ELIMINATION OF UNDESIRABLE MATERIALS FROM RAPESEED (BRASSICA NAPUS) SEEDS BY AQUEOUS SOILUTION TREATMENTS

BY

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ABSTRACT

Rapeseeds were soaked in tap water, hot water and alkaline soln. (2% NaOH). Appreciable changes in some of the physical and chemical characteristics of its cake and oil were noticed. Some changes in total and individual amounts of saturated and unsaturated fatty acids were also pointed out. The amount of erucic acid ($C_{22:1}$) was reduced by using these various soaking treatments and the highest reduction reached 86.7% of the original amounts after soaking of rapseeds in hot water for 24 hrs.

The hydrocarbon (C_{24}) was the predominant one in the hydrocarbon fractions while Stigmasterol and B-sitosterol were the main sterols and a great increase was noticed in the amount of B-sitosterol after dipping in tap water.

Different soaking treatments of rapeseeds showed a slight effect on meal glucosinolate compounds and the highest decrease of its myrosinase enzyme activity arised after soaking of rapeseeds in alkaline soln. (2%) for 36 hrs. i.e. 29.55%.

INTRODUCTION

The plant of rapeseed has the ability to grow and survive under different conditions, besides, it contains relatively high content of triglycerides and less amounts of free fatty acids (Bhatty and Sosulski, 1972). On the other hand, its meal is also rich in some important amino acids (El-Nockrashy *et al.*, 1977). Downey (1983), stated that rapeseed oil provides annually over 8% of the world's

vegetable oil. Even though, Egyptian authorities are still suspecious for using this oil for edible uses. The crop was successfully cultivated in Egypt. However, the resulted oil and meal from rapeseed have been said to contain some undesirable materials which might be toxic to human and ruminants e.g. erucic acid and glucosinolate compounds.

Thomasson and Boldingh (1955), demonstrated that erucic acid in rapeseed oil was responsible for retarding growth in rats. Christophersen and Bramer (1972), evidenced that erucic acid ($C_{22:1}$) or its metabolites inhibit the oxidation of other fatty acids of rapeseed oil. Fenwick and Curtis (1980), mentioned that oil contains erucic acid might lead to deposition of fat in heart muscle tissue.

Kramer et al. (1983) and Swkeldam et al. (1983), stated that rapeseed oil differs in its individual fatty acid contents comparing with other known semidrying oils. Such phenomenon might be regarded as imbalance in fatty acids distribution in edible rapeseed oil.

Fenwick and Curtis (1980), mentioned that the glucosinolates of rapeseed are concentrated in the meal and might reach 8% by weight in Brassica napus varieties and 3-4% in Brassica campestris. They added that the myrosinase enzyme decomposes the glucosinolate contents to other new products e.g. hydroxynitrile and episulphides which are more toxic than the original one.

Radwan and Lu (1976), reported that the detoxification of meal glucosinolate compounds and inactivation of its content of myrosinase enzyme are very important step to solve the problem of goitrogenic and growth inhibitory factors. Sosulski et al. (1972), found that the aqueous leaching of glucosinolates from rapeseed by using alkaline was more effective than that of acid treatment. Ballester et al. (1977) and Rauchberger et al. (1979), stated that water washing of rapeseed meal had improved growth promoting quality of rapeseed meal and reduced its goitrogenicity since over 90% of the isothiocyanate compounds are soluble in water.

Moustafa et al. (1986), studied the effect of different soaking treatments on soybean seeds. Allam et al. (1987), stated that the soaking treatments decreased trypsin inactivating inhibitor (TIA) to a great extent.

The effect of soaking treatments on oil and cake of rapeseed will be considered during this investigation.

MATERIALS AND METHODS

Materials:

Rapeseed (Brassica napus, Variety Lesira 145) sample were obtained from Agric. Res. Center, Oil crops Department, Giza.

Mustard seeds (Black) were obtained from Fac. of Medicine, Giza, to prepare myrosinase enzyme which was used for determination of glucosinolate compounds (Rauchberger et al., 1979).

Pure D(+) glucose was purchased from B.D.H. Co.

Standard normal fatty acids, erucic acid, hydrocarbons, squalene, and sterols, A.R. grade were obtained from Koch Light Laboratories, England.

Treatments of rapeseeds:

Three different soaking treatments were applied as follows:

Rapeseeds were soaked in container of tap water (25°C) for 12, 24 and 36 hrs. The seeds in another treatment were soaked in hot water (70°C) for 6, 12 and 24 hrs. The third treatment was accomplished with 2% sodium hydroxide soln. at room temperature for 12, 24 and 36 hrs., then washed several times with tap water to remove the alkali. In all cases, the seeds were air dried, then ground and kept for further investigation.

The oil was isolated from the ground seeds by using n-hexane according to the method reported by Rady et al., (1987).

Analysis of crude oil:

The refractive index, specific gravity, acid value, peroxide value, saponification value and the amount of the unsaponifiable matter were determined in the oil samples from different treatments as reported by A.O.A.C. (1980).

Determination of fatty acids and unsaponifiable matter:

Methylation of fatty acids was achieved as mentioned by Anon (1966), then subjected to G.L.C. analysis using a Pye Unicam instrument (model 104). The nitrogen, hydrogen and air flow rates were 60, 60 and 30 ml/min. respectively, the chart speed was one cm/min., attenuation was 50×10^{-2}, column and detector temp. were 200°C and 250°C. Standard methyl esters of the abovementioned fatty acids were used

as standard authentic samples. The amount of each individual fatty acid was determined according to Nelson et al., (1969).

Hydrocarbons and sterols from different samples were determined by G.L.C. with the same equipment. The flow rates of the different gases were: nitrogen 30 ml/min., hydrogen 33 ml/min. and air 330 ml/min., the chart speed was 2 cm/min. and the attenuation was $32x10^{-2}$. Qualitative identification was carried out by comparing the relative retention time with the standard authentic samples. The quantitative analysis was carried out on the basis of peak area measurements.

Analysis of meal:

Moisture, oil, protein and crude fibre contents of the meal samples obtained from different treatments were determined according to A.O.A.C. (1980).

Total soluble carbohydrates were estimated according to the method described by Helbert and Brown (1959).

Cation amounts were determined as reported by Rowe (1973) using a Pye Unicam atomic absorption spectrophotometer, Model (1900).

Estimation of glucosinolate and the activity of myrosinase enzyme were carried out by measuring the liberated glucose during hydrolysis of glucosinolates by myrosinase enzyme (Rauchberger et al., 1979).

RESULTS AND DISCUSSION

1- Effect of various soaking treatments on chemical and physical properties of rapeseed oil:

The values in table (1) illustrate the physical and chemical properties of rapeseed oil (Brassica napus, Lesira 145).

The values obtained for the control sample are in agreement with those obtained by Kramer et al., (1983); Daun et al., (1986) and Farag et al., (1986).

Different soaking treatments showed no changes in the refractive index of rapeseed oil, while it induced a slight change in specific gravity, saponification value and the unsaponifiable matter. Such results agreed with that reported by Uzzan (1965).

Table (1):Effect of various soaking treatments on physical and chemical properties of rapeseed oil .

Contents	Control	Tap water			Hot water (70°C)			2% NaOH soln.		
		12 hrs.	24 hrs.	36 hrs.	6 hrs.	12 hrs.	24 hrs.	12 hrs.	24 hrs.	36 hrs.
Refractive index / 25°C	1.4693	1.4708	1.4699	1.4700	1.4693	1.4702	1.4705	1.4669	1.4665	1.4685
Specific gravity / 25°C	0.9115	0.9073	0.9071	0.9043	0.9077	0.9013	0.9005	0.9057	0.9066	0.9048
Acid value	0.72	0.40	0.37	0.41	0.82	0.89	0.82	0.50	0.52	0.27
Peroxide value (meq/kg)	3.328	2.780	2.25	2.23	8.53	10.74	10.57	9.92	16.90	17.18
Iodine value	106.95	100.89	94.73	95.45	100.28	99.60	83.90	100.28	94.04	80.84
Saponification value	190.36	194.15	194.43	195.86	193.75	193.64	191.61	189.01	189.37	187.65
Unsaponifiable matter percentage	1.70	1.67	1.63	1.71	1.59	1.63	1.66	1.58	1.51	1.60

The lowest decrease in acid value occurred after soaking in NaOH (2%) for 36 hrs. Such phenomenon resulted from the neutralization process of alkaline soln. with free fatty acids present in untreated seeds. The obtained data are similar to that reported by Bailey (1964). However, soaking in hot water was accompanied with a slight increase in acid values. This slight increase in acid values might be resulted from the partial activation of lipase enzyme leading to more liberation of free fatty acids.

Soaking in hot water (70°C) and in 2% NaOH soln. led to a noticeable increase in peroxide value of the obtained oils. Meanwhile, soaking rapeseed in tap water had induced a slight decrease in the peroxide value of rapeseed oil. These results are almost in agreement with those mentioned by Moustafa et al., (1986).

Iodine value dropped from 106.95 in control sample to 94.45 after soaking in tap water for 12, 24, respectively. The highest decrease in the iodine value occurred in alkaline soln. This decrease in iodine value might be attributed to oxidative rancidity, since this reaction leads to the saturation of double bonds. The presence of unsaturated centres in fatty acid molecules acts as active centres toward oxygen attack, leading to the formation of various derivatives e.g. ketones, aldehydes and hydroperoxides. Therefore, the decrease in iodine values was accompanied with an increase in peroxide value as shown in table (1). Such explanation was in agreement with that introduced by Bailey (1964) and List et al., (1974).

2- Effect of soaking treatments on fatty acid composition of rapeseed oil:

Table (2) illustrates the effect of various soaking treatments on the fatty acids profile of rapeseed oil. Generally, it could be stated that soaking of rapeseeds in tap awter had no effect on both total unsaturated and saturated fatty acids of rapeseed oil while soaking in hot water and 2% NaOH soln. induced some changes in fatty acids content.

The amount of oleic acid in the control sample was relatively high i.e. 76.52%. This value was greatly increased after soaking treatments than that reported by Beadle et al., (1965); Khalil (1978) and Swkeldam et al., (1983), for other semidrying oils. Linoleic was relatively small when compared with other reported values for well known edible oils. However, several authors had claimed that rapeseed oil suffers from imbalancing in distribution of saturated to unsaturated fatty acid content.

Table (2) Relative amounts of fatty acids composition obtained by G.L.C. analysis in rapeseed oil subjected to different soaking treatments .

Fatty acids		R.R.T	Control	Tap water			Hot water			NaOH (2%)	
				12 hrs.	24 hrs.	36 hrs.	6 hrs.	12 hrs.	24 hrs.	24 hrs.	36 hrs.
Undecanoic	(11:0)	0.083	0.83	0.48	0.20	0.21	0.14	0.13	0.03	0.04	0.07
Palmitic	(16:0)	0.47	3.71	4.82	4.25	4.99	4.90	4.11	3.34	3.58	3.54
Palmitoleic	(16:1)	0.54	-	-	-	-	-	-	-	-	-
Stearic	(18:0)	0.90	0.27	0.38	0.51	0.32	0.31	0.29	0.51	0.44	0.45
Oleic	(18:1)	1.00	76.52	77.44	78.22	76.86	76.91	78.35	89.96	79.77	82.73
Linoleic	(18:2)	1.20	10.29	10.36	10.67	11.46	11.58	10.03	4.33	10.04	8.57
Linolenic	(18:3)	1.50	6.13	4.86	4.27	4.61	4.73	5.51	1.37	4.44	3.45
Arachedic	(20:0)	1.70	1.42	1.02	1.27	1.11	1.06	1.18	0.35	1.18	0.87
Erucic	(22:1)	4.30	0.83	0.64	0.61	0.44	0.36	0.39	0.11	0.51	0.32
Total unsaturated fatty acids			93.77	93.30	93.78	93.37	93.59	94.29	95.77	94.76	95.07
Total saturated fatty acids			6.23	6.70	6.22	6.63	6.41	5.71	4.23	5.24	4.93

Table (2) shows that oleic acid ($C_{18:1}$) 76.52% increased to 89.96% after 24 hrs. of continuous dipping in hot water (70°C). On the other hand, linoleic acid ($C_{18:2}$) 10.2% was sharply decreased and reached 4.33% and linolenic behaved in a similar manner.

It is important to note that the percentage of the undesirable erucic acid ($C_{22:1}$) decreased as shown in table (2) from 0.83% in unsoaked seeds (control sample) to less percentage by using various soaking treatments. The highest reduction reached 86.7% by soaking the seeds in hot water (70°C) for 24 hrs.

3- Effect of various soaking treatments on unsaponifiable matter of rapeseed oil:

It is obvious from table (3) that all soaking treatments caused a noticeable changes in the relative percentages of unsaponifiable matter of rapeseed oil. Total hydrocarbons showed a noticeable decrease due to soaking in tap water and 2% NaOH soln. but the rate of this decrease was much higher in tap water. Such results are in agreement with those reported by Bailey (1964) and Khalil (1978). Soaking of rapeseed in hot water (70°C) for 24 hrs. did not change its total hydrocarbons. The predominant hydrocarbon component (C_{24}) increased upon soaking seeds in hot water 70°C and 2% NaOH soln. The hydrocarbon C_{14}, C_{16}, C_{18} and squalene were slightly decreased after all soaking treatments.

It has to be mentioned in this aspect that some new hydrocarbon compounds were detected as a result of various different R.R.T. values while the hydrocarbon C_{31} disappeared.

The major sterol compounds, B-sitosterol and stigmasterol increased after soaking in tap water and 2% NaOH soln. The soaking of rapeseeds in hot water had led to the disappearance of B-sitosterol. Campesterol was noticeably increased in all soaking treatments. The obtained results are in conscident with those reported by Khalil (1978).

4- Effect of various soaking treatments on chemical composition of rapeseed meal:

The obtained results in table (4) indicate that various soaking treatments caused slight changes in moisture, protein and crude fibre contents of rapeseed meal. These results are almost in agreement with those reported by Bhatty and Sosulski et al. (1972), Bell and Jeffers (1976) and Fenwick and Curtis (1980).

Table (3): Effect of various soaking treatments for 24 hrs. on unsaponifiable matter components of rapeseed oil.

Components	R.R.T.*	Control	Tap water	Hot water (70°C)	2% NaOH
1- Decane C_{10}	0.03	--	--	1.14	2.93
2- Dodencane C_{17}	0.11	0.54	0.17	1.76	T
3- Unknown (1)	0.13	0.77	0.26	0.21	0.17
4- Tetradecance C_{14}	0.15	0.86	0.32	0.10	0.36
5- Hexadecane C_{16}	0.25	2.79	0.66	0.24	0.87
6- Octadecane C_{18}	0.33	1.63	0.39	0.31	0.44
7- Eicosane C_{20}	0.41	1.34	0.86	0.26	1.59
8- Unknown (2)	0.45	--	0.44	1.27	0.26
9- n-docosane C_{22}	0.45	--	2.21	0.29	0.26
10- n-tetracosane C_{24}	0.48	46.48	37.69	65.51	49.76
11- n-pentacosane C_{25}	0.51	0.45	0.09	--	0.44
12- Unknown (3)	0.53	0.59	1.26	3.76	0.84
13- n-hexacosane C_{26}	0.55	2.56	0.88	1.80	2.84
14- n-octacosane C_{28}	0.60	1.19	0.44	0.45	1.60
15- Unknown (4)	0.62	--	--	2.98	0.19
16- n-nonacosane C_{29}	0.66	0.45	0.63	0.15	0.28
17- Unknown (5)	0.67	--	1.09	Traces	0.77
18- Squalene	0.68	0.62	0.31	0.19	0.55
19- n-triacontane C_{30}	0.70	1.13	0.08	0.06	1.30
20- n-untriacontane C_{31}	0.72	2.23	--	--	--
21- Unknown (6)	0.74	5.47	0.25	0.86	2.84
22- n-dotriacontene C_{32}	0.76	3.74	0.22	0.73	1.76
23- n-dotriacontane C_{32}	0.79	11.41	1.05	2.35	5.01
24- Cholesterol	0.82	--	0.35	--	0.73
25- Unknown (7)	0.86	--	--	9.03	--
26- Campesterol	0.89	1.69	2.76	2.43	2.68
27- Stigmasterol	0.92	5.74	13.69	2.74	7.61
28- B-Sitosterol	1.00	8.32	33.54	--	13.92
29- -7-stigmasterol	1.25	--	--	1.38	--
Total hydrocarbon		84.25	49.66	84.42	75.06
Total sterols		15.75	50.34	15.58	24.94

* R.R.T.: Relative retention time.

Table (4) :Effect of various soaking treatments on chemical composition of rapeseed meal

Contents	Control	Tap water			Hot water (70°C)			2% NaOH sol		
		12 hrs.	24 hrs.	36 hrs.	6 hrs.	12 hrs.	24 hrs.	12 hrs.	24 hrs.	36 hrs.
Moisture (%)	9.28	9.20	8.92	8.96	9.10	9.18	9.05	9.13	8.89	8.97
Ash (%)	7.04	6.73	6.79	6.90	5.89	5.25	5.39	8.35	8.35	8.48
Crude fibre (%)	8.46	7.49	7.62	7.01	7.47	6.59	6.51	7.68	7.69	7.65
Protein (%)	34.63	34.41	34.18	34.29	33.55	33.03	33.56	33.38	33.16	33.12
Reducing and non-reducing (%) sugars	4.60	4.32	3.21	3.05	1.41	0.96	0.96	3.68	1.25	0.93
Minerals (%)										
Na	0.022	0.021	0.020	0.020	0.020	0.020	0.019	0.041	0.056	0.094
K	0.440	0.440	0.430	0.410	0.420	0.415	0.410	0.435	0.430	0.430
Zn	0.160	0.158	0.157	0.152	0.156	0.155	0.153	0.159	0.159	0.156
Cu	0.037	0.036	0.034	0.034	0.035	0.033	0.031	0.035	0.035	0.036
Mn	0.080	0.078	0.077	0.075	0.077	0.076	0.073	0.080	0.078	0.078

Soaking of rapeseed in either tap water or hot water (70°C) caused a noticeable decrease in ash content which might be attributed to leaching of some elements from rapeseed in water. On the contrary, ash content was increased after soaking in alkaline soln. owing to the increase in Na content in the meal after dipping in 2% NaOH soln.

All soaking treatments had induced a gradual decrease in reducing and non reducing sugars with increasing the period of soaking treatments. The reduction of percentage in these sugars was much higher in samples soaked in hot water, followed by alkaline and tap water, respectively. This means that the decrease in reducing and non-reducing sugars might be attributed to the partial dissolving of these sugars in soaking media.

5- Effect of various soaking treatments on total glucosinolates and myrosinase enzyme activity:

Soaking treatment in tap water induced a gradual decrease in myrosinase enzyme activity with increasing soaking periods i.e. from 1.76 g/100 g in the control sample to 1.64, 1.52 and 1.40 g/100 g for 12, 24 and 35 hrs. which equals a reduction percentage of 6.82%, 13.64% and 20.45%, respectively. The amounts of glucosinolate compounds were also decreased from 1.88 g/100 g in control smaple to 1.68 g/100 g in meal. Soaking in 2% NaOH soln. greatly decreased myrosinase enzyme activity and total glucosinolate compounds more than that reported after soaking in hot and tap water. Such results are almost in agreement with those stated by Bhatty and Sosulski (1972), Moustafa et al., (1986) and Allam et al., (1987).

Table (5): Effect of various soaking treatments on total glucosinolate compounds and myrosinase enzyme activity of rapeseed meal.

Contents	Control sample	Tap water			Hot water			2% NaOH soln.		
		12 hrs.	24 hrs.	36 hrs.	6 hrs.	12 hrs.	24 hrs.	12 hrs.	24 hrs.	36 hrs.
Total glucosinolates (g/100g)	1.88	1.76	1.70	1.68	1.67	1.65	1.56	1.71	1.60	1.48
Reduction (%)		6.38	9.57	10.64	11.17	12.23	17.02	9.04	14.89	21.28
Myrosinase enzyme	1.76	1.64	1.52	1.40	1.56	1.55	1.28	1.44	1.28	1.24
Reduction (%)		6.82	13.64	20.45	11.36	11.93	27.27	18.18	27.27	29.55

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تحليل بعض المواد الغير مرغوبة
من بذور اللفت (اللفت) بالعمليات المائية

مصطفى كمال صبرى شاته — صلاح مصطفى محمود سعيد
على حسن راض — خليل حلمى الخواص

نقع بذور اللفت (التلخم) فى ماء الصنور والماء الساخن (٧٠م) وكذلك نوملت بمحلول صودا كاوية (٦٪) - لمدد متفاوتة - بهدف دراسة تأثير هذه المعاملات على كل من الزيت والكسب المتبقى من محاولة تحسين نوعيتها بالاضافة الى تحليل نشاط انزيم الميروزينيز لتحليل المركبات السامة التى تنتج من تحليل بعض مركبات الجلوكوسينولات بتأثير هذا الانزيم.

أدت معاملات النقع بالماء والقلوى الى تغييرات واضحة فى بعض الخواص الطبيعية والكيماوية فى كلا من الزيت والكسب . وقد نسبت هذه المعاملات فى بعض التغيرات فى النسبة الكلية وكذلك مقررات الاحماض الدهنية المشبعة والغير مشبعة.

أظهرت النتائج أن هناك انخفاض ملحوظ فى حمض الاريوسيك (C_{22} :1) العمر مرغوب نتيجة المعاملات المختلفة وقد أدت معاملة النقع بالماء الساخن لمدة ٢٤ ساعة الى أقصى انخفاض (٧ر٨٦٪).

أظهرت النتائج أن المركب الليجنوسيرك (C_{24}) هو المكون السائد فى الهيدروكربونات بينما المركب بيتا سيتوستيرول B -sitosterol هو المكون الاساسى للاستيرولات وقد أدى نقع البذور بماء الحنفية الى زيادة واضحة فى ذلك المكون الاستيرولى .

أدت معاملات النقع المختلفة الى تغييرات بسيطة فى مركبات الجلوكوسينولات وكذلك نشاط انزيم الميروزينيز بالبذرة .

وقد أدت المعاملة بالقلوى (٢٪) لمدة ٣٦ ساعة الى أقصى انخفاض فى النشاط الانزيمى (٥٥ر٢٩ ٪) .