Food Chemistry: X 22 (2024) 101418

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Physicochemical and nutritional profiles of wild adlay (*Coix lacryma-jobi Linn*) accessions by GC, FTIR, and spectrophotometer

Rauf Ahmad [a], Muhammad Liaquat [a,**], Shehla Sammi [a], Jehad S. Al-Hawadi [b], Muhammad Jahangir [a], Amer Mumtaz [c], Imran Khan [a], Mohammad K. Okla [d], Ibrahim A. Alaraidh [d], Hamada AbdElgawad [e], Ke Liu [f], Matthew Tom Harrison [f], Shah Saud [g,**], Shah Hassan [h], Taufiq Nawaz [i], Mo Zhu [j,k,l], Haitao Liu [m], Muhammad Adnan [n], Abdul Sadiq [o], Tanzeel Ur Rahman [p], Basem H. Asghari [q], Shah Fahad [i,q,*]

[a] Department of Food Sciences and Technology, The University of Haripur, Haripur, Pakistan
[b] Faculty of Science, Zarqa University, Zarqa 13110, Jordan.
[c] National Agricultural Research Centre, Park Road, Chak Shezad, Islamabad, Pakistan
[d] Botany and Microbiology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[e] Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, 62521 Beni-Suef, Egypt
[f] Tasmanian Institute of Agriculture, University of Tasmania, Burnie 7250, Tasmania, Australia
[g] College of Life Science, Linyi University, Linyi, Shandong 276000, China
[h] Department of Agricultural Extension Education & Communication, The University of Agriculture, Peshawar, 25130, Khyber Pakhtunkhwa, Pakistan
[i] Department of Biology and Microbiology, South Dakota State University, Brookings, SD 57007, USA
[j] College of Life Sciences, Henan Normal University, Xinxiang 453007, PR China
[k] Henan International Joint Laboratory of Agricultural Microbial Ecology and Technology, Henan Normal University, Xinxiang 453007, PR China
[l] Xinxiang Key Laboratory of Plant Stress Biology, Xinxiang 453000, PR China
[m] College of Resources and Environment, Henan Agricultural University, Zhengzhou 450002, PR China
[n] Department of Agriculture, The University of Swabi, Khyber-Pakhtunkhwa, Pakistan
[o] Department of Pharmacy, Faculty of Biological Sciences, University of Malakand, Chakdara 18000, KP, Pakistan
[p] Department of Hotel Management and Tourism, University of Swabi, Khyber-Pakhtunkhwa. Pakistan
[q] Department of Agronomy, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa 23200, Pakistan

ARTICLE INFO

Keywords:
Indigenous adlay
Functional groups
Triglyceride
Minerals
Pakistan

ABSTRACT

Purpose of current study was to determine physicochemical, triglyceride composition, and functional groups of wild adlay accessions (brown, black, yellow, grey, green, off white, and purple) to find out its scope as cereal crop. Triglycerides, minerals and functional groups were determined through Gas chromatography, spectrophotometer and Fourier Transform Infrared (FTIR) spectrophotometer respectively. Results revealed variation among bulk densities, specific densities, percent empty spaces, and corresponding grain counts per 10 g of sample are useful in distinguishing brown, black, yellow, grey, green, off white, and purple wild adlay accessions. Specific density and grain count per 10 g sample was significantly related. No statistical relationship exists among the pronounced physical characteristics. Brown adlay expressed the highest protein, fat, and fiber contents 15.82%, 4.76% and 2.37% respectively. Protein, fat, ash, and fiber percent contents were found comparable to cultivated adlay. Spectrophotometric analysis revealed macro elements including phosphorus, potassium, calcium, and sodium in the range 0.3% - 2.2% and micro elements boron, iron, copper, zinc, and manganese in the range 1.6 mg/kg - 20.8 mg/kg. Gas chromatography showed polyunsaturated fatty acids (PUFA) constitute the primary fraction (39% ± 7.2) of wild adlay triglycerides. Linoleic and palmitic acids were present as prominent fatty acids, 43.5% ±1.4 and 26.3% ±1.4 respectively. Infra-red frequencies distinguished functional groups in narrow band and fingerprint region of protein in association with out of plane region leading to

* Corresponding author at: Department of Agronomy, Abdul Wali Khan University Mardan, Khyber Pakhtunkhwa 23200, Pakistan.
** Corresponding author.
E-mail addresses: mliaquat@uoh.edu.pk (M. Liaquat), shela.sammi@uoh.edu.pk (S. Sammi), jalhawadi@zu.edu.jo (J.S. Al-Hawadi), m.jahangir@uoh.edu.pk (M. Jahangir), imrankhan572@uoh.edu.pk (I. Khan), malokla@ksu.edu.sa (M.K. Okla), Ialaraidh@ksu.edu.sa (I.A. Alaraidh), hamada.abdelgawad@uantwerpen.be (H. AbdElgawad), ke.liu@utas.edu.au (K. Liu), matthew.harrison@utas.edu.au (M.T. Harrison), saudhort@gmail.com (S. Saud), Taufiq.Nawaz@jacks.sdstate.edu (T. Nawaz), zhumo@htu.edu.cn (M. Zhu), liuhaitaoky@henau.edu.cn (H. Liu), shah_fahad80@yahoo.com (S. Fahad).

https://doi.org/10.1016/j.fochx.2024.101418
Received 9 March 2024; Received in revised form 23 April 2024; Accepted 25 April 2024
Available online 30 April 2024

structural differences among adlay accessions. Comparison of major distinguishing vibrational frequencies among different flours indicated black adlay containing highest functional groups appeared promising for varietal development.

1. Introduction

In south Asia, 20% to 30% children are under protein malnutrition while one billion people are deficient in dietary protein around the world (Ghosh, Suri, & Uauy, 2012). Cereal supply ensures >60% of our daily intake food. Further, increased dietary protein intake should be fulfilled from plant proteins (Andriansyah et al., 2019; Moughan, 2021). The grain part of ancient plant *Coix lacryma L.*, called adlay is rich in nutrients with numerous therapeutic and nutraceuticals functions. Research progress on functional ingredients on adlay supports the notion that adlay may be one of the best functional foods. It is used in traditional Chinese medicine (TCM) for >17 chronic diseases and is known as "the king of Gramineae plants" in lieu of its functional characteristics (Li, 2020; Weng et al., 2022). Physicochemical composition, bioactivity, processing, application, functionality, and safety aspects of adlay, besides phytochemistry and health-promoting effects are well documented (Igbokwe et al., 2020; Devaraj, Jeepipalli, & Xu, 2020). Research on the chemical composition of adlay began in the 1960s, and >70 compounds, including lipids, sterols, and phenols, have been isolated and their dynamics for health have been reviewed (Zeng et al., 2022). Initial research was focused on adlay proteins, amino acids, and vitamins, but in recent years, studies have surged to investigate functional ingredients of adlay. Further, the action mechanism of adlay oils, polysaccharides, phenols, phytosterols, Kanglaite, Coixenolide coixol, Coixolin, naringenin, lactam, and resistant starch for combating diseases have been revealed (Deng, Ying, Yang, Lin, & Chen, 2017; Soni et al., 2023; Zeng et al., 2022). China is a pioneer in using adlay as healthy food, and many studies on the functional ingredients of adlay have been conducted (Soni et al., 2023; Zeng et al., 2022). Cultivated adlay (*Coix lachryma-jobi* L. var. ma-yuen Stapf) represents a seasonal crop grown in various regions across the globe. In contrast, indigenous adlay (*Coix lachryma* L.) is a wild perennial grass distinguished by 9–11 different colors grains with stony hard-shelled texture (Andriansyah et al., 2019; Xi et al., 2016). The indigenous adlay variety is relatively rare and poorly understood in Pakistan, primarily found at altitudes 500 m or higher, including the Margalla hills region. Additionally, it is colloquially referred to as "Tesbih daana" due to its use in crafting prayer beads.

Adaly type, habitat, biotransformation processes (e.g., fermentation), and measurement techniques significantly influence the functional ingredients (Hou et al., 2018; Wen et al., 2020; Xi et al., 2016). Further, literature pertaining to stony hard-shelled adlay, particularly within the context of indigenous adlay, is limited. Keeping in view these aspects, physicochemical, mineral, triglyceride composition and functional group characteristics of wild adlay accessions has been investigated using gas chromatography with Flame Ionization detector (GC-FID), atomic absorption, and Fourier Transform Infrared (FT-IR) spectroscopy. Statistical tests such as LSD and principal component analysis were employed to elucidate the differences and classify the accessions with similarities among the characteristics. The insights obtained are significant for the indigenous adlay supply chain, and its applications in functional foods.

2. Experimental sections

2.1. Materials

Indigenous adlay accessions were collected from various outlets, including the Plant Genetic Resources Institute, National Herbarium, National Agricultural Research Centre (NARC), and Margalla hills in Islamabad. Collections were carried out during the period January–February 2021. Subsequently, samples were subjected to a series of preparation steps including drying, cleaning, and sorting of the grains based on their morphological features comprising of size, shape, appearance, and color as shown in supplementary Fig. S1. Prior to the milling, the grains were subjected to winnowing, moistening, and manual pounding in a laboratory-scale Mortar and Pestle until their shells became pliable. Following air drying, the resulting flour was successively milled using a China mill and then a Cyclone Mill until it could pass through a 100-mesh sieve. The flours were labeled with acronyms corresponding to the grain colors, such as BLK (black), PRP (purple), BRN (brown), YLW (yellow), GRY (grey), OWT (off white), and GRN (green), and were stored in airtight polythene containers for subsequent analysis (Andriansyah et al., 2019; Xi et al., 2016).

2.2. Physical characteristics determination of adlay

A comprehensive analysis of the physical attributes of indigenous adlay, consisting of texture, bulk density, specific density, bulk porosity (representing % empty spaces), 1000-grains weight, bead dimensions (length, breadth, thickness), length-to-breadth ratio (lbr) indicative of shape, and the quality index (lbr/t) was performed following routine procedures (Ahmad, Gurmani, & Khan, 2019). Specific density, (g/mL^3) was determined using isobutane and cyclohexane. Moisture (%) was assessed using a digital grain moisture tester from Kett®, Germany, designated as PB 1D2. The calibration process was carried out with a standard plate supplied by the PB 1D2 Tester, with the plate maintaining a precise weight of 20 g, accurate to within ±0.1 g, as provided by the instrument's specifications.

2.3. Physicochemical, triglycerides composition and functional groups determination

The primary instrumentations used in this study included Gas chromatograph with Flame Ionization Detector (GCFID 7890 A system, USA), a Fourier Transform Infrared (FTIR) spectrophotometer (Brüker Vertex 30, Germany), an Atomic Absorption Spectrophotometer (Varian, USA), and NMR analyzer (4000 Oxford, USA), UV–vis 1800 Shimadzu, Japan, and a muffle furnace. For triglycerides analysis, a Supelco © 37-components mixture of fatty acid methyl esters (FAME) standard was procured from Aldrich. De-ionized water was consistently employed throughout the analysis.

2.3.1. Physicochemical parameters measurement

Defatting of adlay flour (100 g) was performed using n-hexane in a Soxhlet extraction assembly. Crude fat on a dry weight basis was determined following standard procedures outlined (OMA, 2023). Additionally, the fat content was also quantified using Nuclear Magnetic Resonance (NMR) to compare absolute values with the estimated oil content. To determine the protein content in the adlay flour, Kjeldahl standard method was employed. This method utilized the SH220N Graphite digester and K9840 auto Kjeldahl distillation unit, (Hanon Instrument, China). The protein content was calculated using conversion factor 6.25.

$$\%\text{Protein} = \text{N} \times 6.25 \times 100.$$

where N represents the number of moles of sample calculated as follows:

$$\text{N} = \text{Xmoles}/1000\ \text{cm}^3 \times (\text{Vsmp} - \text{Vblk})\ \text{cm}^3/\text{mg} \times 14\ \text{g}/\text{moles} \times 100.$$

2.3.2. Ash, crude fiber, and mineral determination

Briefly, defatted flour (50 g) was digested in 1.25% H_2SO_4 for 30 min and subsequently with 1.25% NaOH in the same manner. After filtration, residues were dried, weighed, and transformed to ash employing muffle furnace following standard AOAC method (OMA, 2023). Crude fiber was calculated by equation.

$$\%\text{Crude fiber} = \frac{\text{Weight of residue} - \text{Weight of ash}}{\text{Weight of sample}} x\, 100$$

Minerals P, K, Ca, Na, and B and Fe, Cu, Zn, Mn, were determined following standard method (OMA, 2023). Briefly, after acid digestion, Fe, Cu, Mn, and Zn were estimated using Atomic Absorbance Spectrophotometer (Varian®) while P, K, B, Ca, and Na absorbance was measured on Shimadzu® Spectrophotometer (UV-1800) at wavelength $\lambda = 410$ nm using KH_2PO_4 as standard following working line Y = 0.082×; $R^2 = 0.998$ with Limit of Determination (LoD) = 0.5 ppm. Carbohydrate was determined as the difference between total weight (100%), subtracting % protein, oil, moisture, and digestible fiber/ash.

2.3.3. Triglyceride composition determination

The analytical sample was prepared following established FAME (fatty acid methyl esterification) method outlined (Ratnayake, Hansen, & Kennedy, 2006) with some minor modifications. Briefly, methylating agent was prepared by dissolving Sodium methoxide powder (CH_3NaO = 10 g) portion wise in 500 mL methanol (½ N). adlay flour (0.2 g) was vigorously shaken with 2 mL petroleum ether in a plastic capped capsule container for 10 min. Subsequently, 1.0 mL methylating agent was added to the resulting supernatant (0.5 mL) and vigorously shaken. To this solution, 1.0 mL NaCl solution (10%) in methanol was added before its application onto the Agilent® system 7890 A. For FAME testing, GCFID method was validated using a capillary column HP-5. The analytical conditions involved injector and detector temperatures maintained at 210 °C and 220 °C, respectively, split ratio 1:5, Helium flow rate set 1 mL/s, and ramping was initiated at 150 °C for 1 min, followed by increase @ 10 °C/min until 210 °C, and hold for 1.0 min.

2.3.4. Functional groups/ bonds frequencies determination in adlay flour using FTIR

Infrared spectra were obtained using a Fourier Transform Infrared (FTIR) spectrometer (Brüker Vertex 30 model), equipped with an attenuated total reflectance (ATR) accessory. The spectra were recorded over the frequency range 410 cm^{-1} to 3670 cm^{-1} (Timilsena, Vongsvivut, Adhikari, & Adhikari, 2017; Abdul & Che, 2011).

2.3.5. Statistical analysis

Level of significance with each assay in triplicate, least square design (LSD) was calculated among means. Minitab software was used for statistical analysis. PCA (Principal component analysis) was conducted to ensure linear combination of variables (Huq et al., 2020)

3. Results and discussion

3.1. Physical characteristics of indigenous adlay accessions

Significant variations were observed among physical characteristics of stony hard and hard shell texture grains. Bead length, width, color, appearance, and shape were significantly different. Grain shapes ranged from irregular to oval and visible in different colors including purple (PRP), black (BLK), yellow (YLW), brown (BRN), grey (GRY), off white (OWT), and green (GRN). Stony hard-shelled grains were prevalent in PRP, BLK, BRN, and OWT, while hard-shelled grains were less common in YLW, GRY, and GRN, based on both weight-to-weight (*w*/w) basis and grain count criteria. Bead average length was 0.86 cm (± 0.062), with the longest beads found in BLK (0.95 cm), and followed by GRY and YLW (0.78 cm). Bead breadth, weight, shape (length to breadth ratio-lbr), and volume ranged from 0.631 cm to 0.8 cm, 0.08 g to 0.29 g, 1.08 to 1.33, and 0.12mL^3 to 0.392mL^3 respectively, as illustrated in Table 1.

The observed lengths, widths, and weights are comparable to three Chinese wild adlay accessions (Xi et al., 2016). Grain shape is independent of both length and breadth, and the influence of breadth on volume is significantly greater than that of length. The observed lengths, widths, and weights are comparable to three Chinese wild adlay accessions (Xi et al., 2016). Furthermore, a good relationship was found between bead volume and oil content. Average bulk density 0.45 g/mL (± 0.19) was highest in BRN, GRN, and PRP whereas the average specific density was 0.745` g/mL (± 0.35) corresponding to flour average density 0.583 g/m^3 (± 0.19) as indicated in Table 1.

The pronounced differences in densities and % empty spaces are useful for differentiation among indigenous adlay accessions. These density characteristics are closely linked to the texture of the seed coat, a significant factor influencing the milling quality of cereals (Andriansyah et al., 2019; Xi et al., 2016). Among physical characteristics, bulk density, and grain count per 10 g of sample were observed inversely related as shown in Fig. 1.

The readily apparent relationship can be explained as lower density grains tend to occupy greater volume, resulting in higher grain count per unit weight. This phenomenon is closely linked to differences in the percentage of empty spaces (bulk porosity) and both bulk and individual grain densities. Bulk porosity which measures the % empty spaces was 53.89% (± 7.41) and comparable with the average milling recovery % of adlay which is 45% (Mendoza, Sabellano Jr., Baco, Nabua, & Pantallano, 2015). The wider range of specific density values compare to flour density indicates variations in grain filling and highlights disparities among the individual grains. Furthermore, lower flour densities are associated with increased flakes recovery during the milling process (Mendoza et al., 2015).

3.2. Physicochemical, triglycerides and functional groups of indigenous adlay accessions

3.2.1. Physicochemical characterization

adlay oil plays a significant role in various physiological and nutritional attributes in addition to the general factors associated with oil in cereals (Hou et al., 2018; Vujic & Acanski, 2012; Zeng et al., 2022). Average oil contents among different accessions of indigenous adlay determined through NMR and hexane were not different (4.5% ± 0.3) as illustrated in Table 2.

This similarity in oil values by two different methods supports the accuracy of the measurement methodology employed. Different oil content among indigenous adlay grains are due to measurement methodologies, physical, geographical, and other inherent factors as earlier reported (Ding et al., 2020; Liaotrakoon, Liaotrakoon, Wongsaengtham, & Rodsiri, 2014; Xu et al., 2017; Zhang et al., 2022). Black and white cultivars exhibit distinct oil contents, whereas minimal variations in oil content between whole grain flour and degermed flour was noted (Xu et al., 2017). Ding et al. (2020) also reported significant differences in oil content among cultivated adlay varieties. Furthermore, defatted flour of indigenous adlay revealed an average protein content of 14.66% with the highest protein of 15.82% observed in BRN grains as illustrated in Table 2., which is higher as compared to 13.78% protein content found in the commercial locally grown wheat variety, named 'Laasani 2008'. The differences in protein content among adlay accessions seems linked to color variations within the adlay grains, specifically transitioning from GRN to BRN. GRN adlay tends to be relatively softer which implies immature grains might have lower protein content (Ding et al., 2020; Xu et al., 2017). Prior research has also highlighted substantial differences in protein content among various cultivated whole grain adlay (ma-yun Stapf) varieties which are widely consumed in China and Taiwan (Xi et al., 2016). However, no significant differences were detected among different compartments within grain (Ding et al., 2020; Liu et al., 2015). Variations observed can be attributed to several factors, including adlay type, agro-ecological influences (Gene x Environment interaction),

Table 1
Physical characteristics of indigenous adlay accessions.

Parameters description	Indigenous adlay collections (1000 grains each)							
	PRP	BLK	BRN	GRN	GRY	YLW	OWT	Av: ±STDev
Shell texture	Stony	Stony	Stony	Hard	Hard	Stony	Hard	NA
Appearance*	Shiny pears,	Shiny, rounded	Shiny, irregular	Shiny, irregular	Shiny, oval	Shiny, rounded	Shiny, pears	NA
Grain count	35^{a}	63^{b}	60^{c}	68^{d}	105^{e}	95^{f}	144^{g}	82 ± 36.03
Length (cm)	0.95^{a}	0.84^{c}	0.84^{c}	0.91^{b}	0.81^{d}	0.78^{e}	0.91^{b}	0.86 ± 0.062
Breadth (cm)	0.7^{c}	0.63^{e}	0.65^{d}	0.8^{a}	0.75^{b}	0.65^{d}	0.75^{b}	0.71 ± 0.064
Shape (lbr)	1.2^{b}	1.33^{a}	1.29^{a}	1.14^{c}	1.08^{c}	1.19^{b}	1.22^{b}	1.04 ± 0.46
bead dia(mm)	0.2^{a}	0.2^{a}	0.2^{a}	0.2^{a}	0.2^{a}	0.2^{a}	0.2^{a}	0.2
bead vol mm^{3}	0.295^{a}	0.262^{b}	0.264^{b}	0.286^{a}	0.255^{b}	0.245^{b}	0.287^{a}	0.27 ± 0.02
Weight (g)	0.286^{a}	0.159^{b}	0.17^{c}	0.15^{c}	0.1^{d}	0.11^{d}	0.07^{e}	0.15 ± 0.07
BD (g/ml^{3})	0.624^{a}	0.61^{a}	1.035^{b}	0.863^{c}	0.234^{d}	0.34^{e}	0.185^{f}	0.45 ± 0.19
SD (g/mL^{3}) †	1.1^{a}	1.02^{a}	1.035^{a}	0.863^{b}	0.445^{c}	0.56^{d}	0.19^{e}	0.745 ± 0.35
GD (g/ml^{3})	0.031^{a}	0.02^{b}	0.02^{b}	0.013^{c}	0.004^{d}	0.006d	0.001^{d}	0.014 ± 0.011
FD (g/mL^{3})	0.667^{b}	0.966^{a}	0.588^{b}	0.5^{c}	0.5^{c}	0.476^{c}	0.385^{d}	0.58 ± 0.19
BP (%)	63.6^{a}	63.6^{a}	45.6^{b}	45.6^{b}	52.73^{c}	52.6^{c}	53.5^{c}	53.89 ± 7.41

Results are average of three replicates; BD stands for bulk density; GD – grain density; SD-specific density, FD-flour density, BP- bulk porosity; across row, values in the cells with similar alphabets suffixes are comparable at α ≤ (0.05); † stands for Isobutane and n-hexane use as medium for SD determination. * lbr ≤ 2.0 = rounded shape; 2 ≤ lbr ≥3, and lbr ≥ 3.0 = slender shape.

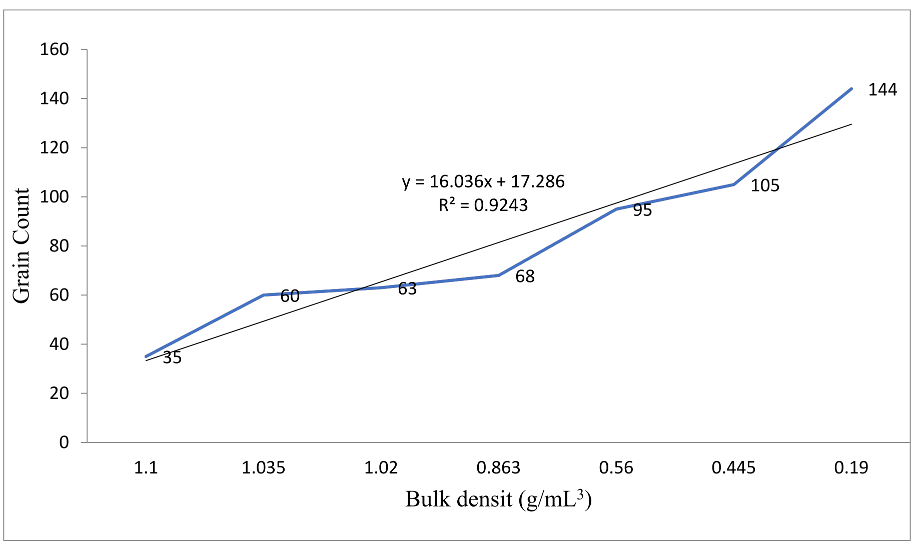

Fig. 1. Relationship between bulk density and corresponding grain count per 10 g adlay.

Table 2
Physicochemical characteristics of indigenous adlay accessions.

Proximate Parameter		Indigenous adlay accessions							
		PRP	BLK	BRN	GRN	GRY	YLW	OWT	Av: ±STDEV
Moisture (%)		11.9^{c}	11.9^{c}	12.2^{b}	13.2^{a}	11.6^{d}	12.2^{b}	12^{b}	12.4 ± 0.51
protein (%)		15.57^{b}	15.63^{b}	15.8^{a}	12.01^{e}	15.58^{b}	14.30^{c}	13.73^{d}	14.66 ± 1.4
Oil (%)	NMR	4.29^{a}	4.51^{a}	4.78^{a}	4.13^{b}	4.63^{b}	4.77^{b}	4.07^{c}	4.45 ± 0.294
	Extract	4.31^{c}	4.57^{b}	4.76^{a}	4.07^{d}	4.69^{b}	4.71^{b}	4.19^{c}	4.5 ± 0.3
Fiber (%)		1.79^{e}	1.98^{b}	2.37^{a}	1.94^{b}	1.97^{b}	2.21^{d}	1.88^{e}	2.02 ± 0.2
Ash (%)		2.6^{a}	2.0b	1.88^{c}	2.63^{a}	2.0^{b}	1.95^{b}	2.63^{a}	2.24 ± 0.36
P (%)		0.232^{c}	0.2^{d}	0.2^{d}	0.3^{a}	0.27^{b}	0.23^{c}	0.25^{c}	0.3 ± 0.04
K (%)		0.34^{c}	0.3^{c}	0.28^{d}	0.48^{b}	0.64^{a}	0.42^{b}	0.68^{a}	0.45 ± 0.16
Ca (%)		1.2^{c}	0.9^{d}	1.3^{c}	2.1^{a}	1.5^{b}	2.2^{a}	0.6^{e}	1.4 ± 0.6
Na (%)		0.28^{a}	0.16^{b}	0.28^{a}	0.16^{b}	0.17^{b}	0.21^{c}	0.2^{c}	0.21 ± 0.052
B (mg/Kg)		3.0^{c}	4.0^{a}	3.9^{a}	3.41^{b}	3.0^{b}	2.0^{d}	3.25^{b}	3.2 ± 0.7
Fe (mg/Kg)		2.9^{a}	1.011^{e}	2.3^{b}	1.67^{c}	1.045^{e}	1.44^{d}	1.0^{e}	1.6 ± 0.73
Cu(mg/Kg)		43.53^{a}	8.00^{e}	37.0^{b}	34.5^{c}	15.3^{d}	6.64^{f}	0.56^{g}	20.8 ± 17.18
Zn (mg/Kg		36.623^{a}	22.157^{b}	1.27^{f}	13.24^{d}	9.2^{e}	20.37^{c}	20.37^{c}	17.61 ± 11.21
Mn (mg/Kg		5.8^{c}	1.56^{e}	8.3^{a}	7.06^{b}	2.04^{d}	1.78^{e}	BDL	4.4 ± 3.0

Results are an average of three replicates. Values in the cells with similar alphabets suffix are comparable at α ≤ (0.05). NMR stands for nuclear magnetic resonance.

genetic effects, biotransformation by fermentation, and the specific techniques employed for measurement (He et al., 2020; Wen et al., 2020; Xu et al., 2017; Zhang et al., 2022; Zhao, Gong, Huang, Yu, & Lu, 2010). These factors collectively contribute to the differences in protein contents. Moisture and ash are considered crucial as they directly affect stability and storage of food. Average ash value determined on a dry weight basis in indigenous adlay accessions was 2.24% ±0.36 equally highest in OWT and GRN grains followed by PRP (2.6%) as shown in Table 2. Relatively higher ash contents are probably due to accessions hailing to Margalla hills areas in Pakistan. The fiber contents (2.02 ± 0.2) were found similar to the previously reported fiber content of 2.3% in cultivated adlay and are found maximum in BRN adlay seeds.

Indigenous adlay accessions contained both macro and micro minerals. GRN adlay exhibited` highest P (0.3%), followed by GRY (0.27%) and OWT (0.25%). These differences, however, were not statistically significant. It is worth noting that phosphorus levels have not been previously reported in hard-shelled adlay genotypes. Additionally, levels of K in OWT (0.68%), and Ca in YLW (2.2%) were notably higher than the highest Na content (0.21%). These findings suggest the potential advantages of promoting indigenous adlay for their mineral contents. Boron is recognized as a crucial mineral influencing shell hardening which in turn impacts milling quality (Fatima et al., 2018). BLK adlay contained highest B (4 mg/kg), followed by BRN, GRN, and OWT varieties, respectively. The Boron levels 2.0–4.0 mg/kg were found comparable to Indian cultivars. In contrast P, K, Ca, and Na contents (0.3% to 2.2%) were notably higher than those typically found in cultivated adlay (Hore & Rathi, 2008). These variations can be attributed to differences in soil chemistry, adlay type, habitat, extraction method, and measurement techniques (Coludo & Janairo, 2015; He et al., 2020; Hu et al., 2012; Zhang et al., 2022; Zhao et al., 2010). Fe, Mn, Cu, and Zn contents have been reported for the first time in this study in hard-shelled wild adlay acession. Previously only Fe, Cu, and Mn in soft-shelled adlay was determined while Zn remained undetected in genotypes (Hore & Rathi, 2008). Considering the strong relationship among the physicochemical characteristics of indigenous adlay accessions as indicated in Table 2, multivariate analysis was conducted using the statistical tool ‘Principal Components Analysis’ (PCA). PCA involves the generation of linear combinations of the variables, resulting in principal directions that are equal in number to the original variables. These principal directions are represented by vectors in the PCA-Biplot, as depicted in Fig. 2.

In the analysis, variables PRP and BLK adlay accessions were identified as PC1 and PC2, respectively. Notably, YLW clustered with BLK while GRY clustered with PRP distantly apart from others as shown in Fig. 2. These clusters accounted for a substantial proportion of the data variation, contributing nearly equally 52.7% and 43.7%, respectively. Such observation arises from the fact that these variables are more closely aligned with the axes and exhibit similar vector lengths. In contrast, parameters Zn and Cu appeared distant apart in the analysis. The PCA-Biplot presented in Fig. 2 represents the distribution of characteristics. It delineates these features into scattered positive loadings, as indicated by eigenvalues and eigenvectors, primarily driven by protein content. Conversely, negative loadings are associated with extracted oil content, Fe, and Na. Additionally, parameters such as ash, B, and Mn contents also play roles in shaping the data distribution within the biplot. Furthermore, the variables GRN, OWT, and BRN exhibit comparable vector lengths, and the angles between them in the biplot convey their inherent correlations in the multivariate space, as depicted in PCA-Biplot Fig. 2.

3.2.2. *Triglyceride composition wild adlay accessions*

In indigenous adlay accessions, short and medium chain fatty acids have not been detected in wild adlay accessions. Among the nine identified fatty acids, saturated fatty acids (SFAs) collectively constituted a significant portion (33.81% ±13.58%) of the total, with palmitic acid (C16:0) being the predominant SFA (26.3% ±4), followed by lignoceric (C24:0), behenic (C22:0), and arachidic (C20:0) acids, as illustrated in Table 3 while fatty acids chromatograms shown in the supplementary Figures (Fig. S1- S8)

As anticipated, unsaturated fatty acids (USFAs) expressed the dominant portion (53.1% ± 8.74) of the wild adlay triglycerides composition. The USFAs fraction mainly consists of PUFAs, with linoleic acid (C18:2n6C) being the major contributor (38.73% ±7.2) followed by MUFAs representing 7.42% ± 4.4 of the total USFAs. Oleic acid (C18:1n9C) was present as being the prominent MUFAs (4.2% ±0.63) among the adlay accessions followed by eicosenoic acid (C20:1n9C) 3.13% ±0.4. Notably, other unsaturated fatty acids including palmitoleic (C16:1n9C) and eicosenoic acid (C20:1n9C) were present only in the YLW adlay oils. Our results are slightly different from previously studies regarding the quantitative fractions of unsaturated fatty acids (85.1%)

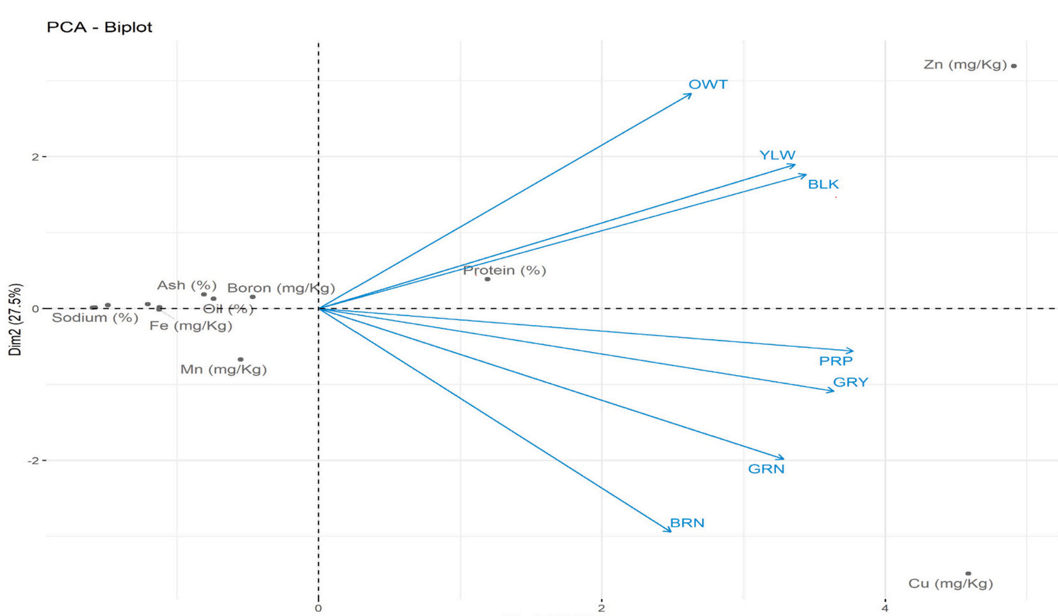

Fig. 2. PCA -Biplot constructed by physiochemical characteristics of indigenous adlay.

Table 3
Fatty acid profile of Indigenous adlay oils.

FAs (% av.: ±STDEV)	BLK	BRN	GRN	OWT	GRY	YLW	PRP	Av ± SDEV
Palmitic (C16:0) (26.3 ± 4)	24.5[d]	27.5[c]	29.7[b]	30.1[a]	22[e]	20.6[f]	29.6[b]	26.3 ± 1.4
Palmitolic (C16:1)	–	–	–	–	–	2.02	–	2.02 ± 0.0
Oleic (C18:1n9C (4.2 ± 0.63)	3.7[e]	3.92[d]	4.2[c]	4.79[b]	3.5[f]	3.7[e]	5.2[a]	4.2 ± 1.4
Linoleic(C18:2n6C)43.5 ± 1.4	52.0[a]	43.5[c]	37.6[d]	34.7[e]	51[b]	52.5[a]	33.0[f]	43.5 ± 1.4
Linolenic(C18:3n9C)	1.09[c]	0.99[c]	1.04[c]	1.01[c]	4.1[b]	5.8[a]	–	2.4 ± 1.4
Arachidic (C20:0)	–	2.35[c]	–	–	2.8[b]	1.31[d]	4.81[a]	2.82 ± 1.5
eicosenoic (C20:1) (3.13 ± 4)	3.8[b]	–	1.96[d]	2.62[c]	–	2.1[d]	11.4[a]	4.4 ± 1.4
Behenic (C22:0)	3.1[d]	4.65[b]	3.71[c]	4.99[a]	–	2.6[e]	5.3[a]	4.06 ± 1.1
Lignoceric(C24:0)	4.7[f]	7.52[d]	9.89[c]	10.9[a]	3.5[g]	7.2[e]	10.62[b]	7.8 ± 2.9
Solvent Extract Oil (%)	2.51[b]	2.91[a]	1.75[e]	1.91[d]	2.6[a]	2.68[a]	2.2[c]	2.4 ± 0.43
ΣSFAs	32.3	39.67	43.3	45.99	38.3	31.71	5.40	33.8 ± 13.58
ΣUSFAs	60.59	48.41	44.8	43.12	58.6	66.12	49.6	53.04 ± 8.7
ΣMUFA	6.5	3.92	6.16	7.41	3.5	7.82	16.6	7.42 ± 4.4
ΣPUFA	6.29	44.04	38.64	35.71	55.1	58.3	33.0	38.73 ± 7.2
n-3UFAs	1.09	0.99	1.04	1.01	4.1	5.8	–	2.38 ± 2.1
Ratio of ΣUSFAs/ ΣSFAs	1.88	1.2	1.1	0.93	1.53	2.08	0.98	1.4 ± 0.45

SFAs stands for saturated fatty acids, PUFAs stands for polyunsaturated fatty acid, MUFAs for mono saturated fatty acids. Results are average of three replicates; Values in the cells across rows with similar alphabets suffixes are comparable at $\alpha \leq (0.05)$.

and their respective individual constituents (Ni et al., 2021) that might be due to measurement technique, adlay type and habitat (Zhang et al., 2022; He et al., 2020; Hou et al., 2018; Hu et al., 2012; Yang et al., 2017; Peng et al., 2010). A significant observation was the higher amount of PUFA (∑PUFAs) 38.73% ±7.2 which makes up a significant proportion of the wild adlay oil triglyceride composition in most accessions. The ∑PUFAs was 58.3% in YLW followed by GRY (55.1%), BRN (44.04%), GRN (38.04%), and PRP adlay (33.0%). Similar results have been reported earlier in cultivated adlay (Ni et al., 2021). Yang et al. (2017) found Triolein (1.04%) as the most abundant constituent in cultivated adlay oil from Zhejiang province, China.

To comprehensively analyze the characteristics of nine identified fatty acids and their derivatives ΣUSFAs/ΣSFAs and ΣPUFA/ΣMUFA, PCA was carried out. YLW, GRY, and BLK adlay accessions were notably identified as PC1, PC2, and PC3 respectively, with BRN, GRN, and OWT adlay clustering together with GRY. YLW and GRY vectors are parallel to the axes and equal in length within the multivariate space, thereby making maximal and nearly equal contributions to the overall variation accounting for >80% of the total (42% and 39% respectively), as indicated in the PCA-Biplot Fig. 3.

The angle of PC3 (BLK) signifies its influence. Although it is not parallel to the axes and does not contribute to the same extent as PC1 or PC2, its vector length is substantial enough to warrant consideration in the sum up of variation. Moreover, PC1, PC2, and PC3 distribute characteristics into compact positive loadings as revealed by linolenic acid, oil contents, long-chain saturated fatty acids (lignoceric, behenic, and arachidic acids), and the ratio of ΣUSFAs/ΣSFAs. In contrast, other factors exhibit scattered loadings, with ΣMUFA in quadrant Q1, linoleic acid in Q3, and ΣPUFA in Q4. ΣUSFAs and ΣSFAs are distant apart from the rest of the data points. The angles between vectors YLW, GRY, BRN, GRN, and OWT represent their respective correlation coefficients in the multivariate space, with increased angles indicating proportional decreases in correlation as revealed by the reduced correlation associated with BLK adlay and the complete lack of correlation denoted by a wider angle for PRP adlay.

3.2.3. *Functional groups identification in adlay flours using FTIR*

Fourier Transform Infrared (FTIR) is used for qualitative and quantitative assay with minimal sample preparation to outlook vibration of bonds within functional group leading to the metabolic fingerprint out of the molecule (Kuhnen et al., 2010). Comparative IR spectra of indigenous adlay accessions flours have been produced in Fig. 4.

For simplicity, each spectrum in Fig. 4 has been viewed by two distinct frequency (υ = cm^{-1}) ranges according to placement and application. Frequencies possessing well-defined origin and distributed evenly among the accessions are illustrated in Table 4.

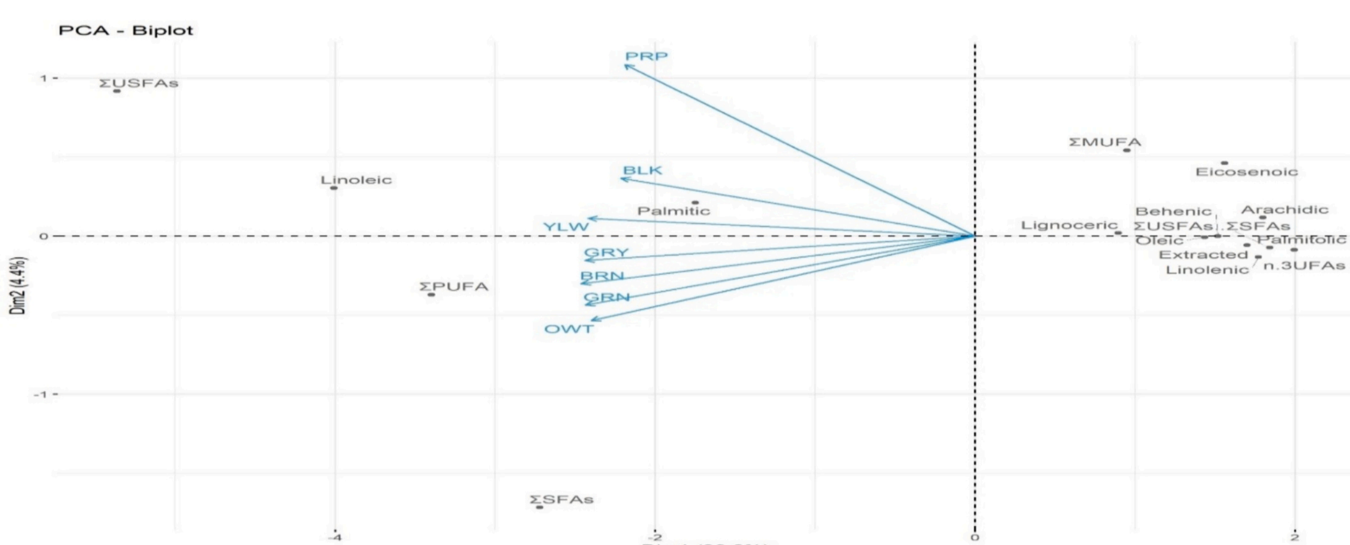

Fig. 3. PCA – Biplot constructed by indegebous adlay triglycerides fatty acids.

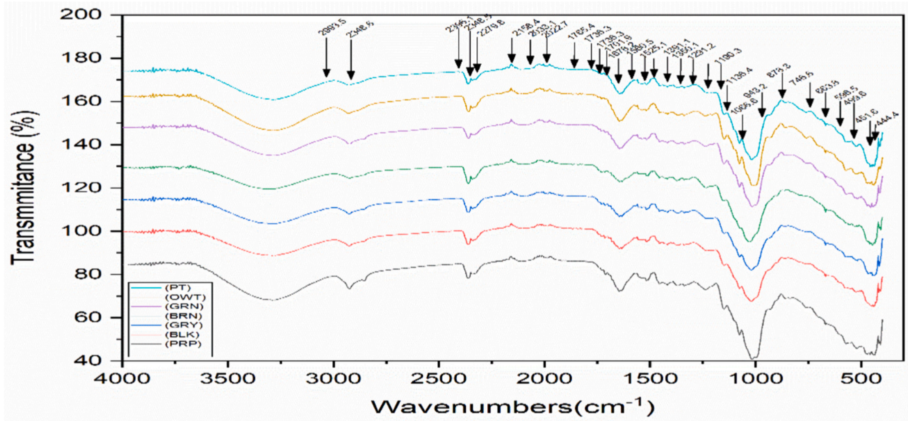

Fig. 4. FTIR spectra of indigenous `adlay black, purple, off-white, grey, green, yellow, and brown accessions flours. Arrow (→) indicates position where peaks give impression. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
Functional groups/ bonds originated by common frequency (stretching/ bending vibrations) through FTIR analysis of indigenous adlay flours.

Peak (s) at animated cm^{-1}	Origin relevant to peak (s)	Functional group (s) reached out	Indigenous adlay grains flours						
			PRP	YLW	OWT	BLK	GRY	GRN	BRN
			Brief of 2348/49–3350/ 3635 cm^{-1} originating peaks						
3350/3653	bold/alone/broader or semi rounded peak *sym.* Stretch - H bonded OH	Aliphatic or / Aryl Alcohols/ carboxylic/ carbohydrate known window	Peak appearance (broader/ wider) shape (semi global/ rounded) bold and alone found uniformly and equally in all collection evident H-bonded OH group symmetric stretch pertinent to -COOH; and or carbohydrates; and or R-OH/ Ar-OH						
~ 3000	-CH_3; >CH_2 symmetric stretching	Well known υ (defined Basic skeleton)	2965; 2994	same separated band type				at 2963	same bands
2863/65	aldehydic H symmetric stretch shoulder type	typical defined υ HC═O near R- stretch				shoulder type			
2348/49	O=C=O stretch	CO_2 indication as background contamination	CO_2 presence without any detectable difference in samples indicates background atmospheric CO_2 interception, that may be avoided by T at <2%. It is a well-defined window						

Vibration 3350–3653 cm^{-1} is characteristic peak of carboxylic acid; aldehyde/ ketone, and or carbohydrates; and or aliphatic/ acrylic alcohol (s) or esters and or other related compounds since it is single, broader/wider, or bold, or semi-global/rounded shape (Ali, Al-Hattab, & Al-Hydary, 2015; Abdul & Che, 2011). ~3000cm[1] hailing to —CH_3 group, or > CH_2 group symmetrical stretch (C—H bond) evident basic skeleton (Timilsena et al., 2017). Other such common vibrations observed are 2348/2398 cm^{-1}, 1719/1721 cm^{-1}, 1702/1709 cm^{-1}, 1677/1678 cm^{-1}, 1134/1140 cm^{-1}, and 1088/1069 cm^{-1}. Nevertheless, such frequencies are not usable as they are evenly distributed across all accessions.

Vibrations expressed unevenly and elaborative only in combination with other frequencies or originating multiple functional groups pertaining to FPR region in association with OOP or NBR region are sub classifiable. Such frequencies are categorical to a functional group (s) reached out inclusively in multiple samples or exclusively being alone in a sample, are elaborative only when multiple functional groups are considered simultaneously. For example, 1134/1144 cm^{-1} and 1069/1066 cm^{-1} is interpretable in link with the FPR or intermingle with OOP region (U at 738 cm^{-1} and 687 cm^{-1}; ortho 738 cm^{-1}) and 1,3,5-tri substituted (U at 687 cm^{-1} and 720 cm^{-1} and 878 cm^{-1}) is important, are illustrated in supplementary Table S1. These determents vibrations give an insight into FPR (1500 cm^{-1} -1678 cm^{-1}) and NBR (1188/1199 cm^{-1} - 1577 cm^{-1}) in association with combination bands ("Comb band") and or overtones (1600–2000 cm^{-1}). Additionally, 631/632 cm^{-1} to 943/945 cm^{-1} ("OOP") intermingles with the FPR region pose ring substitutions, isomerism. The comparative results of this data information are summarized in the 'remarks column' of supplementary Table S2.

If a vibration does not distinctly belong to a particular group, it is categorized as such (Ni et al., 2021). 1134/44 cm^{-1} and 1069/66 cm^{-1} (ester group C—O bond stretching vibration) can be interpreted in conjunction with the FPR or OOP region (Abdul & Che, 2011). Similarly, frequency 1677/1678 cm^{-1} exhibited evenly except PRP wild adlay suggests esters, dienes, trienes, or saturated alcohol groups. The frequency 2158/2159 cm^{-1} is common across all flours and indicates isothiocyanate (-SCN) group (Ali et al., 2015). The vibration 2149 cm^{-1} expressed by BLK adlay only, marks R-N + ≡C- or isonitrile group whereas 1686 cm^{-1} expressed by GRY adlay flour only, indicate the R-HC=N- group (Ali et al., 2015). Both PRP and GRY adlay flours exhibited distinguishing vibration 2863/2865 cm^{-1} stands for H-C=O group, as elaborated in the remark's column in in supplementary Table S2.

Frequencies differences 1492/1493 cm^{-1} (~1500 cm^{-1}) - 656 cm^{-1} (serial number 26–35 supplementary Table S2) profoundly discriminated flours in FPR or NBR in association with Comb band, and or overtones attributed of resonance and ring 'OOP' bending/scissoring, stretching (ring characteristics) as well as position isomerism (arene). Ring C═C bond has typical association with 680 cm^{-1} -710 cm^{-1} and 720 cm^{-1}-760 cm^{-1}. vibrations 745 cm^{-1}/ 747 cm^{-1}, 876 cm^{-1}/ 878 cm^{-1}, 877 cm^{-1}/ 905 cm^{-1}, 687 cm^{-1}, 738 cm^{-1}, and 410 cm^{-1}/419 cm^{-1} linked to the absence of well-defined peaks beyond 3000 cm^{-1} strongly suggest ring C═C bonds (arene). This is reaffirmed by the absence of vibration 895 cm^{-1} - 915 cm^{-1} and 985 cm^{-1} - 995 cm^{-1} attributed to alkene C═C bond vibrations as illustrated in Supplementary Table S2. Vibrations 738 cm^{-1} and 687 cm^{-1} indicate ortho substitution whereas 720 cm^{-1} and 878 cm^{-1} are significant in 1,3,5-trisubstituted compounds.

With this point of view, PRP and YLW adlay flours expressed ortho/ para substitutions or combination of both in lieu of vibration 745 cm^{-1}/

747 cm^{-1} or more specifically, 1,2,3,4 or 1,2,4,5 or 1,2,3,5 or 1,3,4,5-tetra substitutions attributed to 876 cm^{-1}/ 878 cm^{-1} frequency. OWT and BLK accessions exhibit a slight difference due to frequency 845 cm^{-1}/849 cm^{-1} (indicative of para substitution) and, in some cases, both ortho/para substitutions. Moreover, OWT and BLK adlay flours are distinguishable by 877 cm^{-1}/ 905 cm^{-1} signifying penta substitutions, as well as 410 cm^{-1}/ 419 cm^{-1} pointing hexa- substitutions (Ali et al., 2015). In BRN adlay, the combination of 687 cm^{-1} and 738 cm^{-1} suggests mono-substitution, 738 cm^{-1} correspond to ortho and/or 1,3,5-tri substitutions or 687 cm^{-1} also indicates alkyl iodide (R-I stretch). GRY flour stands out with its unique absorbance 878 cm^{-1} signifying 1,2,3,4 or 1,2,3,5 or 1,3,4,5-tetra substitutions and/or penta- substitution. In the GRN flour spectrum, 744 cm^{-1} suggests ortho isomerism whereas 827 cm^{-1}/828 cm^{-1} indicates para isomerism and/or 1,2,3,4 or 1,2,3,5 or 1,3,4,5-tetra substitutions. The background frequency 880 cm^{-1} further supports penta-substitutions. If resonance, and or bonded hydrogen or other factors are considered same and equally among the accessions, then functional groups/ bonds are proportional to the number of vibration (s) frequency present in a spectrum. Consequently, BLK adlay expressed higher vibrations (53) corresponding to the equivalent number of functional groups followed by PRP and YLW (46 each); OWT, GRN and BRN (43 each) and GRY (42) respectively.

Cereal grains primarily comprise starch and protein as their main constituents, accompanied by minor components such as lipids, non-starch carbohydrates, phytic acid, vitamins, and minerals.During the processing and storage of cereal products, physical interactions and chemical reactions take place among these constituents, which determine their quality, storage stability, and nutritional value. In wild adlay we were looking for such groups representing proteins, starch, lipids and others minors constituents present in cereals. Functional groups representing, carboxylic acid (COOH) or triglycerides (ester), aldehyde/ ketone (C=O), and or carbohydrates; aliphatic/ acrylic alcohol (s) or esters (RCOOR), anol amine representing protein/antioxidants and or other related compounds such as dienes, trienes, which are precursor of vitamin E. We also found that black adaly contained Ar- C≡N or nitrile group which used in pharmaceutical industry as antimicrobial agent. These are few functional groups authors detected in in black adlay seeds which we think are important functional groups in black adlay suggesting black adlay for varietal development.

4. Conclusion

Physicochemical characteristics of seven wild adlay accessions were found statistically not different from each other. Higher protein, fiber and oil contents were noted in brown adlay contained. Spectrophotometer analysis showed presence of both macro mineral including phosphorus, potassium, calcium, and sodium and micro minerals boron, iron, copper, zinc, and manganese. Fatty acid profile of indigenous accessions composed of mainly unsaturated fatty acids (53.1%) with linoleic acid as the major fatty acid (38.73%) followed by oleic acid (4.2%). Most of the wild adlay accessions were found to have important functional groups including, carboxyl, carbonyl (aldehyde/ketone) group, aliphatic/ acrylic alcohol (s) or esters, anol amine, dienes, trienes, nitrile or isonitrile and isothiocyanate groups representing carbohydrates, proteins starch and non-starch with maximum in black adlay (53) suggesting this accession for varietal development.

Ethics approval and consent to participate

We all declare that manuscripts reporting studies do not involve any human participants, human data, or human tissue. So, it is not applicable.

Funding

Researchers Supporting Project number (RSP2024R176) King Saud University, Riyadh, Saudi Arabia.

CRediT authorship contribution statement

Rauf Ahmad: Methodology, Investigation, Formal analysis, Data curation. **Shehla Sammi:** Methodology, Funding acquisition, Formal analysis, Data curation. **Jehad S. Al-Hawadi:** Resources, Project administration. **Amer Mumtaz:** Visualization, Validation, Resources. **Imran Khan:** Formal analysis, Data curation. **Mohammad K. Okla:** Funding acquisition, Formal analysis. **Ibrahim A. Alaraidh:** Methodology, Funding acquisition, Formal analysis. **Hamada AbdElgawad:** Writing – original draft, Visualization. **Ke Liu:** Funding acquisition, Data curation. **Matthew Tom Harrison:** Writing – review & editing, Visualization. **Taufiq Nawaz:** Project administration, Methodology. **Mo Zhu:** Funding acquisition, Data curation. **Haitao Liu:** Project administration, Funding acquisition, Formal analysis. **Muhammad Adnan:** Software, Resources, Project administration. **Abdul Sadiq:** Project administration, Methodology, Data curation. **Tanzeel Ur Rahman:** Methodology, Project administration, Resources. **Basem H. Asghari:** Project administration, Validation, Visualization. **Shah Fahad:** Writing – review & editing, Validation, Software.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

The authors extend their appreciation to the Researchers Supporting Project number (RSP2024R176) King Saud University, Riyadh, Saud Arabia.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101418.

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