

REPORT

Physicochemical and Antimicrobial properties of canola (*Brassica napus* L.) seed oil

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Abstract: Canola oil has been used in the Pakistan for the treatment of various diseases and skin infections. Oil was extracted with n-hexane from the seeds of canola (*Brassica napus* L.) and was evaluated for free fatty acid value. Four microorganisms namely; *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas originals*, and *Klebsiella pneumonia*, has known to cause some infections treatable with these oils were investigated. The results showed that all oil shown inhibitory effects against *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, and *Pseudomonas originals* but no inhibitory effects was found against *Staphylococcus aureus*.

Keywords: Canola oil, fatty acid, anti-bacterial activity, pod position.

INTRODUCTION

Brassica napus is belong to family Brassicaceae family and an annual crop. Canola plant growth and development is continuous but can be divided into easily identifiable growth stages. Canola has been introduced after nutritional up-gradation of oil by genetic alteration of *Brassica* cultivars (*Brassica campestris* and *Brassica napus*) and mustard (*Brassica juncea*) having less than 2 % erucic acid in the oil and 30µm (Bhowmik, 2003). It was originally bred from rapeseed in Canada. It contains almost 40-45% oil content and 36-40% protein in meal (Gan *et al.*, 2004; Amin and Khalil, 2005; Aboki *et al.*, 2012). In general seed expansion is separated into three steps: In first stage, weight and size of seed is small and starch and ethanol soluble compounds accounts for most of dry matter, in second stage, seed size enlargement take place as well as storage oil and proteins deposits increased at the end of this phase. Starch, glucose and fructose are utilized in this process. Stage 3 is mostly related to deposition of oil and protein in fixed amount. Sugars are transferred from hull toward seed to maintain this growth. In the seeds, the impartial lipid increases from 20 % of the total lipids in phase 1-93% at maturity. The amount of structural lipids decrease as the storage lipids amount increases (Batool *et al.*, 2013; Mustafa *et al.*, 2015). The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) suggest an average limit (55g per capita) for daily intake of lipid, dietary lipid play major role in growth and development (Scarath and Tang, 2006; Mustafa *et al.*, 2015) and

conversion of 20 to 30% fat to energy as ensure good health. Oil content is generally shown as percentage of entire seed that comprised of 10 to 20% omega-3 fatty acid, <0.1% erucic acid, 59 to 62% oleic acid, 18 to 22% linoleic acid and 10 to 12% linolenic acid. Canola oil has lowest point of saturated (7%) of any common edible oil and high in mono and poly-unsaturated fatty acids (93%). Plant oil showed high degree of antimycotic and antibacterial activity (Wagh *et al.*, 2007; De and Shruti, 2014; Anywar and Claude, 2015). Palm kernel and oil were tested antimicrobial effects against different microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger* (Ekwenye and Ijeomah, 2005). Ginger oil physicochemical parameters and antimicrobial activities were analyzed by Abitogun and Badejo, (2010). They focused their study against *Klebsiella pneumoniae*, *Strephylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Physicochemical characterization, antimicrobial activity and toxicity analysis of seed oil of *Swietenia mahagoni* (Majid *et al.*, 2004). Present research work looked into characterization of the canola seed oil and to check antimicrobial properties of the oil.

MATERIALS AND METHODS

This study was carried out at Agronomic Research Area of PMAS AAU, Rawalpindi. The area was prepared and recommended doses of fertilizers were applied at the time of last ploughing. *Brassica napus* variety Bulbul 2000 seeds were used and seeds were sown in a completely

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randomized design with three replicates. When pods appear on main stem labelling was done on the day of emergence and starting from the lowest point. Labelling was considered as a first treatment and which started on mid of February. Likewise twelve treatments were recorded on twelve different dates on the basis of the emergence of pods. About 1000 pods were labelled on each date and labelling continued till the end of flowering. This experiment had 12 treatments and three replications. All the plants with labelled pods were harvested at maturity. These labelled pods were thrashed manually and seed from each position were separately analyzed for oil, moisture, ash and fatty acids.

Moisture content

Moisture content of seeds was analyzed by the method according to AOAC, 1990. In this method 10g seed from each treatment taken in pre weighed petri dish, kept in oven at 500°C for overnight and weight Petri dish and sample was taken after cooling.

Ash content

For total ash content 2g sample of seeds was taken in pre weighed crucible and shifted to furnace at 300°C for 1 hour. Then the sample were cooled in desiccator and weight was taken after cooling. Analysis of total ash was done by a method, AOAC 1990.

Oil extraction

The oven dried seeds were ground with blender and 5 gm of sample was put in thumble and place in extractor. Three hundred ml n-haxane poured into a round bottom flask of soxhelet apparatus and heated at 60°C. This was allowed to continue for 8 hour. At the end of the extraction, the resulting mixture containing the oil was heated to recover solvent from the oil (AOAC, 1990).

Fatty acids

Fatty acid composition, was analyzed by using gas chromatography of methyl esters. Ten µl of the oils were taken in falcon tube after this 2000 µl of n-hexane and 200 µl of KOH were added. Then it was vortexed for 2 minutes and centrifuged. It was centrifuged at 1000 RPM for 10 minutes at the temperature of 30 °C degrees. Then hexane layer was collected for the analysis. Fatty acid methyl ester (FAME) was prepared by treating 10 mg of lipid with two mL hexane followed by the addition of 0.2 mL of 2M methanolic KOH. The tube was vortex for 2 min at room temperature after a light centrifugation aliquot of the hexane layer was collected for GC analysis and GC analysis was performed on a Perkin Elmer, Clarus 500 series. The chromatogram was collected from GC analysis used for identification of different fatty acid compounds present in *Brassica napus* seed oil.

Antimicrobial activity of canola oil

Antimicrobial activity was tested against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas originals*

and *Staphylococcus epidermidis* by agar well diffusion method. For this purpose inoculums of selected microorganism were prepared in sterilized Lauria Bertini (LB) media and than then kept in shaker incubator at 37°C for 24 hours. Lauria Bertini (LB) media were prepared and autoclaved and then poured in Petri plates and then 75 µl of each sample was pipette in well. All petri plates were incubated at 37°C for 24 hours. After 24, hours zones of inhibitions were measured (Hemaiswarya *et al.*, 2009).

Data were analyzed using the Mstat-C When analysis of variance showed significant treatment effects, DMRT was applied to compare the means at $P = 0.05$.

RESULTS

Moisture content is the quantity of water contained a sample on a volumetric or gravimetric basis. Data concerning moisture content exhibited statistically significant ($P < 0.05$) differences among different pod position (Table 1). The results of mean analysis showed the superiority of the pod position twelve. Results showed that pod position one and two were non significant, same as seven and eight. Changes in moisture content depend on different factors i.e., climatic conditions. Variation among different pod position in case of moisture content was 1.33%. Ash content refers to the mineral content of the sample. The mineral content depends on many factors, such as the variety and the climate. Mean analysis of ash content (Table 1) showed significant differences among different pod position. Though, pod position one and twelve (with ash content 2.24% and 4.12%, respectively) had lowest and highest ash content and other pod position stood between them. Variation of ash content with in a plant was 3.64%.

Seeds of canola plant are used to extract edible oil fit for human consumption as it has lesser erucic acid than traditional rapeseed oils, while cake fed to livestock contains reduced levels of the toxic glucosinolates. It is depicted from results that lower pods accumulated comparatively higher oil content than upper pods. The oil content in seeds obtained from pod one was significantly higher than those seeds of rest of pod positions (Table 1). All pod position significantly ($P < 0.05$) differed in terms of oil content. Differences among different pod positions oil content may be attributed to pod positions and temperature during pod formation, maturity.

Canola oil is mainly comprised on saturated and unsaturated fatty acids. Canola oil is low in saturated fats, high in monounsaturated fat, and has a beneficial omega-3 fatty acid profile. Statistical differences in present study were recorded among pod positions for oleic acid (Table 2). The maximum oleic acid (65.22%) was exhibited by the pod position eight while pod position twelve gave the minimum oleic acid (56.13%). Differences between pod

Table 1: Effect of pod position on moisture, ash and oil content of Brassica seed

Pod position	oil content (%)	Moisture content (%)	Ash content (%)
1	49.56A	3.920B	2.240H
2	48.48B	3.827B	2.440G
3	48.29B	3.560C	2.733F
4	47.09C	2.700D	2.850F
5	46.93C	2.690E	3.077E
6	45.05E	2.370F	3.213DE
7	43.87F	2.160G	3.283DE
8	43.01F	2.117G	3.420CD
9	42.06G	3.243D	3.550BC
10	41.94G	3.570C	3.740B
11	44.96D	3.823B	3.950A
12	45.12D	4.113A	4.123A

Any two means carrying the same letter in a column are non-significantly different at $p=0.05$ by DMRT.

Table 2: Effect of pod position on monounsaturated and polyunsaturated fatty acid of canola oil

Pod position	Oleic acid (18:1) (%)	Linoleic acid (18:2) (ω 6) (%)	Alpha Linolenic acid (18:3) (ω 3) (%)
1	58.31H	16.85D	9.173BC
2	59.47G	16.13E	10.28A
3	60.16F	16.08E	9.037C
4	61.20E	15.79E	8.619D
5	62.15D	15.34F	8.415DE
6	63.40C	14.12G	8.227E
7	64.37B	14.32G	7.372F
8	65.22A	13.48H	7.181F
9	57.65I	17.20C	7.433F
10	57.15J	18.26B	8.363DE
11	56.45K	19.13A	9.212BC
12	56.13L	16.57D	9.507B

Any two means carrying the same letter in a column are non-significantly different at $p=0.05$ by DM.RT

positions for oleic acid may be attributed to their genetic potential in addition to effects of environmental variables throughout seed growth and maturation. Increased in oleic acid content may be due to high temperature at the time of seed expansion besides other environmental factors. Linoleic acid is an unsaturated omega-6 fatty acid and constitutes 21 % of canola oil. Significant ($p<0.05$) differences were observed among pod positions for linoleic acid (table 2). The maximum (19.13%) linoleic acid was obtained from the eleven pod position while the minimum (13.48%) was accumulated by pod position eight, lowest and highest pod positions depicted 68.89% difference. This Linolenic acid has been assessed for its role in cardiovascular health. Data regarding linolenic acid exhibited statistically significant ($p<0.05$) differences among pod position (table 2). Linolenic acid highest amount (10.28%) was observed at lowest pod position while linolenic lowest value (7.18%) was recorded at upper pod position.

Antimicrobial activity of oil of different pod positions was tested against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas*

originals results were given in table 3. According to results obtained in the present study it was observed that all oil shown inhibitory affects against *Klebsiella pneumoniae*, *Staphylococcus epidermidis* but no inhibitory affects was found against *Staphylococcus aureus*.

DISCUSSION

Moisture content of canola also responded to temperature stress but the extent of the difference was small. These results were in agreement with Gecgal *et al.* (2006) who observed that moisture content turns down after 15 days flowering period to maturity in safflower. The higher oil yield in the lower pods seeds may be due to longer time period available for oil filling as compared to formation of small and less developed seeds in upper pods (Chauhan *et al.*, 2010). Oil accumulation is considered having inverse relationship with temperature and that of protein content. Oil proportion in the pod decreased with the phase of silique growth likewise, in soybean central capsules had seeds with less oil content than seeds from lateral capsules (Gularia *et al.*, 2008; Ghassemi-Golezani

Table 3: Anti-bacterial Activity of canola oil

Pod position	Treatment	<i>Klebsiella pneumoniae</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Pseudomonas originals</i>
1	24:2	6.2±0.17	-	5.5±0.34	4.5±0.17
2	28:2	6.5±0.14	-	7.0±0.23	4.0±0.201
3	3:3	6.5±0.28	-	-	5.7±0.31
4	5:3	-	-	6.5±0.20	6.0±0.37
5	7:3	6.5±0.37	-	6.0±0.28	7.5±0.21
6	10:3	7.0±0.16	-	6.0±0.23	-
7	13:3	6.5±0.23	-	5.5±0.11	5.5±0.26
8	17:3	7.5±0.14	-	5.0±0.23	5.2±0.11
9	20:3	6.0±0.20	-	5.5±0.28	7.5±0.46
10	22:3	-	-	5.5±0.14	6.0±0.28
11	25:3	6.0±0.25	-	-	6.5±0.23
12	27:3	5.0±0.15	-	5.0±0.39	5.0±0.11

Data are given as mean of inhibition (mm) of three readings. (-) indicates no inhibition zone.

and Lotfi, 2013). Gupta *et al.* (2009) observed higher oil yield in peripheral whorls than central whorls in sunflower (Chung *et al.*, 1995).

Oleic acid decreased with low temperature and low rainfall but increased at high temperature (Pritchard *et al.*, 2000). During pod growth temperature changes were not steady thus oleic acid accumulation responded in the same fashion. Munshi and Kumari, (2006) observed an increase in the content of oleic acid in the seeds from siliqua position 27 and above as compared with lower end. The synthesis of linoleic acid from oleic acid, during the seed expansion is interrupted, because of the inhibition of oleate desaturase activity due to high temperature. These results are in line with Aslam *et al.*, (2009) and Pritchard *et al.*, (2000), concluded that linoleic acid content decreased as temperature increased. Baydar and Sabri (2005) concluded that linoleic acid increased significantly during seed maturing processes in sunflower seed. However, Gupta *et al.* (2009) observed higher linoleic acid content in inner whorls of sunflower head. Linolenic acid is a carboxylic acid with an 18-carbon chain and three cis double bonds. Results of linolenic acid similar with Aslam *et al.* (2009) and they found that linolenic acid decreased with increase in temperature.

According to literature oils *Pongamia pinnate* have pronounced activity against *Staphylococcus aureus* (Wagh *et al.*, 2007) and palm oil also showed inhibitory zone against *Staphylococcus aureus* (Ekwenye and Ijeomh, 2005). Naz and Asghari (2013) findings show that *Lantana camra* extracts effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These results are similar to Abitogun and Badejo. (2010). Ginger oil showed inhibitory effects against *Klebsiella pneumoniae*.

CONCLUSION

Pod position and temperature fluctuation influence on seed oil, moisture and fatty acids content. Our results show that highest amount linoleic acid was observed at lowest pod position while oleic acid content increased with increasing pod position. Canola oil display inhibitory effect against *Klebsiella pneumoniae*, *Staphylococcus epidermidis* and *Pseudomonas originals* but no inhibition found against *Staphylococcus aureus*.

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