

## COMPOSITIONAL ANALYSIS, FUNCTIONAL PROPERTIES AND MICROSTRUCTURE OF COOKIES FORTIFIED WITH OLIVE LEAVES POWDER

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### ABSTRACT

Olive leaves (OL) are a valuable source of minerals, fibers, and bioactive compounds with functional properties. The study aimed to develop fortified cookies with olive leaves powder (OLP) at varying concentrations (0, 20, 40, 60, 80, and 100 g kg<sup>-1</sup> flour). Fine wheat flour (FWF), OLP and composite flours were analyzed for proximate composition, mineral content, functional properties and SEM analysis. Fortified cookies were evaluated for physicochemical, sensory, mineral, and microstructural attributes. Results showed that OLP contains high concentrations of ash, fiber, fat, calcium, iron, and zinc but lower moisture, protein, nitrogen-free extract (NFE), and potassium as compared to FWF. Functional properties such as water and oil absorption capacity were high in OLP, while swelling, foaming capacity, and foaming stability were lower in OLP. In composite flours, increasing OLP levels significantly reduced wet gluten from 30.02% to 17.97% and dry gluten from 9.10% to 6.84%. SEM analysis revealed irregular protein matrices with large granules of starch, and rough fiber structures. As the concentration of OLP increased in fortified cookies, a significant reduction was observed in width, spread ratio and NFE content while increase was observed in thickness, hardness, moisture, ash, fiber, fat, protein, and minerals contents. Sensory evaluation indicated that cookies fortified with 6% OLP were highly acceptable, offering enhanced nutritional benefits and prolonged shelf life.

**Keywords:** Olive leaves powder, functional properties, fortified cookies, SEM analysis, sensory evaluation

### INTRODUCTION

Food fortification is a strategy that employs locally available and accessible resources, either plant-based or animal-derived, containing significant levels of essential micronutrients, to enhance the nutritional value of another food (Uvere *et al.*, 2010). This approach supports economic growth by fostering the utilization of sustainable local resources, promoting self-reliance, and generating market opportunities for locally produced foods (Riaz *et al.*, 2022; Burchi *et al.*, 2011). The olive leaves, pomace, mill wastewater and olive stones are considered as the waste or byproduct of the olive (Gullón *et al.*, 2020). Olive leaves are a rich source of fiber content, minerals, antioxidant, flavonoids and phenolic content (Asmaey *et al.*, 2024). Whole leaves of olive and their extracts are commonly utilized as food supplements. Olive leaf extracts can be utilized as natural additives in food for their healthy and technological properties, with the objective to develop functional foods and to enhance the shelf-life of the product (Difonzo *et al.*, 2019). The chemical composition of olive leaves varies depending on several factors, including olive cultivar, climatic conditions, tree age, and wood content. Olive leaves contain 76.4-92.7g/100g of organic matter, 6.31-10.9g/100g of low levels of crude protein, adequate number of amino acids (89.9 g/100 g total nitrogen) and non-protein nitrogen associated with the cell wall is significant yet variable, accounting for 35.4-49.2g/100g of total nitrogen. Similarly, olive leaves contain 2.28-9.75g/100g of crude fat (Rahmanian *et al.*, 2015). The scientific community has paid special attention to utilizing olive wastes and byproducts such as leaves as they are rich in bioactive chemicals that can improve the functional qualities of packaging systems and lengthen the shelf life of food (Selim *et al.*, 2022).

Cookie is a small sweet taste cereal-based baked food. They are formed from dough of wheat flour (Miller, 2016; Manley, 2011). Cookies are prepared by mixing certain ingredients such as wheat flour, sugar, baking soda, eggs, water, ghee and salt and are blended in a mixer in a specific ratio and kneaded by hand or machine to get find dough. The baking sector also enhances its production with the passage of time worldwide in last decades like other sectors by producing certain varieties of baked goods (Anjali *et al.*, 2019). The olive leaves are ever green leaves and is cultivated in more than 41 nations. The economic importance of the olive dates back to the period of Bronze Age (3600 BC) (Barazani *et al.*, 2023). The study was designed to prepare cookies with fortification of olive leaves powder, in order to enhance the nutritional, functional and antioxidant capacity of prepared cookies.

### MATERIAL AND METHODS

Fine wheat flour (FWF) of Persabak variety was purchased from new Ahmad flour mill khazana sugar mill road, Peshawar. Olive leaves (OL) of Kalamata variety were obtained from the olive orchard of Agriculture Research Institute (ARI) Tarnab Peshawar. Olive leaves were pretreated, dried and grinded to produce powder using electric grinder (Ewulo *et al.*, 2017). The olive leaves powder (OLP) was passed through sieve (50 mesh) to get uniform particle size. The prepared FWF and OLP was stored in clean polyethylene bags.

#### Preparation of Cookies

Cookies were prepared according to the standard method of Riaz *et al.*, (2021) with a little modification in the spreading of dough sheets and formation of uniform cookies size, while baking time and temperature were remains constant. Ingredients for preparation of cookies were FWF (500g), grinded sugar (250g), Shortening (250g), egg (3 No's), baking powder (5g) and Baking Soda (5g). Treatments were prepared by replacing FWF with OLP in different proportions such as 2, 4, 6, 8 and 10% respectively.

#### Proximate composition

Moisture, ash content, crude fiber, crude protein, crude fat and NFE content of FWF, OLP and fortified cookies were analyzed by the method of AACC (2010).

#### Mineral analysis and gluten content

Mineral content of FWF, OLP and fortified cookie were determined by atomic absorption and spectrophotometer with the standard method of AACC (2010). Glutomatic instrument was used to measure the gluten content of the dough according to the standard method for gluten determination (AACC, 2010). The content left on the sieve after centrifugation is gluten Index (GI). Dry gluten was obtained by drying of wet gluten. The value of dry and wet gluten was recorded in the percentage.

#### Functional Properties

Water and oil absorption capacity were determined by the method of Chandra *et al.*, (2014) was used.

**Swelling power**

Swelling power of FWF and OLP were determined by the method as reported by **Qazi et al., (2014)**.

$$\text{Swelling power} = (\text{Weight of sediment})/(\text{Weight of flour-weight of dried supernatant}) \text{ -----Eq-1}$$

**Foaming Capacity (FC) and Foaming Stability (FS)**

The foam capacity (FC) and Foam stability (FS) was determined with certain modification by using the method used by **Narayana and Narasinga (1982)**.

$$\text{Foam Capacity (\%)} = (\text{VA-VB})/\text{VB} \times 100 \text{ -----Eq-2}$$

Where, VA = Volume of foam after whipping, VB = Volume of foam before whipping

$$\text{Foam Stability (\%)} = (\text{VA-VB})/\text{VB} \times 100 \text{ -----Eq-3}$$

**Physical Characteristics and Scan Electron Microscopy (SEM) Analysis**

Fortified cookies were analyzed for thickness, width, spread ratio and hardness using the method of **AACC (2010)**, While Scan Electron Microscopy Model No. SM5910, JEOL, made in Japan was used for SEM analysis.

**Color analysis**

Color of the fortified cookies were carried out by using Hunter Lab (Colorimeter) fitted with optical sensor (Hunter Associates Laboratory Inc., Reston, VA, USA) on the basis of CIE L\*, a\*, b\* color system. L\* values measure black to white (0–100), a\* values measure redness or greenness when positive, and b\* values measure yellowness when positive (**Aljobair, 2022**).

**Sensory Evaluation of Cookie**

Sensory evaluation of fortified cookies was determined by the method of **Larmond (1997)** using 9-point hedonic scale by semi trained judges from faculty and

students of the department of Food Science and Technology. The data were taken in triplicate and mean score value of judges were determined.

**Statistical Analysis**

All the sample were taken in triplicate and were analyzed statistically by Statistix 8.1 and R-studio software. Significance level within the treatments, was determined by Analysis of variance (ANOVA) with LSD test at  $p \leq (0.05)$  using CRD design with the standard method of **Steel et al., (1997)**. Origin 2024b software and MS Excel was used for graphical representation of data and standard deviation.

**RESULTS AND DISCUSSION**

**Proximate composition, mineral content and functional properties of FWF and OLP**

Proximate composition of both FWF and OLP are shown in Table 1. Results revealed that OLP contains moisture content (8.03%), Ash (5.23%), protein (10.28%), fiber (55.65%), fat (3.60%) and NFE (17.79%) while that of FWF were 11.15% moisture, 1.34% ash, 12.48% protein, 2.40% fiber, 1.49% fat and 71.14% NFE content. Results showed that OLP contain considerable amount of fiber, fat and ash content as compared with FWF. **Muhammad et al., (2023)** also analyses FWF for moisture content (11.01%), protein (10%), ash (1.34%), fat (1.06%) and NFE (74.43%) while moisture content (13.38%) at of dried olive leave at 40 °C temperature was determined by **Núñez-Gómez et al., (2023)**. This difference between the moisture range of both FWF and OLP is because of different nature, drying condition, humidity and weather condition as well. The higher ash content in food sometimes is not suitable for making bread because the dough volume is reduced. But in case of bakery such as cookies, biscuit and pasta higher ash food are more appealing in term of sensory properties (**Miller, 2016**).

**Table 1** Proximate composition, mineral content and functional properties of FWF and OLP

Treatment	Moisture (%)	Ash (%)	Protein (%)	Fiber (%)	Fat (%)	NFE (%)
FWF	11.15 ± 0.05	1.34 ± 0.06	12.48 ± 0.41	2.40 ± 0.44	1.49 ± 0.03	71.14 ± 0.50
OLP	8.03 ± 0.33	5.23 ± 0.25	10.28 ± 1.08	55.65 ± 0.36	3.60 ± 0.65	17.79 ± 1.78
Mineral content (mg/100g) of Fine Wheat Flour and Olive Leaves Powder						
	Ca	K	Fe	Zn		
FWF	4.45±0.03	33.92±0.01	0.047±0.001	0.260±0.65		
OLP	15.30±0.05	23.12±0.05	28.00±0.05	21.52±0.02		
Functional properties of FWF and OLP						
	WAC (%)	OAC (%)	SP (%)	FC (%)	FS (%)	
FWF	8.35±0.02	5.80±0.12	8.80±0.16	12.85±0.08	8.95±0.02	
OLP	33.16±0.11	12.25±0.07	6.20±0.26	0.02±0.02	2.21±0.12	

FWF=fine wheat flour, OLP=Olive leaves powder, WAC: water absorption capacity, OAC, oil absorption capacity, SP, swelling power, FC, foaming capacity, FS, foaming stability, along with a standard deviation (±SD).

The value of crude fiber for olive leave powder in previous study was recorded as 10.02% (**Cayan and Erener 2015**). Similar studies were also carried out on the fiber content of olive leaves powders which were 57.90% and 54.08% respectively (**Shokery et al., 2017; Faccioli et al., 2021**). The fiber rich food products are considered to be beneficial as they are helpful in reducing the risk of certain harmful diseases like diabetes, heart diseases, obesity and cancer (colorectal) (**Liu et al.,2011**). The higher fiber content in the OLP make it as a beneficial ingredient for baking of cookies in its formulation. **Faccioli et al., (2021)** also measured the value of dried olive flour and they observed the fat of 1.60% and 8.80% while NFE (22.42%). As olive leave is highly nutritive in term of containing rich protein, fiber, fat and carbohydrates which make a food product a functional food.

**Mineral content of FWF and OLP**

Minerals of FWF were Ca (4.45), K (33.92), Fe (0.047) and Zn (0.260) while OLP were Ca (15.30), K (23.12), Fe (28) and Zn (21.52) as shown in Table 1. Results showed that OLP contain high amount of Ca, Fe and Zn as compared to FWF. **Muhammad et al., (2023)** also analyzed FWF for mineral analysis such as Ca 4.42 mg/100 g, 33.91 mg/100g of potassium and Fe (0.044 mg/100g). **De-Oliveira et al., (2023)** worked on the various varieties of the olive leaves and analyzed the leave for macro and micro nutrients such as Ca (8.04 to 15.29 mg/100g), K (23.12 mg/100g), 22-31 mg/100g of Fe content and 15-62 mg/100g of Zn content. Calcium and potassium are the important micronutrient and plays a key role in the growth, formation of bones (**Janati et al., 2012**). **Lalas et al., (2017)** showed the level of Zn up to 3.8mg/100g of the dry powder. Meanwhile common results were also shared by **Hassanein (2018)** which was 2.6-5.8mg100<sup>-1</sup> g).

**Functional properties of FWF and OLP**

Functional properties of FWF and OLP shows its suitability for application in various baked goods. Results showed that the FWF has less water absorption capacity 8.38%, Oil absorption capacity 5.85% and have higher swelling power 8.80%, foaming capacity (FC) 12.85% and foaming stability (FS) 8.95 in contrast to the OLP which showed higher WAC (33.16%) and OAC (12.25%) as shown in Table 1. Results showed that swelling power of OLP was 6.18%, FC was 06.02% and FS were 2.21% respectively. Higher level of WAC of the OLP is due to a fibrous nature which absorbs more water whereas the FWF have lower level of WAC because of the bran which is removed during the refining process (**De-Oliveira et al., 2023**). As OLP have lower carbohydrates and higher fiber level so that its WAC is also higher (**Kinsella 1979**). Lower WAC is due to rigid structure of starch molecules while high WAC means loosely compact structure of starch (**Dauda et al., 2018**). FWF exhibit lower WAC than whole wheat flour (WWF) because of having low quantity of bran and germ (**Riaz et al., 2022**). Oil absorption capacity (OAC) of the flour is due to its ability to hold oil. This property of FWF and OLP gives indication that at which rate the protein binds with the added fat in the formulations (**Singh et al., 2005**). The binding capacity of OLP is higher because of lower protein but higher fiber content than FWF. While the flour has low hydrophobic proteins which show superior binding of lipids (**Adeleke and Odedeji 2010**). OAC is important parameter of flour as it imparts flavor and texture to the bakery products (**Kumar et al., 2011**). Swelling capacity also played a key role in the formulation of the baked goods like, pasties, cakes and cookies etc. Swelling power of the product is due to the bonding of water molecules by the component of starch such as amylose and amylopectin in certain ratios (**Rasper et al., 1974**). Non-Significance difference was observed in the SP of FWF and OLP. FWF shows lower SP (**Kaur et al., 2012**) due to

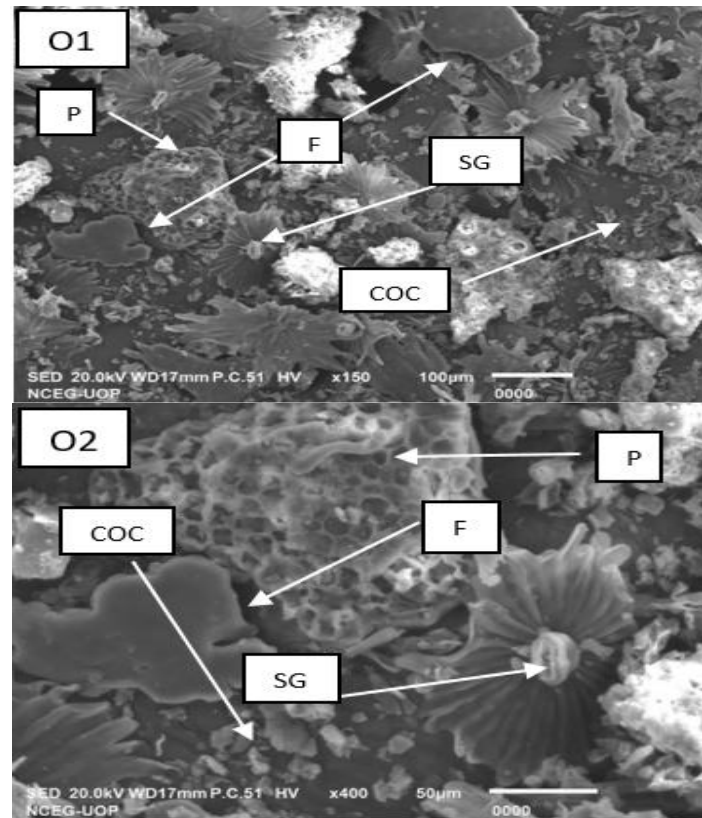
existence of bran and germ portion. SP is also dependent upon the accessibility of water, heat, polysaccharide (starch) and protein (Sui et al., 2006). As the SP depends upon the carbohydrates and other factors so the lower level of SP of OLP is due to less amount of carbohydrates and protein in the OLP.

Foaming capacity (FC) is the ability of material to give foam after thoroughly shaking or mixing. The foaming capacity and stability of the product is due to its composition such as protein content, starch and other surface-active compounds (Appiah et al., 2011). Results showed that foaming capacity of FWF was 12.85% while that of OLP was 6.02% which lower than that of FWF. By using olive leave extract (OLE) in the egg showed enhancement in the FC initially but after a while the FC level starts to decline (Godswill 2019). The foaming capacity of OLP was less in comparison to that of FWF because of having less protein content (Aliyari et al., 2021).

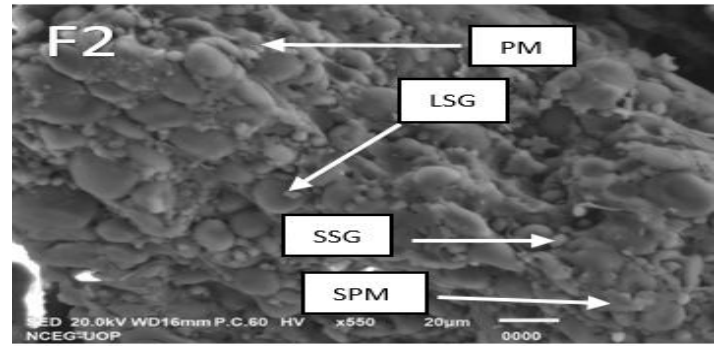
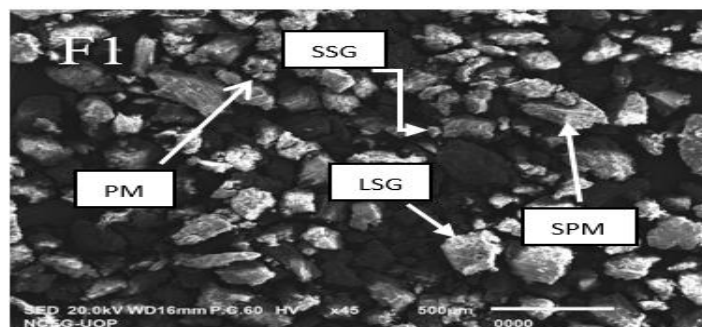
Foaming Stability (FS) is also important factor for evaluating the foam of any substance. Increasing the level of blending with other flour will decrease the foaming stability of flour but in case of FWF, its stability doesn't decline and remained constant (Chandra et al., 2014). Foaming stability is inversely proportional to foaming capacity (Chandra et al., 2014). Similarly using olive leave extract in the egg increases the FS (Aliyari et al., 2021).

**Scan Electron Microscopy (SEM) of FWF and OLP**

The microphotograph of FWF is shown in Figure-1a and figure-1b. it showed a lentil look alike starch granules with multiple sizes distributed throughout the flour. Portion of protein embedded into the granules of starch. In some images irregular shapes such as disk and elongated granules has also been observed (Gutierrez et al., 2002, Saifullah and Qazi 2019). In some images starch granules are embedded in protein matrix as shown in Figure 1a (Parda and Aguilera, 2011).



**Figure 1a** Micrograph of FWF and OLP where F1 & F2 shows the micrograph of FWF and O1 & O2 shows the micrograph of OLP. SSG: smaller starch granules, PM: protein matrix, LSG: larger starch granules, SPM: starch protein matrix, F: fiber, P: protein and COC: calcium oxalate crystal

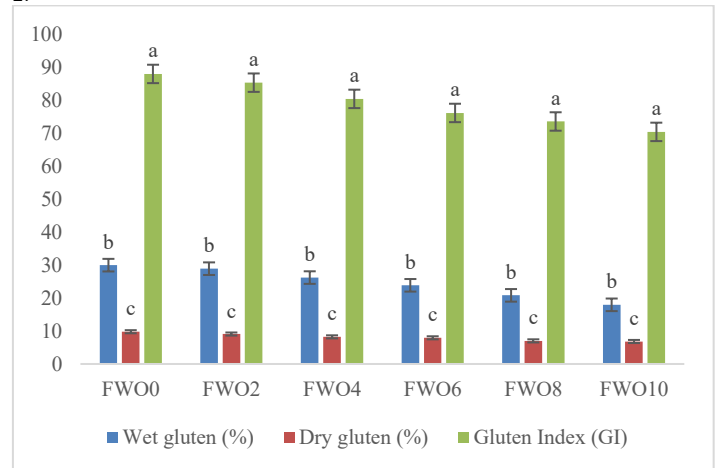


**Figure 1b** Micrograph of FWF and OLP where F1 & F2 shows the micrograph of FWF and O1 & O2 shows the micrograph of OLP. SSG: smaller starch granules, PM: protein matrix, LSG: larger starch granules, SPM: starch protein matrix, F: fiber, P: protein and COC: calcium oxalate crystal.

Micrograph for OLP is shown in the Figure 1a and 1b. The structure in the center resembles a flower-like formation, which could be due to particles aggregating into clusters or a natural morphology of specific compounds or cell fragments within the powder. Olive leaves contain various components such as fibers, waxes, and bioactive compounds, and processing them into powder can lead to the formation of these distinct structures. Riaz et al., (2021) and Dachana et al., (2010) worked on moringa leave powder and they also observe other constituents of the leaves such as fiber, crystals of oxalate, irregular structure and particle sizes in moringa leave powder (Reddy et al., 2005).

**Rheological properties of blended dough**

FWF and OLP were blended for composite flour (FWF 100%, 98% 96%, 94%, 92% and 90% and OLP incorporated with 2, 4, 6, 8 and 10% respectively. Results showed the Gluten content, and GI of FWF and composite flour as shown in Figure 2.



**Figure 2** Gluten content of FWF and composite flour with addition of OLP. FWO<sub>0</sub>: 100% FWF; FWO<sub>2</sub>: 2% OLP; FWO<sub>4</sub>: 4% OLP; FWO<sub>6</sub>: 6% OLP; FWO<sub>8</sub>: 8% OLP; FWO<sub>10</sub>: 10% OLP

Results showed that wet gluten, dry gluten and gluten index (GI) decreases significantly with increasing the concentration of OLP in composite flour. Riaz et al., (2021) develop moringa leave based biscuits and also observed the decreasing trend in the gluten content of composite flour. Hallén et al., (2004) and Javeria et al., (2023) prepared biscuits from wheat flour with incorporation of lentil oat flour and their results showed that gluten content declined to 14.37% from 22.30% with increasing the concentration of lentil oat flour. The reason for reduction of gluten (wet, dry and GI) is because the concentration of wheat flour decreases in the recipe and olive leave powder increases as the olive leave powder lacks gluten content (Hassanein, 2018).

**Physicochemical properties and color analysis of fortified cookie**

Width of the fortified cookies declines with enhancement of olive leave powder according to the Table 2. The decline in width is due to the gluten dilution and reduction of water as the olive leaf powder contains less amount of gluten than that of wheat flour which brings a change in the dimensions of the cookie (Nogueira and Steel, 2018). High fiber, non-wheat flour addition reduces the width of supplemented cookies (Sharif et al., 2009).

Results showed that thickness of fortified cookie increases with increasing the level of OLP. Declines in thickness is due to the hydrophilic nature of the non-wheat flour (Olive leave powder) with high fiber and high protein content, increase thickness of the cookies (Rebellato et al., 2015, Riaz et al., 2021, Miller, 2016).

The Spread ratio of OLP in the cookie decline significantly (P<0.05) as the level of incorporation of OLP increases. Spread ratio of cookies reduces with

supplementation of non-wheat flour such as moringa leaf flour, soy flour, Murrava koenigii leaf powder and legume flour. As the olive leaf powder has high concentration of fiber as compare to the fine wheat flour, so this fiber competes for the water with the starch within the dough and adversely affect spread ratio (Sharif et al., 2009). The lower the spread ratio the higher the raising ability of cookie (Cheng and Bhat 2016, Riaz et al., 2021, Aljobair 2022, Dachana et al., 2010, Kulthe et al., 2014) was observed. Riaz et al., (2022) stated that during

dough mixing process for cookie preparation, a competition starts among the gluten with other ingredients of the wheat flour which might be one to the reason for the decrease in the spread ratio of the final cookie (Hooda and Jood 2005). Higher the spread ratio the higher will be the acceptability of the cookie attributed by consumers.

**Table 2** Physicochemical properties and color analysis of fortified cookie

Parameters	FOC <sub>0</sub>	FOC <sub>2</sub>	FOC <sub>4</sub>	FOC <sub>6</sub>	FOC <sub>8</sub>	FOC <sub>10</sub>
<b>Width</b>	55.35a± 0.04	53.64b ± 0.01	53.00c ± 0.02	52.65d ± 2.52	52.07e± 0.53	51.33f± 1.88
<b>Thickness</b>	8.9f± 0.10	10.35e ± 0.14	11.07d ± 0.10	11.56c ± 0.09	12.09b± 0.02	12.87a ± 0.03
<b>Spread Ratio</b>	6.12a ± 0.01	5.66b± 0.21	5.66b± 0.21	4.26d ± 0.09	4.06e ± 0.06	3.80f± 0.36
<b>Hardness</b>	5.75f ± 0.04	5.99e ± 0.16	6.28d ± 0.04	6.69c± 0.05	6.85b± 0.01	6.99a ± 0.03
Color analysis of fortified cookies						
<b>L*</b>		62.03b±	52.01c±	51.98d±		
	69.19a± 0.16	0.12	0.11	0.03	50.35e± 0.11	49.59f± 0.18
<b>a*</b>	10.51f±	11.62e±	12.96d±	13.03c±		
	0.13	0.12	0.20	0.16	14.52b± 0.30	15.08a± 0.03
<b>b*</b>	32.23a±	27.78b±	26.79c±	26.20d±		
	0.16	0.21	0.17	0.11	26.10e± 0.06	24.36f± 0.10

FOC<sub>0</sub>=control, FOC<sub>2</sub>= 2% olive powder, FOC<sub>4</sub>= 4% olive powder, FOC<sub>6</sub>= 6% olive powder, FOC<sub>8</sub>= 8% olive powder, and FOC<sub>10</sub>=10% olive powder.

The hardness of cookies increased significantly ( $p \leq 0.05$ ) with increasing concentration of OLP. Increase in the hardness of the cookie is due to the presence of high amount of fiber of OLP, due to which the less water is available for gluten hydration (Sharma et al., 2013, Sudha et al., 2007). Another reason for the raise in hardness of the cookies which were reported by the literature is due to raise in the protein content which might be interact with during dough development and baking (McWatters et al., 2003, Khouryeh and Aramouni 2012). Hardness is also depended upon the water and oil absorption of flour which reinforce internal constituent of the dough which increased the hardness of the fortified cookie (Batista et al., 2017).

**Color analysis (L\*, a\* and b\*)**

L\* value indicates the lightness of the cookies which was decreased with increasing concentration of OLP from 69.19 in control sample to 49.59 in FOC<sub>10</sub> sample. Enzymatic browning and caramelization of the sugar or due to onset of Maillard reaction during the baking process at such a high temperature of 150°C browning reactions are the reasons for this decline in the value of L\* (Aljobair 2022). Similarly, L\* value decreased from 62.14 to 28.50 in the cookies supplemented with pitaya peel flour (Ho et al., 2016).

The value of a\* indicates the green and red color. The results revealed that the a\* value of sample FOC<sub>2</sub> (11.62) increased to FOC<sub>10</sub> (15.08) in fortified cookies. The color of the cookies become dark due to the incorporation of chlorophyll which impart green color to the leaves. Chauhan et al., (2016) revealed the similar increasing trend in the value of a\* for the cookies prepared by using amaranth flour, recorded the value of a\* as 6.71-8.36. Chevallier et al., (2000) indicating that the Maillard reaction played the major role in color formation.

Similarly, b\* value decreased to 24.36 in sample FOC<sub>10</sub> from FOC<sub>0</sub> (32.23) as the concentration of OLP increases from 2, 6, 8 and 10%. Decrease in the yellowness of the cookie is might be due to degradation of unstable yellow components during baking process (Chia et al., 2015). Similar decline in the b\* value (28.08-17.83) for the cookies were also observed by Ho et al., (2016) when substituting the wheat flour with pitaya peel flour.

**Proximate composition and minerals content of fortified cookies**

Proximate composition of the control sample as well as the olive leaves powder-based cookie samples were shown in Table.3. Addition of olive leaf powder had a non-significant effect ( $p \leq 0.05$ ) on the moisture level of fortified cookie. Results showed that moisture content in sample FOC<sub>0</sub> was 3.79% which gradually increased to 3.92 in sample FOC<sub>4</sub>. Jan et al., (2015) and Faccioli et al., (2021) developed crackers with the addition of olive leaf flour and recorded a moisture level in the range of (3.71-3.87%). Moisture level of any product is important to measure its shelf life (Bradley 2010). The moisture content increases because the level of fiber increases as fiber have the tendency to absorb more water and tends to retain.

The ash content of the cookies increased from 1.07% in sample FOC<sub>0</sub> to 2.21% in sample FOC<sub>10</sub> with addition of olive leaves powder. The increase in ash content of cookie is due to higher fiber level of olive leaf powder which also enhance the mineral content of the cookies (Riaz et al., 2021, Galla et al., 2017). Increase in the ash content might be due to incorporation of non-wheat flour having higher ash content which might enhance the value of ash content of cookies (Cheng and Bhat 2016; Núñez-Gómez et al., 2023; Dauda et al., 2018).

The crude fiber of the cookies increased with the incorporation of olive leaf powder. This increase in the crude fiber level is due to the enriched level of fiber in the olive leaf powder. The increase in the fiber of the cookies is might be due incorporation of olive leaf powder having higher level of crude fiber than fine wheat (Muhammad et al., 2023). As the level of OLP increases in the cookies the fat content tends to be increased. The increase in the fat content of the cookie is due to higher level of fat in the powder of olive as compare to wheat flour for various baked goods such as cookies, biscuits, crackers and bread by incorporation of non-wheat flour (Ikuomola et al., 2017; Muhammad et al., 2023; Aljobair 2022).

**Table 3** Proximate composition and mineral content of fortified cookies

Treatment	Moisture (%)	Ash (%)	Crude Fiber (%)	Crude Fat (%)	Crude Protein (%)	NFE (%)
FOC <sub>0</sub>	3.79f± 0.11	1.07f± 0.03	1.12f± 0.03	23.05f± 0.40	7.55b± 0.10	63.42a± 0.13
FOC <sub>2</sub>	3.85e± 0.02	1.12e± 0.04	1.55e± 0.03	23.65e± 0.79	7.75a± 0.26	62.08b± 0.02
FOC <sub>4</sub>	3.92d± 0.11	1.35d± 0.05	2.68± 0.09	24.09d± 0.74	7.59b± 0.40	60.02c± 0.01
FOC <sub>6</sub>	4.12c± 0.02	1.79c± 0.13	2.95c± 0.04	24.65c± 0.05	7.60b± 0.01	58.28d± 0.03
FOC <sub>8</sub>	4.41b± 0.01	1.97b± 0.02	3.16± 0.05b	24.97b± 0.02	7.61b± 0.26	57.1e± 0.02
FOC <sub>10</sub>	4.95a± 0.03	2.21a± 0.09	3.65a± 0.12	25.25a± 0.06	7.62b± 0.30	55.3f± 0.02
Mineral Content (mg/100g) of the Cookies fortified with Olive leaf powder						
	<b>Ca</b>	<b>K</b>	<b>Fe</b>	<b>Zn</b>		
FOC <sub>0</sub>	19.75f± 0.02	35.05f± 0.06	1.57f± 0.14	0.90f± 0.20		

FOC <sub>2</sub>	48.20e± 0.13	38.25e± 0.23	1.92e± 0.07	1.10e± 0.05
FOC <sub>4</sub>	55.06d± 0.04	45.72d± 0.47	2.46d± 0.02	1.58d± 0.06
FOC <sub>6</sub>	74.15c± 0.20	48.55c± 0.02	2.97c± 0.03	2.01c± 0.09
FOC <sub>8</sub>	85.24b± 0.10	51.51b± 1.21	3.81b± 0.18	2.53b± 0.15
FOC <sub>10</sub>	105.25a± 0.04	56.17a± 0.92	4.88a± 0.04	3.74a± 0.07

FOC0=control, FOC<sub>2</sub>= 2% olive powder, FOC<sub>4</sub>= 4% olive powder, FOC<sub>6</sub>= 6% olive powder, FOC<sub>8</sub>= 8% olive powder, and FOC<sub>10</sub>=10% olive powder. Values with different letters in the rows show significant difference at 5% level of significance. ± = standard deviation.

Increasing the level of OLP up to 10% enhanced the protein level of FOC<sub>10</sub> (7.63%). Non-significant increase in the protein content of the cookie were also studied by using dried leaf powder of *Tinospora cordifolia*, dried spinach powder and moringa leaf powder (Galla et al., 2017, Sharma et al., 2013; Riaz et al., 2021). protein act as an enzyme, hormones and also it balances the electrolytes of the body by adjusting acid-base equilibrium (Adeola and Ohizua 2018). The value of NFE% decline with increasing concentration of OLP in the cookies, because the Olive leaf powder contains lesser amount of carbohydrate then that of fine wheat flour. Results showed that maximum reduction was observed in the sample FOC<sub>10</sub> having 10% OLP (Muhammad et al., 2023).

**Mineral content of the fortified cookies**

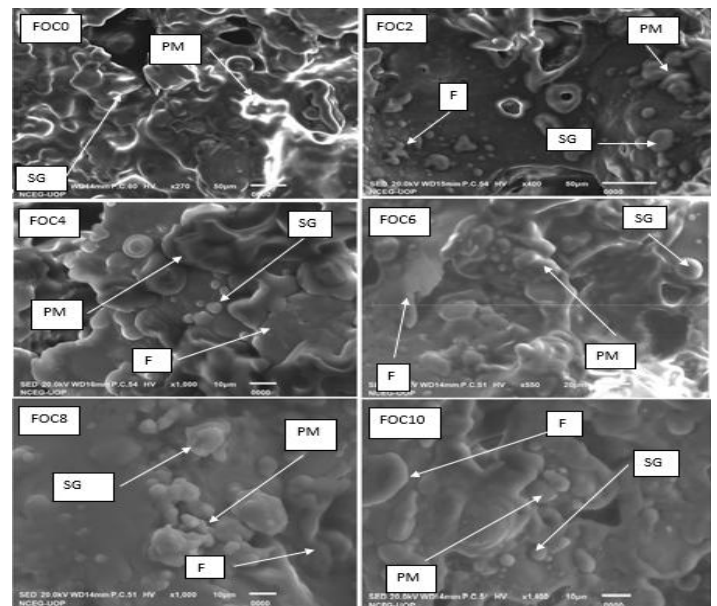
Mineral content of control and fortified cookies are given in Table-3 which showed that calcium content increased from 19.75 to 105.25mg 100<sup>-1</sup>g with increasing the incorporation OLP in the supplemented cookie. Calcium content of cookie increased with the addition of non-wheat flour (Galla et al., 2017; Aljobair, 2022; Muhammad et al., 2023). The K content was high in sample FOC<sub>10</sub> having 10% OLP (56.17 mg100<sup>-1</sup>g) as compared to FOC0 FWF (35.05 100<sup>-1</sup>g). Iron content non-significantly (P<0.05) increased from 1.57 to 4.88 mg100<sup>-1</sup>g. Fe content in supplemented cookies increased by the incorporation of clove powder in the cookie from 8.14 to 8.61 mg100<sup>-1</sup> g (Riaz et al., 2022; Tessera et al., 2015; Javeria et al., 2023, Aljobair 2022). The zinc content increased in the sample having 10% OLP in sample FOC<sub>10</sub> (3.74 mg100<sup>-1</sup>g) from FOC<sub>2</sub> (1.10 mg100<sup>-1</sup>g) having 2% OLP. Riaz et al., (2022) added moringa leaves powders in various concentrations in the biscuits and observed increasing trends in Zn content (1.90 to 2.11 mg100<sup>-1</sup>g). The increase in the Zn content of the cookie sample was might be due to higher level of Zn in the olive leave powder which incorporate its impact in the final product (Tessera et al., 2015).

**SEM analysis of cookies fortified with OLP**

Micrograph of fortified cookies are shown in figure 3. In the control sample where no OLP is used the granules of gelatinized starch can be observed which is embedded the protein matrix. The small pores within the sample can be observed these pores are created during baking process due to expanding of gasses. The gluten observed within the image helps to maintain the dough elasticity and shape whereas the starch gives the bulk and texture to the dough (Saifullah and Qazi, 2019). The SEM images for other samples can be also seen in the figure, which shows that when the concentration of the olive leave powder enhances the starch protein matrix weakens as the level of fiber increases. The image, taken at x400 magnification for the FOC<sub>2</sub> where a porous structure with air pockets throughout the structure can be observed. olive leaf powder may have slightly altered the porosity and pore distribution by changing the dough’s hydration and expansion behavior. The OLP particles appear to be dispersed within the wheat flour matrix, although they may not be easily distinguishable from the flour at this magnification.

Our findings were common to the results of Riaz et al., (2021) who used moringa leaf powder in the biscuits, they also observed that protein matrix disruption of cookies occurs at the highest level of moringa leaves (10%). Rajiv et al., (2012) also stated that when the protein is higher due to hydration the gluten network disrupted, thus starch and protein matrix dose not enwrap. Protein matrix was also disrupted while studying the SEM of multigrain dough by Indrani et al., (2010). The structure of the other cookie can also be observed in the Figure 2 where the structure of the cookies becomes more compact with increasing the Olive leave content from 20g to 100g. The increased level of olive leaf powder leads to a denser, more irregular structure with reduced porosity, likely affecting the cookie's texture and potentially enhancing its nutritional profile due to the added fiber and bioactive compounds. Riaz et al., (2021) also reported these constituents in the cookies sample having moringa leaf powder.

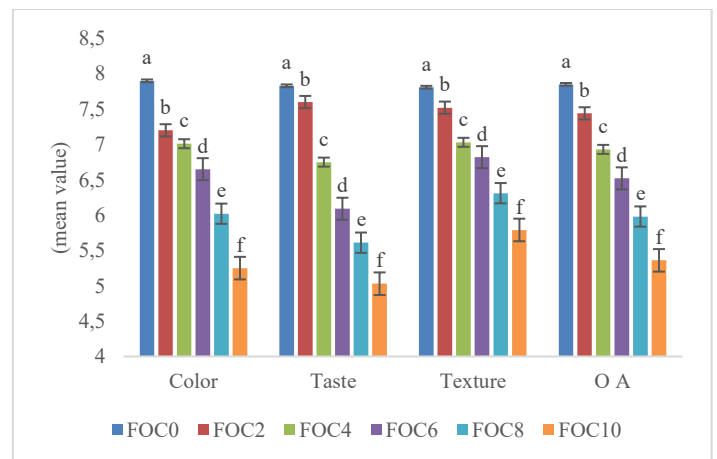
Further increasing the level up-to 6% the SEM image illustrates that a high concentration of olive leaf powder significantly changes the cookie's microstructure, leading to a denser, less porous matrix with rougher textures. This alteration in structure impacts the cookie's physical properties, making it potentially harder and more brittle while offering an enhanced nutritional profile (Srivastava et al., 2014). Similarly, in the sample FOC<sub>8</sub> and FOC<sub>10</sub> having 8 and 10% OLP the images showed much compact structure of the cookies where large particles, low porous nature and other constitutes of irregular shapes were seen. Due to this level of concentration significantly disrupts the continuity of the wheat flour matrix, creating a rough, granular texture (Riaz et al., 2021).



**Figure 3** Micrograph (SEM) of control and fortified cookies with addition of OLP FOC0=control, FOC<sub>2</sub>= 2% OLP, FOC<sub>4</sub>= 4% OLP, FOC<sub>6</sub>= 6% OLP, FOC<sub>8</sub>= 8% OLP, FOC<sub>10</sub>=10% OLP, SG: starch granule, PM: Protein matrix, F, Fiber.

**Sensory evaluation of fortified cookies**

Color is the sensory property which enhances the esthetic sense of any product and also exerts its effect on the taste and flavor. The addition of OLP from FOC<sub>2</sub> (7.90) to FOC<sub>10</sub> (5.25) in the cookies significantly affect the color of the cookies as shown in Figure 4. Riaz et al., (2022) also observed reduction in the color score from 7.86 to 5.71 by using moringa leaf powder. Increasing level of OLP from 6% to 10% has a negative effect on taste of the cookie FOC<sub>6</sub> (7.83) to FOC<sub>10</sub> (5.03). Riaz et al., (2022) also observed decrease in the taste of biscuits (7.73 to 6.36) by enhancing the level of moringa leaf powder. While Sedej et al., (2011) reported that taste of the cookies is might be affected due to the phenolic compounds in the olive leaves powder. Increasing the level of OLP from FOC<sub>2</sub> (2%) to FOC<sub>10</sub>(10%) in the cookies the texture significant decreased from 7.81 to 5.79 (Faccioli et al., 2021). Overall acceptability of cookies showed that increasing the level of OLP from 6% to 10%. The reason for this decline in the acceptability was given by (Ikuomola et al., 2017) is his study on cookies and stated that bitter after taste, darkish brown color, herbaceous aroma and brittle texture decrease the overall likeness of the final product (Muhammad et al., 2023).



**Figure 4** Sensory Evaluation of Cookies fortified with olive leaves powder FOC0=control, FOC<sub>2</sub>= 2% OLP, FOC<sub>4</sub>= 4% OLP, FOC<sub>6</sub>= 6% OLP, FOC<sub>8</sub>= 8% OLP, FOC<sub>10</sub>=10% OLP, OA= overall acceptability

## CONCLUSION

Fortified cookies were developed with the incorporation of olive leaf powder (OLP) at varying concentrations (0, 20, 40, 60, 80, and 100 g/kg flour). In composite flours, increasing OLP levels significantly reduced wet (30.02% to 17.97%) and dry gluten (9.10% to 6.84%), with SEM micrographs indicating compact structures due to fiber content in olive leaves powder. In cookies, higher OLP levels reduced width and spread ratio due to high fiber content which absorb moisture content but increased other parameters such as thickness, hardness, and levels of moisture, ash, fiber, fat, protein, and minerals, while NFE decreased. Results indicate that cookies with 4% OLP were highly acceptable followed by 6% OLP, offering enhanced nutritional benefits and prolonged shelf life. Fortified cookies sample FOC4 (4%) was found best on the basis of sensory evaluation by the panel of judges followed by sample FOC6 (6% of OLP). Industrial application of OLP in preparation of cookies will not only enhance the minerals contents, fiber and bioactive compounds in food supplements but it will also help to reduce environmental pollution by utilization of olive leaves which generate a huge waste during cutting and pruning of olive trees.

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