	53.70^{p} ± 1.31	36.97^{a} ± 1.71	9.73^{o} ± 0.15	115.42^{a} ± 2.00
CITH-A-22	64.53^{n} ± 0.50	26.07^{d} ± 0.60	9.64^{o} ± 0.08	105.36^{cd} ± 0.67
CITH-A-23	64.61^{n} ± 0.56	27.38^{cd} ± 1.03	11.5^{o-j} ± 0.35	107.84^{bc} ± 1.44
Shalimar	71.57^{kl} ± 1.30	20.38^{fg} ± 1.07	11.42^{ij} ± 0.14	101.37^{ef} ± 0.77
Drake	74.45^{f-k} ± 1.49	16.74^{jkl} ± 1.21	10.35^{mn} ± 0.10	97.30^{g-i} ± 0.87
Makhdoom	70.57^{lm} ± 2.02	21.82^{ef} ± 1.40	12.12^{fg} ± 0.12	103.09^{de} ± 1.17
IXL	75.87^{c-h} ± 0.52	16.24^{jk} ± 1.06	11.67^{g-j} ± 0.10	97.67^{f-i} ± 1.54
Pranyaj	65.48^{n} ± 1.25	25.66^{d} ± 0.50	10.29^{n} ± 0.11	105.41^{cd} ± 0.52
CPS	78.82^{a-c} ± 2.06	13.98^{mn} ± 1.04	12.91^{cd} ± 0.12	96.30^{hi} ± 0.50
Primorskij	63.62^{n} ± 1.29	26.89^{d} ± 1.19	9.53^{o} ± 0.11	106.02^{cd} ± 1.15
Nonpareil	58.37^{o} ± 1.54	31.54^{b} ± 1.00	8.91^{p} ± 0.18	109.69^{b} ± 1.05
Waris	63.57^{n} ± 1.27	26.93^{d} ± 1.44	9.54^{o} ± 0.25	106.12^{cd} ± 1.59
Merced	78.71^{a-d} ± 1.49	13.59^{n} ± 1.30	11.99^{f-h} ± 0.25	95.41^{hi} ± 1.16
Minimum	53.70	11.71	8.91	93.96
Maximum	80.81	36.97	14.82	115.42
Mean	71.99	19.95	11.53	100.97
Pr> F	<.0001	<.0001	<.0001	<.0001

SFA: saturated fatty acids; UFA: unsaturated fatty acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Fatty acids are expressed as per cent of total fatty acid. Mean values ± S.D. are average of three independent measurements. Values followed by the same superscript letter within the columns are not significantly different (p=0.05) using Duncan's multiple range test.

The iodine value varied from 93.96 (CITH-A-6) to 115.42 (CITH-A-21) in different almond genotypes under study (Table 3). Iodine Value characterizes the unsaturation level of oil and iodine value is the grams of iodine consumed by 100 g of fat. Thus, a higher iodine value suggests higher unsaturation level of oil. In this study, almost 50% of the almond genotypes had an iodine value more than 100. The higher content of Iodine Value was observed in CITH-A-21 due to the presence of higher content of linoleic acid (36.88%) compared to other genotypes. The findings are in accordance with Maestri *et al.* (2015) and Houmy *et al.* (2016). Maestri *et al.* (2015) reported iodine values ranging from 98.9 to 106.7 in 9 almond genotypes grown in Argentina. Similarly, Houmy *et al.* (2016) observed Iodine values ranging from 98.42 to 103.9 in 4 almond genotypes grown in Eastern Morocco.

Correlation matrix related to interrelationships among the fatty acids and their derivatives of the investigated almond genotypes kernel is shown in Table 4. In this study, statistically significant correlations were not found between the oil content and type of fatty acid and their derivatives. Kodad *et al.* (2011) also observed statistically non-significant correlations between oil and fatty acids content in 73 almond genotypes. Likewise, no statistically significant correlations were observed between linolenic acid and other fatty acids. Similar findings were reported by Sathe *et al.* (2008) in 84 and Gouta *et al.* (2020) in 17 almond genotypes. However, the palmitic acid and stearic acid exhibited

Table 4: Correlations between fatty acids and their derivatives in almond genotypes grown in Western Himalayan region of India

	Oil content	Palmitic Acid	Stearic Acid	Oleic Acid	Linoleic Acid	Linolenic Acid	SFA	MUFA	PUFA	UFA	Iodine value	O/L ratio
Oil content	1.00	0.11	-0.24	0.12	-0.13	0.18	-0.02	0.12	-0.13	0.02	-0.14	0.11
Palmitic Acid		1.00	0.29	-0.67**	0.62**	-0.26	0.91**	-0.67**	0.62**	-0.91**	0.54**	-0.62**
Stearic Acid			1.00	-0.68**	0.67**	0.13	0.66**	-0.68**	0.67**	-0.66**	0.64**	-0.62**
Oleic Acid				1.00	-1.00**	-0.02	-0.82**	1.00**	-1.00**	0.82**	-0.98**	0.95**
Linoleic Acid					1.00	0.04	0.77**	-1.00**	1.00**	-0.77**	0.99**	-0.96**
Linolenic Acid						1.00	-0.15	-0.02	0.05	0.15	0.08	-0.06
SFA							1.00	-0.82**	0.77**	-1.00**	0.70**	-0.76**
MUFA								1.00	-1.00**	0.82**	-0.98**	0.95**
PUFA									1.00	-0.77**	0.99**	-0.96**
UFA										1.00	-0.70**	0.76**
UFA:SFA											-0.67**	0.74**
Iodine value											1.00	-0.95**
O/L ratio												1.00

Correlation is significant at **<0.01. SFA: saturated fatty acids; UFA: unsaturated fatty acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; O/L ratio- Oleic and Linoleic acid ratio

Table 5: Eigenvectors of three principle component axes of almond oil and fatty acids composition

Variables	Principle Component		
	1	2	3
Oil content (%)	-0.04	-0.22	0.83
Palmitic Acid (%)	0.26	-0.46	0.11
Stearic Acid (%)	0.24	0.20	-0.13
Oleic Acid (%)	-0.32	-0.13	0.00
Linoleic Acid (%)	0.32	0.18	-0.01
Linolenic Acid (%)	-0.01	0.54	0.53
SFA	0.30	-0.28	0.03
MUFA	-0.32	-0.13	0.00
PUFA	0.32	0.18	0.00
UFA	-0.30	0.28	-0.04
UFA:SFA	-0.30	0.28	-0.03
Iodine value	0.31	0.25	0.00
O/L ratio	-0.31	-0.16	-0.03
Eigen value	9.11	1.52	1.16
Variation (%)	70.06	11.71	8.93
Cumulative variation (%)	70.06	81.77	90.69

SFA: saturated fatty acids; UFA: unsaturated fatty acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; O/L ratio: Oleic and Linoleic acid ratio

a significantly positive (P<0.05) correlation with linoleic acid (r = 0.62** and r = 0.67**, respectively). A significant positive correlation between palmitic acid and linoleic acid was also reported by Gouta *et al.* (2020). Oleic acid, on the other hand, exhibited highly significant negative (P<0.05) correlations with linoleic acid (r = -1.00**). In almond statistically significant negative correlation between oleic acid and linoleic acid contents has been also reported by Askin *et al*. (2007) (r=-0.92); Sathe *et al.* (2008) (r=-0.99); Karatay *et al.* (2014) (r=-0.99); Csakvari *et al.* (2019) (r=-0.86); Gouta *et al.* (2020) (r=-0.97) and Levent (2022) (r=-0.98). The highly significant negative correlation between oleic and linoleic acid may be driven by the fact that the pool of oleic acid seems to be regulated by its conversion to linoleic acid, most likely as a response of the enzymatic activity of oleic desaturase (Garcia *et al.,* 1992). According to the findings of Kodad *et al.* (2011), the correlation observed between oleic and linoleic acid in the present investigation is genotype-dependent as correlation coefficients larger than 0.71 or less than -0.71 is biologically meaningful and not affected by climatic and environmental variables. Because of the strong association between these two major fatty acids, accurate predictions of total fatty acid composition of almond kernels could be obtained by analyzing only the linoleic acid content. Further, this strong correlation could be used as an index in the breeding programme to improve almond quality (Wang *et al.,* 2019).

Oleic acid and linoleic acid exhibited a highly significant positive and negative correlation with O/L ratio (r = 0.95** and r = -0.96**). Similar results were reported by Karatay *et al.* (2014) in almond. A high O/L ratio increases the resistance of almond kernel/oil to oxidation during storage, processing, and transportation (Zacheo *et al.,* 2000; Zhu *et al.,* 2015). The strong positive correlation between high O/L ratio with almond oil and kernel quality during storage implies that in a breeding program both parameters can be selected together. Consequently, selecting to enhance the O/L ratio aligns with the goal of improving oil quality and kernel storage life. Oleic acid also showed a significant negative (P<0.05) correlation with palmitic acid (r = -67**) and stearic acid (r=-68**). Askin *et al.* (2007); Karatay *et al.* (2014) and Gouta *et al.* (2020) also found a negative association between oleic and palmitic acid in different almond genotypes. Oleic acid and linoleic acid exhibited a highly significant negative and positive correlation with iodine value (r = -

0.98** and r = 0.99**). These associations between different fatty acids observed in this investigation suggest that the selection for one of these fatty acids could positively and negatively modify the content of other fatty acids therefore, correlation knowledge is important for breeders.

Principal component analysis was carried out to determine which components could be liable for almond genotypes differentiation in terms of oil content and fatty acids. The contribution of each PC to the total variance is presented in Table 5. About 70.06 per cent of variation is explained by the first eigenvalue and 81.77 per cent of the variation is explained by the first two eigenvalues. The first three PC explained 90.69 per cent of the total variability observed. Except for oil content and linolenic acid, most of the variables (value 0.24-0.32) were responsible for the separation of PC 1, and PC 2, primarily represented by palmitic and linolenic acid. However, along with oil content, linolenic acid also significantly contributed to the total variability observed in PC 3. Results of the PCA application in this study support the significance of oleic acid, linoleic acid, O/L ratio, and palmitic acid as important parameters that differentiate almond genotypes. The findings of the current investigation are in accordance with those of earlier PCA applications in almonds by Kodad *et al.* (2011, 2014); Yildirim *et al.* (2016) Rapposelli *et al.* (2018).

In the present study, a significant variation was found between different almond genotypes in respect of oil content, fatty acids, and their derivatives. To improve oil stability and nutritional value in almond, genotypes with high oleic acid (C18:1) and low linoleic acid (C18: 2) levels should be selected as parents in a breeding programme and based on oleic acid content, linolenic acid content and their ratio, it can be concluded that the local genotype CITH-A-06 may be of interest for almond breeding programmes with respect to fatty acid composition trait. Further, our study demonstrated that the Kashmir region is suitable for growing almonds with fatty acid composition standards comparable with commercial cultivars of almond grown throughout the world.

Acknowledgment

The authors are grateful to ICAR-Central Institute of Temperate Horticulture, Srinagar, Jammu and Kashmir for all the facilities, financial assistance and resources invested during the study period.

Authors' contribution: K.L. Kumawat: Planned and conducted the investigation, wrote original manuscript; **M.K. Verma:** Collected the almond genotypes used in the investigation; **D. Kumar:** Maintained the almond genotypes used in the investigation; **D.B. Singh:** Provided facilities for investigation and supervised the investigation; **S. Lal:** Helped in the analysis of fatty acids; **J.I. Mir:** Helped in the writing of the manuscript; **O.M. Sharma:** Helped in editing; **W.H. Raja:** Helped in compiling results; **Lal Chand:** Helped in statistical analysis of data.

Funding: The present work is funded by ICAR-Central Institute of Temperate Horticulture, Srinagar, Jammu and Kashmir under the project code IXX01851.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: Not applicable.

Conflict of interest: The authors declare that they have no conflict of interest, financial.

Data availability: Not applicable.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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