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THE EFFECT OF DIFFERENT CLIMATIC ZONES ON TOTAL PHENOLICS AND FATTY ACID PROFILE OF VARIOUS OLIVE CULTIVARS

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The effect of climatic zones i.e., Sangbhatti and Chakwal on fatty acid profile of three olive cultivars (Coratina, Pendallino and Leccino) was investigated. On the basis of two-year data, it was found that olive cultivars and locations significantly influenced the fatty acid content, peroxide value (PV) and total polyphenols. Leccino had higher concentrations of Palmitic acid (17.72%), Stearic acid (3.95%), Oleic acid (31.22%) and Linolenic acid (5.05%), whereas Palmitoleic acid (2.29%) and peroxide (18.50 meq/kg) was high in Coratina, while Linoleic acid (14.85%) and polyphenols (186.79%) were high in Pendallino. Coratina ranked second for Palmitic acid (17.68%), Stearic acid (3.55%), Oleic acid (29.94%), Linoleic acid (14.82%), Linolenic acid (5.01%), while Pendallino was poor. Maximum Palmitic acid (17.37%), Palmitoleic acid (2.52%), Stearic acid (3.65%), Linoleic acid (16.19%), peroxide value (20.81 meq/kg) was noted in Chakwal, whereas Oleic acid (30.07%), Linolenic acid (5.05%), and total polyphenols (139.43 ppm) was recorded in Sangbhatti. These results showed that climate affects the fatty acid and polyphenol contents of olive oil in different cultivars. This study will help in the selection of proper olive cultivars not only in these regions, but other regions of the world as well.
Keywords: *Olea europaea*, fatty acids, peroxide value, polyphenols

INTRODUCTION

Olive (*Olea europaea L.*) belongs to family Oleaceae, a diploid species (2n=46) (Green and Wickens, 1989). It is slow and steady growing tree locally called Zaitoon or Khuna having a long life of about 900 to 1000 years. Olive is one of the most ancient cultivated fruit trees with its domestication started approximately 6500 years ago having played an important socioeconomic role in human history (Loukas and Krimbas, 1983). It originated from Palestine, Lebanon, North West Syria and Cyprus. Olives have been mentioned seven times in the Quran (the Holy Book of Islam) and their health benefits have been propounded in Prophetic medicine of Islam. In the Holy Book 'Quran' the expression ″mubarakatin zaytoonatin″ portrays the olive as being abundant, holy, favorable, providing countless benedictions. Olive oil conveys numerous medical advantages and is known as one of the most highly recommended types of oil by all experts, especially for coronary and arterial health. The oil, when used in cooking or poured over salads, improves digestion and metabolism through its low content of cholesterol. Experts claim that olive oil consumption causes shiny hair growth, prevents dandruff, wrinkles, skin drying and acne, strengthens nails, stops muscle aching, lowers blood pressure and cancels out the effects of alcohol.

Olive trees are quite resistant to prolonged drought period during summer. Furthermore, these trees do well in sandy loam soils. Olive oil is mainly composed of triacylglycerides with small quantities of free fatty acids, glycerol, pigments, aroma compounds, sterols, and phenols. Olive oil contains a high percentage of the mono-unsaturated constituent called oleic acid (C18: 1cis). This particular fatty acid reduces low density lipoproteins (LDL), which are responsible for the formation of atherosclerotic plaque, and increasing high density lipoproteins (HDL) cholesterol. Olive oil also contains omega-6 and omega-3 oils, which are essential fatty acids. The linoleic acid (an omega-6 oil) is about 10% and linolenic acid (an omega-3 oil) is about 1% of the total olive oil.

Various studies have been performed in developed nations to explore the effect of location, altitude, harvesting time and maturity on olive oil quality and composition (Freihat *et al.*, 2008; Lavee and Wodner, 2004; Sweeney *et al.*, 2002). Due to the lack of such information in Pakistan, this study was, therefore, designed to investigate the effect of two climatic zones on oil contents of three high yielding olive cultivars.

MATERIALS AND METHODS

The samples were collected from two ecologically different locations during 2008 and 2009. Oil from each cultivar was

extracted at Pakistan Oilseed Development Board (PODB), Agricultural Research Institute, Tarnab (Peshawar) and analysis was done at the Pakistan Council for Scientific and Industrial Research (PCSIR) Peshawar, according to the method of Association of Official and Analytical Chemistry (AOAC, 2000).

***Preparation and analysis of samples by Gas Chromatography*:** After extraction of oil from olives, the oil sample (0.2 g) was taken in a test tube, containing 0.2 mL of internal standard (C_{15}) in 10 mL Hexane. 15 mL of 0.5N methanolic sodium hydroxide was added and heated at 80°C for 30 minutes. After boiling, 4 mL of the solution was transferred to another test tube containing 5 mL of boron trifluoride (BF_3) in methanol. The tube was sealed and again heated at 80°C for 30 minutes. Saturated NaCl (5 mL) was added upon cooling and the contents of the tube were shaken vigorously. After shaking, the sample was washed three times with 2 mL hexane and excess water was removed with anhydrous sodium sulphate.

Hexane solution contacting methyl-esterified sample (FAME) at 0.1-0.2 µL was injected into the injection port of Gas Chromatography (GC). A reference standard mixture under the same operating conditions was also injected. Palmitic acid (C16:0), Palmitoleic acid (C16:1), Stearic acid (C18:0), Oleic acid (C18:1), Linoleic acid (C18:2), and Linolenic acid (C18:3) content was determined by comparing sample chromatogram with the reference chromatogram. The operating conditions were; flow = 55 mL, injector temperature = 250°C and detector temperature = 300°C.

***Determination of Peroxide value (PV)*:** For PV, 0.5 g of oil sample was taken into a 250 mL Erlenmeyer flask and 30 mL of acetic acid: chloroform (3:2) solution was added. Saturated potassium iodide (0.5 mL) was then added and the solution was shaken for 1 minute. After addition of distilled water (30 mL), the solution was titrated against 0.1N sodium thiosulfate till the disappearance of yellow color. 2 mL of starch solution was added to the sample and titration was continued till the color was discharged. A blank without sample was also titrated against 0.1N sodium thiosulfate. The PV was determined according to the formula i.e.

PV =

Where S, B and N represents sample for titration, blank and normality of sodium thiosulfate solution, respectively.

***Extraction of polyphenolic compounds from oil*:** The extraction of phenolic compounds from olive oil was done by adding 30 mL of methanol/H_2O (80:20) and hexane (30 mL). The contents were mixed using vortex for 2 minutes and further centrifuged at 5000 g for 10 minutes. This extraction process was repeated thrice. The two immiscible phases were then separated by pipette. The separated methanolic phase was then concentrated under vacuum at 30 °C and was dissolved in acetonitrile (10 mL). It was washed twice with hexane (20 mL) and the solution was then concentrated under vacuum at 38°C till dryness. The dried residue was dissolved in MeOH (1 mL) and subjected to colorimetric analyses of total phenols.

***Colorimetric analysis of total polyphenols*:** The concentration of total phenols in the methanolic extract solution was estimated with the Folin-Ciocalteu reagent using gallic acid as calibration standard. 5 mL of water was added to 0.1 mL of aliquot followed by the addition of Folin-Ciocalteu reagent (0.5 mL). After 3 minutes, 1 mL of aqueous Na_2CO_3 solution (35 g/L) was added and mixed vigorously. The content of the tube was allowed to stand at room temperature for 1 hour and absorbance was finally measured at 725 nm. A blank experiment was carried out the same way, but without a sample.

RESULTS

***Palmitic acid (C16:0)*:** The results showed that Palmitic acid (%) differed significantly by years, location and cultivars (Fig. 1a). The concentration of Palmitic acid was higher in 2008 (17.52%) as compared to 2009 (16.22%). Similarly, higher concentration of Palmitic acid (17.20%) was recorded at Chakwal compared to Sangbhatti (16.54%). Similarly, Leccino resulted in higher Palmitic acid (17.72%) followed by Coratina (17.68%), whereas Pendallino resulted in lower concentration of Palmitic acid (15.22 %). According to the interaction between year and location (Y × L), higher Palmitic acid was recorded at Sangbhatti in 2008 (Fig. 3a).

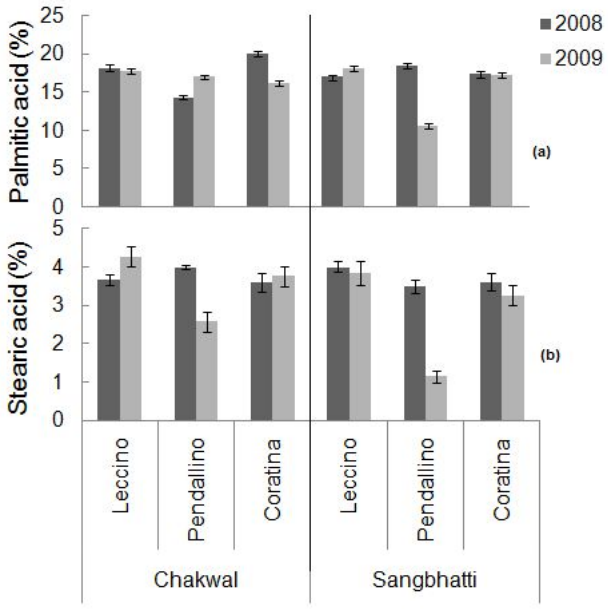

Figure 1. Palmitic acid (a) and Stearic acid (b) content of olive cultivars at different location during 2008 and 2009. Each data point represents mean of triplicate analysis with ±SE.

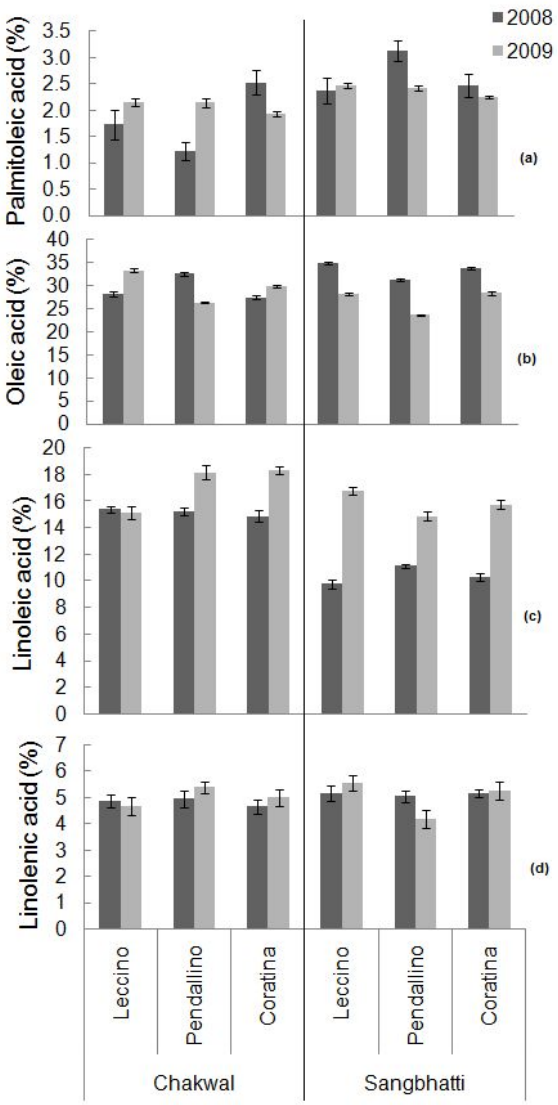

Figure 2. Palmitoleic (a), oleic (b), linoleic (c) and linolenic acid (d) content of olive cultivars at different location during 2008 and 2009. Each data point represents mean of triplicate analysis with ±SE.

The interaction between year and cultivar (Y × C), Coratina resulted in higher Palmitic acid, during 2008 as compared to 2009 (Figure 3b). Whereas the interaction between location, year and cultivar (L × Y × C) indicated that cultivar Coratina when grown at Chakwal resulted in higher concentrations of Palmitic acid during 2008.

***Stearic acid (C18:0)*:** The results indicated that year, location, cultivars and the interaction between Y × C had a significant effect on Stearic acid (%). The concentration of Stearic acid was higher in 2008 (3.73%) as compared to 2009 (3.14%) (Fig. 1b), similarly higher concentration was recorded for Stearic acid (3.65%) at Chakwal compared to Sangbhatti (3.22%). Leccino resulted in higher Stearic acid (3.95%), followed by Coratina (3.55), whereas Pendallino resulted in lower concentration of Stearic acid (2.80 %). Interaction between Y x L indicated that locations yielded different results in different years (Figure 3a). According to the interaction between Y × C, Leccino resulted in higher Stearic acid concentration during 2009 as compared to 2008 (Fig. 3b). Interaction between L × Y × C indicated that olive cultivar Leccino when grown at Chakwal produced higher concentration of Stearic acid during 2009.

***Palmitoleic acid (C16:1)*:** The interaction between L × C; and Y × L × C and location alone had a significant effect on Palmitoleic acid. The concentration of Palmitoleic acid was higher in 2008 (2.24%) as compared to 2009 (2.23%). Similarly, higher concentration of Palmitoleic acid (2.52%) was recorded at Chakwal compared to Sangbhatti (1.95%). Coratina resulted in higher Palmitoleic acid (2.29%), followed by Pendallino (2.23), whereas Leccino resulted in lower concentration of Palmitoleic acid (2.18 %) (Fig. 2a). Interaction between Y × L indicated that locations yielded different results in different years (Fig. 3a). Interaction between L × C revealed that cultivar Pendallino from Sangbhatti resulted higher concentration of palmitoleic acid (Fig. 3c). Interaction between L × Y × C indicated that cultivar Coratina grown at Chakwal had higher concentration of Palmitoleic acid during 2008.

***Oleic acid (C18:1)*:** Statistical analysis showed that cultivars, year and interaction between Y × L, Y × C, L × C and Y × L × C had a significant effect on Oleic acid (%). The concentration of Oleic acid was higher in 2008 (31.26%) as compared to 2009 (28.35%). Similarly, higher concentration of Oleic acid (30.07%) was recorded at Sangbhatti compared to Chakwal (29.67%). Leccino resulted in higher Oleic acid (31.22%), followed by Coratina (29.94), whereas Pendallino resulted in lower concentration of Oleic acid (28.46 %) (Fig. 2b). Interaction between Y × L indicated that locations yielded different results in different years regarding Oleic acid concentration (Figure 3a). According to the interaction between Y × C Pendallino resulted in higher Oleic acid concentration during 2008 as compared to 2009 (Fig. 3b), while the interaction between L × C revealed that olive cultivar Leccino when grown at Sangbhatti produced higher concentrations of Oleic acid (Figure 3d). Interaction between L × Y × C indicated that olive cultivar Coratina when grown at Sangbhatti produced higher concentrations of Oleic acid during 2008.

***Linoleic acid (C18:2)*:** The location and interaction between Y × L and Y × L × C had a significant effect on Linoleic acid content of the olive oil. The concentration of Linoleic acid was higher in 2009 (16.51%) as compared to 2008 (12.78%) (Fig. 2c). Similarly, higher concentrations of Linoleic acid (16.19%) was recorded at Chakwal compared to Sangbhatti (13.09%). Pendallino resulted in higher concentration of Linoleic acid (14.85%), followed by

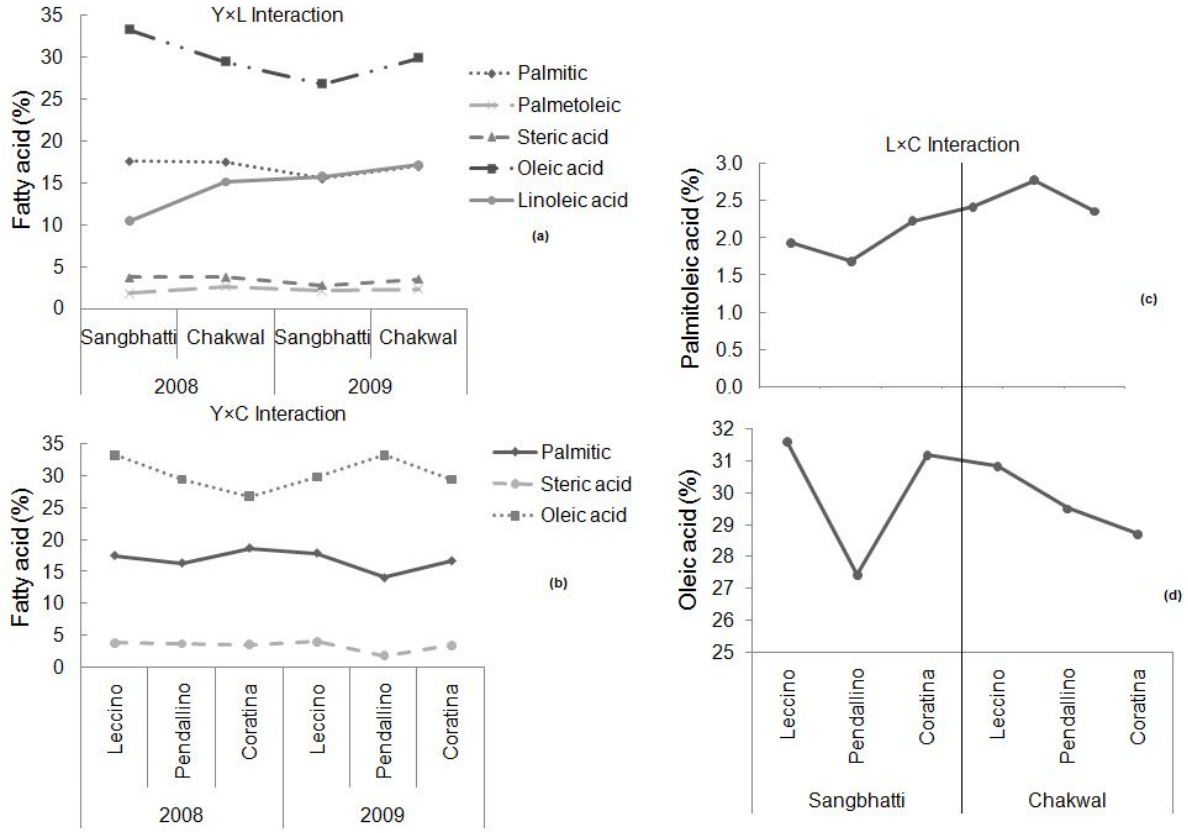

Figure 3. Interaction effect on fatty acid profile of olive cultivars. Interaction between (a) year × location, (b) year × cultivars, and (c,d) location × cultivar.

Coratina (14.82%) whereas Leccino resulted in lower concentration of Linoleic acid (14.27 %). Interaction between Y × L indicated that locations yielded different results in different years (Figure 3a). According to the interaction between Y × C, Coratina resulted in higher Linoleic acid concentration during 2009 as compared to 2008 (Fig. 3b). Interaction between L × Y × C indicated that olive cultivar Coratina when grown at Chakwal produced higher concentrations of Linoleic acid during 2009.

***Linolenic acid (C18:3)*:** The results revealed that location, year, cultivar and their interactions had no significant effect on Linolenic acid content of olive oil. The concentration of Linolenic acid was higher in 2009 (5.00%) as compared to 2008 (4.96%) (Fig. 2d). Similarly, higher concentrations of Linolenic acid (5.05%) was recorded at Sangbhatti compared to Chakwal (4.91%). Leccino resulted in higher concentrations of Linolenic acid (5.05%), followed by Coratina (5.01%).

***Total polyphenol content (ppm)*:** Statistical analysis indicated that location, cultivars and their interaction between Y × L, Y × C and Y × L × C had no significant effect on total phenolic content. Total polyphenols were higher in 2009 (129.64) as compared to 2008 (129.24) (Fig. 4a). Similarly, higher polyphenols (139.43) were recorded at Sangbhatti compared to Chakwal (119.45). The results also showed that Pendallino had higher polyphenols (186.79), followed by Leccino (115.76). The interaction between L × C revealed that olive cultivar Pendallino when grown at Chakwal produced maximum polyphenols (Fig. 5d). Interaction between L × Y × C indicated that olive cultivar Pendallino when grown at Chakwal produced higher polyphenols during 2008.

***Peroxide value (meq/kg)*:** The results indicated that year, location, cultivars and their interactions had a significant effect on PV. The highest PV was observed in 2009 (16.01) as compared to 2008 (15.02) (Fig. 4b). Similarly, higher PV (20.81) was recorded at Chakwal compared to Sangbhatti (10.21). Coratina showed maximum PV (18.50), followed by Leccino (15.32) and Pendallino (12.72). Interaction between Y × L indicated that locations yielded different results in different years (Fig. 5a). According to the interaction between Y × C, Coratina was found to have high levels of PV during 2008 as compared to 2009 (Fig. 5b). Furthermore, the interaction between L × C revealed that cultivar Coratina when grown at Chakwal had high PV (Fig. 5c).

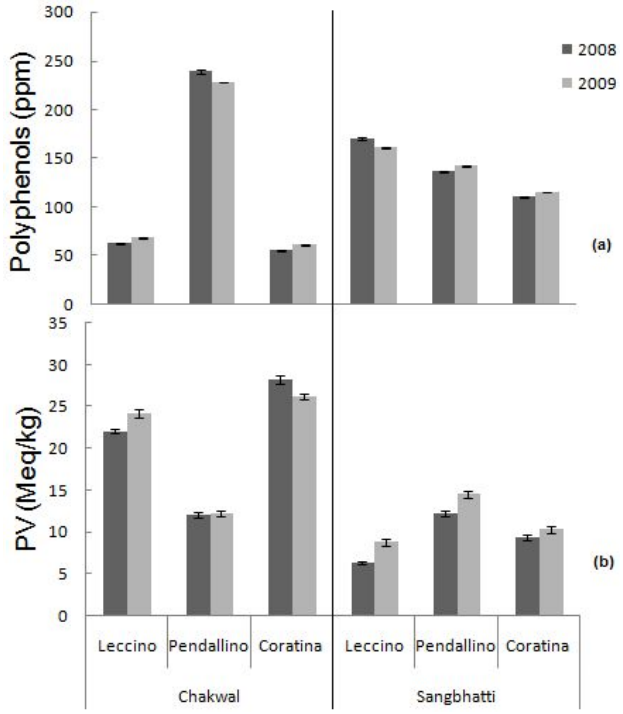

Figure 4. Polyphenols (a) and peroxide value (PV) (b) of olive cultivars at different locations during 2008 and 2009. Each data point represents mean of triplicate analysis with ±SE.

DISCUSSION

Fatty acid profile varies widely in vegetable oils and is an important quality attribute of the oil crops. The composition of fatty acid also influences the stability of oil, for instance poly-unsaturated fatty acids contribute towards the rancidity, odor and flavor of the oil. The importance of fatty acid composition has increased interest to study the oil crops and the effect of various factors i.e., location, maturity, harvesting time etc. on total yield and fatty acid profile.

In this study, the effect of different climatic zones on olive cultivars has been studied. As triglycerides are the main components of olive oil (Williams *et al.*, 1993), so the study was focused on the fatty acid present in triglycerides, which varies markedly among different cultivars (Aguilera *et al.*, 2005). Olive plantation at Chakwal performed well regarding Palmitic acid, Palmitoleic acid, Stearic acid and Linoleic acid as compared to plants grown at Sangbhatti. These differences among the fatty acids might be due to the temperature, relative humidity and altitude of the Chakwal region. Marco D'Imperio *et al.* (2007) also reported that geographical, ecological and agronomical conditions contribute significantly to these differences in the fatty acid content. These differences in low temperatures might increase membrane lipid un-saturation (Martinez-Rivas *et al.*, 2000) in order to maintain membrane fluidity (Qureshi and Ahmad, 2012). It was also reported that Palmitoleic acid

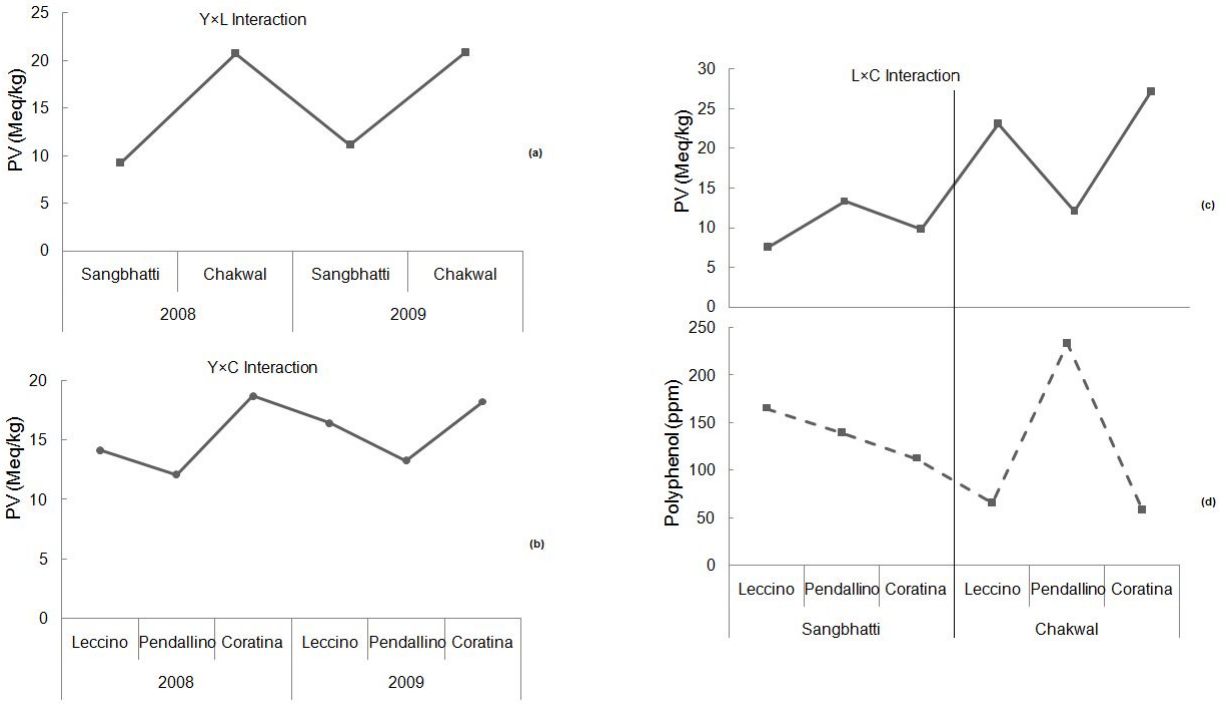

Figure 5. Interaction effect on polyphenols and peroxide value (PV) in olive cultivars. Interaction between (a) year × location, (b) year × cultivars and (c,d) location × cultivar.

content increases with an increasing altitude (Stefanoudaki *et al.*, 1999).

Higher content of Oleic acid and Linolenic acid was recorded at Sangbhatti as compared to plants grown at Chakwal. Sangbhatti is famous for wet summer and that might be the reason for the variation in the fatty acid contents. These findings are in accordance with the findings of Romero *et al.* (2003) and Salas *et al.* (1997). Both these reports suggested that wet summers produce lower levels of Oleic acid and higher C16/C18 ratios.

The highest content of polyphenols was observed in Sangbhatti as compared to Chakwal depending on various factors (such as cultivar, climate and environmental factors, ripeness and processing, after storage of the oil). These results show that the climatic conditions, in particular the rainfall during growth influenced the concentration of phenolic compounds. These results are similar to Pannelli *et al.* (1993) who observed that climatic factors, in particular precipitation influences the quality of olive oil.

The PV of the oil was below 20 meq of oxygen/kg, which is an acceptable limit for quality of virgin olive oil (International Olive Oil Council, 2003). However, the limit was exceeded at Chakwal, which may be attributed due to the environmental conditions of that area. The variation in PV can be explained by dissimilarity in the activity of lipoxygenase enzyme in these cultivars. These results are in agreement with Ramezani-Kharazi report (Kharazi, 2008).

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