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Using Salicylic Acid Treatment of Stored Canola Seed to Decrease the Adversely Effects on Oil quality under Long-Term Storage, High Storage Temperature and Seed Moisture Contents

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Abstract

This study was carried out on canola seeds stored under different temperatures, seed moisture contents, salicylic acid concentrations for 24 months classified into 4 equal periods. The main objectives of this research were to study the effects of the studied 4 factors and their interactions on fatty acid compositions, acidity and free fatty acids (FFA) of the extracted oil, besides test the effect of treating seeds with salicylic acid to reduce the adversely storage conditions on oil compositions and quality. The obtained results showed that around 10%, 46% and 43% reductions in oleic, linoleic and linolenic acids as a result of stored seeds under 24 months and 30C compared with 6 months and 10C ,with significantly increasing in oil acidity and FFA. Also, under the 24 months of the 16% moisture stored seeds highly significantly reductions in oil composition and quality, where percentages of saturated fatty acids (palmitic and stearic) increased and unsaturated fatty acids (oleic, linoleic and linolenic acids) decreased by 23.6%, 47.3% and 49.6%, respectively and both acidity and FFA(%) increased by around 87% compared with the 7% moisture seed stored for 6 months. As seed moisture and storage temperature increased significantly reducing in unsaturated fatty acids and increasing in saturated fatty acids and acidity and FFA. Treating canola seeds with 15 or 30 uM salicylic acid caused in significantly increasing in oleic, linoleic and linolenic acids and decreasing palmitic and stearic acids (%) besides decreasing the acidity and FFA of the extracted oil from the seeds stored under the unfavorable conditions.

Keywords: acidity, canola seed, fatty acids, free fatty acids, oil quality, salicylic acid, storage

1. Introduction

Storage conditions of seeds especially oilseeds highly affected the quality of these seeds and their composition from fatty acids. Fatty acids are important component in the oil where oil quality related to the kinds of the fatty acids and its number of double bonds, accordingly the stability of oil during seed storage depends on fatty acids of oil. (Martini & Anon, 2005; Merello et al., 2004).

The period of storage and storage temperature are the most important factors affecting the concentrations of fatty acids in the stored oil seeds (Neg & Anderson, 2005), and moisture of the stored seeds is the most important factors affecting the quantity and quality of the oil extracted from these seeds (Anderson & Lingnert, 1998; Chen & Ahn, 1998).

As increasing the time of the sunflower seeds storage there has been an increase in the percentage of free fatty acids in oil (Ghasemnezhad & Honermeier, 2007). Unfavorable Storage temperature cause changes in the quality of the oil through an oxidation and a change in the oil fatty acid concentration (Krasucki et al., 2002), especially if stored canola seed moisture (%) is more than 12.5% (Tys & Rybacki, 2001), also these unfavorable storage conditions decrease the number of carbon atoms of the fatty acids resulting free fatty acids (Graham, 2008) and high oil acidity (Miquel & Browse,1995; Sathya et al., 2009, Cassells et al., 2007; Reuss & Cassells., 2003).

Salicylic acid affects the donor of electrons and causes inhibition of peroxidase enzyme, and increase H_2O_2 accumulation and it works to inhibit the growth of pathogens on seeds (Clarke et al., 2001; Chen et al., 1995).

Salicylic acid affects the mechanism of plant to protect against harmful diseases and pests and therefore makes the protection of plants and seeds (Borsani et al., 2001; Pieters et al., 1998).

Mode of action of Salicylic Acid may be due to its role in the resistance to pathogens by inducing the production of pathogenesis-related proteins (Van Huijsduijnen et al., 1986). It is involved in the systemic acquired resistance in which a pathogenic attack on one part of the plant induces resistance in other parts. The signal can also move to nearby plants by salicylic acid being converted to the volatile ester, methyl salicylate (Taiz & Eduardo, 2002).

The main objectives of this research were to study the effects of different storage periods, temperature levels and salicylic acid treatments on the oil compositions from fatty acids and oil acidity and free fatty acids (FFA) of canola seeds with different moisture contents.

2. Materials and Methods

This study was conducted in the labs of Arid Land Agriculture Department, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University.

2.1 Experimental Design and Treatments

Canola (*Brassica napus* L.) fresh harvested seed samples (100 g/sample) Pactole cv. were sealed in 200 ml glass flasks were distributed in a split split split plot design with 4 replications where the storage temperatures (10, 20, and 30°C) were the mail plot treatments , 4 storage periods (6,12,18 and 24 months) were the sub-plot treatments, 4 seed moisture contents (7, 10, 13 and 16%) were the sub sub-plot treatments while 3 salicylic acid concentrations (0.0,15 and 30 uM) were the sub sub sub-plot treatments. Seeds were stored under the previous treatments in 4 environmental chambers for the experimental time. Moisture content samples were maintained using potassium hydroxide solutions according to Solomon (1951).

After each stored period, seeds were dried to constant weight at 60-70°C using a precision vacuum oven and subsequently crushed and milled to obtain the seed flour.

Oil was extracted with N- Hexane (60°C) for 6 hours in a Soxhlet extractor according to AOAC (2000) and the canola seed oil composition from the saturated and unsaturated fatty acids were determined by Gas Chromatography according to AOCS (1990). Methyl esters of fatty acids were extracted with hexane, and then 1 ul aliquots of extracts were injected into a Gas Chromatography (Varian 3800). The column used was a supelcowax 10 fused-silica capillary column (30 m x 0.32 nm i.d.; Supelco, Bellefonte, PA).The carrier gas was helium, and the total gas flow rate was 20 ml/min. The injector, oven, and detector temperatures were 240, 190 and 260 °C, respectively. The determined saturated fatty acids were palmitic and stearic acids and the unsaturated fatty acids were oleic, linoleic and linolenic acids. Oil acidity was determined by the acid base titration technique (ASTM, 2003), a standard solution of one mole potassium hydroxide solution was used. Free fatty acids (FFA) contents were determined by the method of Modified FFA as described by AOCS (2004).

2.2 Statistical Analysis

Statistical analysis of the obtained data was performed using the analysis of variance and mean separation significance test by BLSD according to El-Nakhlawy 2010) using SAS program (SAS 2001).

3. Results and Discussion

According to analysis of variance, the main 4 factors (storage period, temperature, seed moisture and salicylic acid treatments) besides all 2-factor interactions were significant, while the 3 factors and 4-factors interactions were not significant so, the results and discussion will concentrate on the 2-factor interactions effects.

3.1 Effect of the Interaction Between Storage Period and Temperature

The present data in Table(1) showed that saturated fatty acids (palmitic and stearic acids) means were highest in the seed stored under the 24 months with 30°C and 20°C also under the 18 months storage with 30°C without significant differences between these 2 treatments. Palmitic acid (%) were 8.46, 8.05 and 8.11% and stearic acid (%) were 5.65 , 4.92 and 5.19% under the last treatments, respectively , while the treatments of the interaction between 6 or 12 months and 20 and 30°C resulted the lowest palmitic and stearic (%). As for unsaturated fatty acids response to the interaction between storage period and temperature, results of Table (1) illustrated that 6 months storage and 10°C or 20°C treatments were the highest in oleic acid (60.80% and 60.33%, respectively), while treatments of 24 months with 10, 20 and 30°C and the treatment of 18 month storage with 30°C were the lowest in oleic acid with around 10% decreasing. Concerning, linoleic acid contents, only the treatment of 6 month +10°C was the significantly higher than the other treatments. No significant differences were shown between all interaction treatments between 24 months or 18 months and temperatures. Around 46% reduction in linoleic acid concentration occurred as the effect of 24 storage month +30°C compared with 6 month +10°C. Also, Linolenic acid significantly decreased as temperature increased more than 20°C under 6 months, and under the long term storage (24 months) with 20°C by around 43%. Oil acidity and free fatty acids (FFA) significantly increased under the storage periods 6, 12, 18 months if temperature increased more than 20°C, but under 24 months if temperature increased more than 10°C. Acidity increased from 0.98 under (6 months +10°C) to 1.62 and 1.97 under 24 months and 20 and 30°C, respectively. Also, FFA increased significantly by around doubling under the Last treatment.

Our results showed that increasing storage temperature especially under the long term storage caused an adversely effects on canola seed stored oil through deceasing the concentrations of unsaturated fatty acids, and increasing acidity and free fatty acids in the stored seed oil, and these results confirmed with the results of Ghasemnezhad & Homermeier (2007).

Table 1. Means of fatty acids (%), acidity (mg KOH/g) and free fatty acids (%) of the oil produced from canola seed stored under the interaction between storage period and storage temperature

Storage Period (month)	Temp. (°C)	Fatty acids (%)					Acidity (mg KOH/g)	FFA (%)
		Palmitic	Stearic	Oleic	Linoleic	Linolenic		
6	10	4.50	2.81	60.80	17.85	8.32	0.98	0.49
	20	4.61	1.93	60.33	15.52	8.41	1.08	0.50
	30	5.96	3.08	56.71	14.13	7.19	1.30	0.60
12	10	4.91	2.89	58.71	15.29	7.55	0.95	0.48
	20	5.16	3.45	57.52	14.16	6.87	1.26	0.63
	30	5.92	3.92	55.10	13.30	6.25	1.39	0.70
18	10	6.46	3.81	53.71	11.07	6.00	1.18	0.59
	20	6.75	4.26	52.17	10.21	5.67	1.48	0.74
	30	8.11	5.19	50.55	10.18	5.25	1.59	0.80
24	10	6.91	4.32	52.32	10.04	5.22	1.62	0.81
	20	8.05	4.92	48.90	10.45	4.78	1.78	0.89
	30	8.46	5.65	48.24	9.65	4.37	1.97	0.99
BLSD(0.05)		0.68	0.77	1.78	1.63	0.54	0.20	0.05

3.2 Effect of the Interaction Between Storage Period and Seed Moisture Content

As shown in Table 2, significant effects were found for the storage period x seed moisture content interaction on all studied oil characteristics. Stored canola seeds with the same moisture content significantly different under different storage period in all studied characteristics. Generally, as storage period increased, quality of canola oil adversely affected. Reduction of oleic, linoleic and linolenic acids were 23.6%, 47.3% and 49.6%, respectively under the storage treatment of (24 storage period +16% seed moisture) compared with (6 month storage + 7% moisture content). Also acidity and FFA of the extracted oil increased with 87% more than the storage treatment of (6 month storage + 7% moisture content).

The adversely effects of high moisture content of stored seeds under the long storage periods (more than 6 months) on the canola oil may be due to the embryo activity resulting from increasing seed moisture content and as storage period increased, these unfavorable storage conditions caused hydrolysis of the triglycerides by enzymes that increasing in activity with the activity of micro-organisms that attack the seeds (Sathya et al., 2009, Cassells et al., 2007 , Reuss & Pratt, 2001; Reuss & Cassells, 2003).

Unfavorable storage conditions lead to an oxidation reaction and hydrolysis of the double bonds of the unsaturated fatty acids in oil, which leads to a change in their proportions in oil and increase the free fatty acids and acidity of the oil (Canakcii, 2007).

Table 2. Means of fatty acids (%), acidity (mg KOH/g) and free fatty acids (%) of the oil produced from canola seed stored under the interaction between storage period and seed moisture content

Storage Period (month)	Seed Moisture Content (%)	Fatty acids (%)					Acidity (mg KOH/g)	FFA (%)
		Palmitic	Stearic	Oleic	Linoleic	Linolenic		
6	7	4.50	2.04	61.59	16.90	8.56	0.35	0.17
	10	4.68	2.26	60.51	16.37	8.31	0.91	0.45
	13	5.23	3.01	58.30	15.36	7.71	1.33	0.70
	16	5.68	3.25	56.72	14.69	7.25	1.85	0.93
12	7	5.08	2.83	59.89	15.50	7.67	0.42	0.21
	10	5.25	2.96	57.27	15.18	7.21	0.84	0.41
	13	5.40	3.68	56.22	13.81	6.67	1.41	0.70
	16	5.58	4.21	55.06	12.50	6.02	2.14	1.07
18	7	5,74	3.72	54.32	11.95	6.08	0.59	0.30
	10	6.23	4.22	52.98	11.30	5.90	1.21	0.60
	13	8.26	4.67	51.24	10.49	5.47	1.74	0.87
	16	8.20	5.08	50.03	9.44	5.11	2.13	1.07
24	7	6.17	4.13	52.49	11.06	5.38	0.60	0.30
	10	6.91	4.73	50.95	10.44	4.89	1.49	0.74
	13	8.62	5.19	48.84	9.80	4.57	2.33	1.17
	16	9.53	5.81	47.02	8.90	4.31	2.73	1.37
BLSD(0.05)		0.82	0.27	0.58	0.42	0.26	0.16	0.04

3.3 Effect of the Interaction Between Storage Temperature and Seed Moisture Content

Palmitic acid means (Table 3) showed no significance between 7 and 10% seed moisture contents under any storage temperatures, but significant differences were showed for palmitic and stearic acids of the oil extracted from the same canola seeds stored under different temperatures, where as temperature increased both acids significantly increased.

The unsaturated fatty acids responded with an opposite trend of saturated fatty acids, where their means decreased as increasing temperature and seed moisture levels. Unsaturated fatty acids (%) of the canola seeds stored significantly decreased when high moisture seeds subjected to high temperature, where oleic acid (%) significantly decreased with rate of 3.5% and 6.7% , linoleic acid decreased by 7.6% and 23.2% and linolenic acid decreased with 6.8% and 16.2% by increasing temperature into 20°C and 30°C, respectively.

Acidity and FFA (%) significantly increased as temperature + seed moisture increased. The highest acidity and FFA were 2.41 and 1.20% under (30°C + 16% seed moisture).

The effects of the temperature and seed moisture interaction on the oil quality may be discussed as the storage of high seed moisture under high temperature encourage the bio-activity of seed embryo, accordingly, embryo respiration rate increased caused a high activity of the oxidation and hydrolysis enzymes so, the fatty acid proportion changed and FFA and acidity of the extracted oil increased causing bad effects on oil quality, as stated by Tys and Rybacki (2001); Chen and Ahn (1998); Neg and Anderson (2005).

Table 3. Means of fatty acids (%), acidity (mg KOH/g) and free fatty acids (%) of the oil produced from canola seed stored under the interaction between storage temperature and seed moisture content

Storage Temperature (°C)	Seed Moisture Content (%)	Fatty acids (%)					Acidity (mgKOH/g)	FFA (%)
		Palmitic	Stearic	Oleic	Linoleic	Linolenic		
10	7	4.78	2.70	59.08	14.94	7.52	0.35	0.18
	10	5.16	2.97	57.23	14.17	7.06	0.96	0.48
	13	6.03	3.87	55.18	13.14	6.49	1.46	0.73
	16	6.82	4.28	54.05	11.99	6.02	1.95	0.98
20	7	5.34	3.1	57.00	13.80	7.01	0.48	0.24
	10	5.75	3.36	55.49	13.46	6.70	1.14	0.57
	13	6.47	3.83	53.97	12.48	6.20	1.71	0.86
	16	7.00	4.37	52.45	11.52	5.81	2.27	1.14
30	7	6.00	3.73	55.12	12.83	6.30	0.64	0.32
	10	6.39	4.29	53.56	12.33	5.98	1.23	0.61
	13	8.13	4.70	51.80	11.47	5.62	1.92	0.96
	16	8.02	5.18	49.68	10.53	5.20	2.41	1.20
BLSD(0.05)		0.50	0.17	0.38	0.27	0.17	0.10	0.03

3.4 Effect of the Interaction Between Storage Period and Salicylic Acid Treatment

Using salicylic acid to treat canola seeds stored especially for 18 and 24 months keep the palmitic acid concentration in the oil without significant differences versus under 6 or 12 months storage periods. Significantly differences were found between palmitic acid and stearic acid contents under 0.0 salicylic (SA) in the 12, 18 and 24 months compared with using 15 or 30 uM SA treatments (Table 4).

Table 4. Means of fatty acids (%), acidity (mg KOH/g) and free fatty acids (%) of the oil produced from canola seed stored under the interaction between storage period and salicylic acid treatment

Storage Period (month)	Salicylic acid (uM)	Fatty acids (%)					Acidity (mgKOH/g)	FFA (%)
		Palmitic	Stearic	Oleic	Linoleic	Linolenic		
6	0.0	4.11	3.63	55.24	13.51	6.48	0.34	0.18
	15	4.46	2.18	61.69	17.19	8.83	0.34	0.17
	30	4.49	2.18	60.91	16.79	8.63	0.37	0.19
12	0.0	5.44	5.03	50.94	10.73	4.87	1.11	0.55
	15	4.66	2.64	60.33	16.01	7.93	0.47	0.24
	30	4.89	2.59	60.16	16.01	7.88	0.59	0.30
18	0.0	5.82	5.63	42.68	6.02	3.12	1.11	0.55
	15	4.47	3.22	57.30	13.26	6.94	0.39	0.29
	30	4.44	3.42	56.45	13.11	6.86	0.43	0.21
24	0.0	6.17	5.79	37.73	5.07	2.49	1.31	0.65
	15	4.90	3.68	56.28	12.77	6.25	0.60	0.30
	30	4.81	3.83	55.46	12.31	5.62	0.71	0.36
BLSD(0.05)		1.18	0.91	4.70	1.77	1.05	0.10	0.02

As for unsaturated fatty acids, statistical comparisons between the means showed that treating canola seeds with 15 or 30 uM SA under 18 and 24 months storage caused significance increasing in oleic, linoleic and linolenic acids (%) compared with no SA treatment under the long – term storage. Oleic acid (%) increased by 34% and 49%, linoleic acid (%) increased by 120% and 152%, also linolenic acid increased by 122% and 151% as a result of treating seeds with 15 uM SA under 18 and 24 months storage periods, respectively (Table 4).

Positive effects of treating canola stored seeds by SA on oil acidity and FFA (%), where significant decreasing in both were found as canola seeds treated with 15 or 30 uM SA.

The positive effects of treating canola seeds with SA on oil quality especially under the unfavorable storage conditions may be due the effect of salicylic acid on the donor of electrons and causes inhibition of lipids hydrolysis and oxidation enzymes (Clarke et al., 2001 , Chen et al., 1995, Zhang et al., 2002 , Radwan et al., 2006; El-Saidy & El-Hai, 2011).

3.5 Effect of the Interaction Between Storage Temperature and Salicylic Acid

Palmitic and stearic acid (%) significantly decreased by treating canola seeds with 15 or 30 uM SA, especially as temperature of storage increased. Unsaturated fatty acids (%) positively responded to treat canola seed by SA, especially under high storage temperatures (20°C or 30°C). Under 30°C, oleic, linoleic and linolenic acids (%) increased by around 35%, 99% and 122%, respectively as a result of seed treated by 15uM SA.

The positive effect of SA on quality of oil extracted from canola seed stored in unfavorable conditions was reflected also in decreasing oil acidity and FFA (%) by around 50% under 20 and 30°C.

Salicylic acid affects the donor of electrons and causes inhibition of peroxides enzyme, and increase H_2O_2 accumulation and it works to inhibit the growth of pathogens on seeds under the high storage temperature (Clarke et al., 2001; Chen et al., 1995; Rawshan et al., 2010).

Table 5. Means of fatty acids (%), acidity (mg KOH/g) and free fatty acids (%) of the oil produced from canola seed stored under the interaction between storage temperature and Salicylic acid

Storage Temperature (°C)	Salicylic acid (uM)	Fatty acids (%)					Acidity (mgKOH/g)	FFA (%)
		Palmitic	Stearic	Oleic	Linoleic	Linolenic		
10	0.0	5.01	2.96	58.83	10.29	4.94	0.98	0.49
	15	4.98	2.83	59.86	15.18	7.61	0.89	0.45
	30	4.90	2.95	59.47	15.21	7.78	0.97	0.49
20	0.0	6.60	3.42	50.14	8.97	4.55	1.99	1.00
	15	4.81	2.79	58.89	14.82	7.65	1.06	0.53
	30	5.02	2.97	58.15	14.66	7.10	1.16	0.58
30	0.0	7.48	5.98	42.97	7.25	3.24	2.35	1.18
	15	5.25	3.12	57.87	14.41	7.21	1.12	0.56
	30	5.52	3.20	57.26	13.92	6.98	1.12	0.56
BLSD(0.05)		0.46	0.15	0.33	0.24	0.15	0.09	0.02

3.6 Effect of the Interaction Between Seed Moisture Content and Salicylic Acid

As shown in Table (6), generally as stored seed moisture (%) increased significantly adverse effects were pronounced in oil constituents and quality where saturated fatty acids (%) significantly increased and unsaturated fatty acids decreased, besides the significance increasing in oil acidity and FFA (%).

As the high moisture seeds treated with SA (15 or 30 uM), palmitic and stearic acids (%) significantly decreased up to their levels in the 7% seed moisture or less. Significantly increasing in the percentages of oleic, linoleic and linolenic acids were shown as seeds were treated with SA.

Acidity and FFA (%) of canola seed oil significantly decreased as a result of highly moisture seeds were treated with SA.

These results may be due to the protection effects of SA on the high moisture seeds from the infection by microorganisms, accordingly, the bad effects of the high activity of the hydrolysis and oxidative enzymes decreased so, Low acidity and FFA (%) were shown in the extracted oil (Radwan et al., 2006, Pieterse et al., 1995, Zhang et al., 2002, Chen et al., 1995), also our results confirmed with the results obtained by Borsani et al. (2001) and Pieters et al. (1996) which stated that salicylic acid affects the mechanism of plant to protect against harmful diseases and pests and therefore makes the protection of plants and seeds.

Table 6. Means of fatty acids (%), acidity (mg KOH/g) and free fatty acids (%) of the oil produced from canola seed stored under the interaction between Salicylic acid and seed moisture content

Salicylic acid (uM)	Seed Moisture Content (%)	Fatty acids (%)					Acidity	FFA
		Palmitic	Stearic	Oleic	Linoleic	Linolenic	(mgKOH/g)	(%)
0.0	7	5.78	4.25	57.00	11.52	5.66	0.61	0.30
	10	6.57	4.92	51.79	10.35	4.88	1.46	0.73
	13	10.51	6.24	46.12	8.01	3.76	2.48	1.24
	16	11.29	7.27	40.59	5.45	2.66	3.47	1.73
15	7	4.56	2.60	59.41	15.12	7.67	0.46	0.23
	10	4.77	2.79	59.05	14.96	7.53	0.86	0.43
	13	4.97	3.00	58.68	14.65	7.41	1.25	0.62
	16	5.09	3.25	58.35	14.49	7.35	1.51	0.75
30	7	4.78	2.69	58.79	14.93	7.51	0.41	0.21
	10	4.97	2.91	58.45	14.65	7.34	1.02	0.51
	13	5.17	3.16	58.06	14.43	7.14	1.39	0.69
	16	5.22	3.15	57.93	14.42	7.19	1.59	0.79
BLSD(0.05)		0.87	0.22	0.47	0.34	0.21	0.13	0.03

4. Conclusion

The main conclusion of this study is the positive effects of treating canola seeds with 15 or 30 uM salicylic acid to decreasing the adversely effects of the unfavorable storage conditions especially on the oil composition from unsaturated, saturated fatty acids and oil acidity and percentage of free fatty acids.

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