Effect of mechanical extraction parameters on the yield and quality of tobacco (*Nicotiana tabacum* L.) seed oil

M. Sannino^{1,4} · L. del Piano² · Massimo Abet² · S. Baiano² · M. Crimaldi¹ · F. Modestia² · F. Raimo³ · G. Ricciardiello¹ · S. Faugno¹

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Abstract The aim of this study was to investigate how the combination of extraction parameters, such as extraction temperature seeds preheating and screw rotation speed, influenced the yield and chemical quality of tobacco seed oil (TSO). For its peculiar properties, TSO can be used for several purposes, as raw material in the manufacturing of soap, paints, resins, lubricants, biofuels and also as edible oil. TSO was obtained using a mechanical screw press and the quality of the oil was evaluated by monitoring the free fatty acids (FFA), the peroxide value (PV), the spectroscopic indices K_{232} , K_{270} and ΔK and the fatty acid composition. The maximum extraction yield, expressed as percent of oil mechanically extracted respect to the oil content in the seeds, determined by solvent extraction, was obtained with the combination of the highest extraction temperature, the slowest screw rotation speed and seeds preheating. Under these conditions yield was $80.28 \pm 0.33\%$ (w/w), 25% higher than the lowest yield obtained among investigated conditions. The extraction temperature and seed preheating showed a significant effect on FFA, on spectroscopic indices K_{232} , K_{270} and ΔK values. The average values of these parameters slightly increased rising the temperature and in presence of

preheating, the screw rotation speed did not affect the chemical characteristic tested. In the extraction conditions investigated no significant changes in PV and fatty acids composition of oil were observed.

Keywords Tobacco seeds · Tobacco oil chemical quality · Mechanical oil extraction · Fatty acids composition

Introduction

Tobacco (*Nicotiana tabacum* L.) is the major non-food agricultural crop in the world with a production extension higher than four million hectares in the whole world (Poltronieri 2016). Tobacco is cultivated mainly for its leaves, used for smoking products. Considering the harmfulness of smoking to human health, there is a strong anti-tobacco policy that threatens its production. However, the economic lifeline of millions of people in the world depends on tobacco, therefore this crop could be sustained by taking advantage of its potential for alternative uses. So, in order to sustain the crop, it is necessary to intensify the research efforts towards channeling tobacco into non-conventional and economically viable alternative uses (Poltronieri 2016).

Recently, alternative uses of tobacco have been revalued, especially as oleaginous plant. When untopped, tobacco can be also considered an oilseed crop because seeds are rich in oil that is free of nicotine (Stanisavljevic et al. 2009). The oil content of tobacco seeds varies between 30 and 43% on weight basis and it is comparable to mustard, safflower and sunflower (del Piano et al. 2014; Giannelos et al. 2002). TSO has a low proportion of saturated fatty acids that is considered as a positive nutritional factor compared to vegetable oils with an high

✉ S. Faugno
faugno@unina.it

¹ Department of Agriculture, University of Naples Federico II, Via Università 100, 80055 Portici, NA, Italy

² Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, CREA-FRC, Caserta, CE, Italy

³ Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, CREA-ORT, Pontecagnano, SA, Italy

⁴ Department of Agriculture, Tuscia University, 01100 Viterbo, VT, Italy

concentration of saturated fat. The main fatty acids (FA) in TSO are linoleic acid (65–75%), oleic acid (10–16%), palmitic acid (8–11%) and stearic acid (2–3%) (del Piano et al. 2014; Frega et al. 1991; Giannelos et al. 2002). In Italy and some Eastern European countries, during the first half of last century, TSO has been used as raw material in the manufacturing of soap, paints, lubricants and fuel and also as edible oil (Balbi 1959). According to recent studies (Srinivas et al. 2013; Usta et al. 2011), tobacco seed oil may be used for the production of high quality biodiesel. Ogunniyi and Odotoye (2007) and Patel et al. (2008) showed that TSO, in the field of industrial coatings, is an excellent raw material for the production of alkyd resins that do not turn yellow, contributing with the use of less polluting technologies to reduce the environmental impact of this sector. Furthermore, the TSO extraction process generates a solid by-product (TS cake) that might be utilized for animal feed, being rich in protein (20–25%) and fiber but devoid of alkaloids (Abbas et al. 2008; Frega et al. 1991; Rossi et al. 2007). Also fresh and dry biomass represents another valuable product of tobacco plant as it can be used for production electricity and biogas (Poltronieri 2016), as well as stalks can be used as raw materials for paper industry (Shakhes et al. 2011). The use of the protein cake, and the biomass associated to seed oil production are essential for a sustainable tobacco crop.

Currently, the oil is mainly extracted from seeds by press extraction and solvent extraction. Press extraction, respect to solvent extraction techniques, seems to be more acceptable for health, safety, economic and environmental reasons (Yusuf et al. 2014). Using press extraction, Stanisavljevic et al. (2009) achieved an oil yield of 32.9 g/100 g and Faugno et al. (2016) achieved an oil yield of 34.0 g/100 g. The aim of this study was to investigate the effect of the combination of extraction parameters, such as extraction temperature, seeds preheating and screw speed rotation, on yield and quality of tobacco seeds oil.

Materials and methods

Tobacco seed material

In this study, dried tobacco seeds were procured from prior PON-Enerbiochem and BioPolis experimental trails. Seeds were cleaned to remove impurities with a pneumatic separator, then stored in a warehouse at temperature between 15 and 24 °C (Faugno et al. 2016; Galloway 1976; Karaj and Muller 2011). Sample seed moisture content was kept constant to 8% on weight basis. Moisture seed content was determined by standard hot-air oven (Binder, BD115, Germany) method at 105 ± 1 °C to constant weight (Faugno et al. 2016; Garnayak et al. 2008; Pradhan et al.

2011). The oil content of untreated samples was $43.77 \pm 0.58\%$ (w/w) and it was determined by Accelerated Solvent Extraction utilizing an ASE®200 System Dionex.

Mechanical oil extraction procedure

Tobacco oil was extracted from seeds with a mechanical screw press, powered by 2.2 kW electric motor. Different sensors were installed for measuring temperature (RTDs, Pt100) and rotational speed (rev counter) of the screw (Faugno et al. 2016; Karaj and Muller 2011). The sensors were connected to a data-logger (National Instruments cDAQ-9171) and data transferred via data-logging software (National Instruments Lab VIEW 2014) to a laboratory computer. The pure oil yields were calculated with the methodology used by Faugno et al. (2016). The extraction conditions were evaluated because they caused a variation of oil extraction temperature that might alter the chemical quality of seed oil (Kraljic et al. 2013). In order to evaluate the effect of mechanical extraction parameters on chemical quality of TSO, the following conditions were considered:

1. Two different initial temperatures of extraction: 50 °C (T50) and 70 °C (T70) of head press (Faugno et al. 2016; Tambunan et al. 2012);
2. Thermal pretreatment of seeds: yes (H) or not (NH). The seeds were dried for 1 h at 50 °C and then processed (Faugno et al. 2016; Galloway 1976; Singh and Bargale 2000);
3. Two different rotational speeds of the screw: 22 rpm (ω_{22}) and 32 rpm (ω_{32}) (Faugno et al. 2016; Karaj and Muller 2011).

In this study, two different experimental setups were carried out with combinations of three different parameters, utilizing an 8 mm nozzle size (Faugno et al. 2016). In Setup 1 the temperature of the press was kept constant at 50 °C, whereas the pretreatment was varied in combination with screw speed. In Setup 2, the temperature was constantly at 70 °C and the combinations between screw speed and pretreatment were the same as Setup 1. For each sample, 1000 g of seeds were used and three replications were done (Faugno et al. 2016; Stanisavljevic et al. 2009; Unquiche et al. 2008). The extracted TSO was collected and stored in light-proof glass containers and centrifuged in a ALC laboratory centrifuge (ALC T535 PK130R) at 3500 rpm for 20 min in order to remove components that settled during storage (Unquiche et al. 2008). The solid by-product (TS cake) was stored in vacuum packages (Faugno et al. 2016; Unquiche et al. 2008), at 4 °C in the dark.

Chemical experimental procedure

Free fatty acids content

Free fatty acid content, expressed as percent of oleic acid, was determined according to the AOAC method 940.28 (AOAC International 2000).

Peroxide value

The peroxide value was determined according to the AOAC method 965.33 (AOAC International 2000).

Spectroscopic indices

The spectroscopic indices K_{232} and K_{270} in the UV region were determined with a spectrophotometer DU 64 Beckman according to the European Official Method of Analysis on the characteristics of olive oil and olive-residue oil, Commission Implementing Regulation (EU) No 299/2013-Annex I).

Fatty acid composition

Gas chromatographic (GC) analysis of fatty acids was performed following the method described by International Olive Oil Council (2015). The fatty acid methyl esters (FAME) were prepared by trans esterification of oil with 2 N KOH in methanol and *n*-hexane. GC analysis of FAME were performed in a Perkin Elmer Clarus 680 gas chromatograph equipped with a SQ 8 Mass Spectrometer, a Combipal Autosampler, a Restek capillary column (30 m, 0.32 mm ID, film thickness 0.25 μ m), a split injector at 250 °C, and quadrupole of mass spectrometer at 200 °C.). The programmed temperature was: 40–165 °C, at 6 °C/min, 165–250 °C, at 3 °C/min and then 5 min at 250 °C. Helium was used as carrier gas and a 1:50 split ratio was used. Data acquisition was realized in scan mode and mass spectra were acquired over the *m/z* range 40–400 (Dodds et al. 2005). The fatty acid methyl esters peaks were identified comparing their retention times with those of a 37 component FAME mix (Supelco) and confirming the fragmentation spectra utilizing NIST 2002 database. The relative percentage of the fatty acid was calculated on the basis of the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil sample. Each result presents the mean and the standard deviation for a minimum of three analyses.

Statistical analysis

R-Studio statistical software (version 3.1.2) was used for statistical analysis by factorial analysis of variance

(ANOVA, with Tuckey's HSD multiple comparison) with level of significance set up at $p \leq 0.05$.

Results and discussion

Table 1 shows the yield of pure oil and the oil extracted temperature in different setups. The highest tobacco seeds oil yield was 80.28 ± 0.33 (w/w), expressed as percent of oil extracted respect to the oil content in the seeds, determined by solvent extraction, with average extracted oil temperature recorded of 58.3 ± 1.53 °C. This result was achieved with Setup 2, with the combination of high extraction temperature, slow rotational screw speed and thermal seed pretreatment. The lowest oil yield was $64.01 \pm 2.96\%$ (w/w) using Setup 1 with the combination of low extraction temperature, fast rotational screw speed and no thermal seeds pretreatment. These results are in accordance to Karaj and Muller (2011), Tambunan et al. (2012) and Faugno et al. (2016).

Chemical oil quality

The mean values of main quality parameters of TSO are reported in Table 2. For all treatments, FFA and PV are within the Codex Alimentarius limits for cold pressed fats and oils, respectively 2%, expressed as oleic acid, equivalent to an acidity value of 4 mg KOH g⁻¹ fat or oil, and 15 meq of active oxygen kg⁻¹ fat or oil (Codex Stan 19-1981). The value of free fatty acid observed was similar to those cited in the literature for tobacco seed oil (Abbas et al. 2008; Rahman et al. 1977).

Table 1 Oil yield and extracted oil temperature in different experimental setups

	O _y [% (w/w)]	T _o [°C]
Setup 1		
T50_H_ω32	69.49 ± 1.98	51.3 ± 2.52
T50_H_ω22	77.49 ± 0.79	52.0 ± 1.73
T50_NH_ω32	64.01 ± 2.96	47.7 ± 1.53
T50_NH_ω22	76.58 ± 1.98	48.3 ± 1.53
Setup 2		
T70_H_ω32	73.83 ± 1.03	56.3 ± 0.58
T70_H_ω22	80.28 ± 0.33	58.3 ± 1.53
T70_NH_ω32	74.12 ± 2.83	53.7 ± 1.15
T70_NH_ω22	77.61 ± 1.19	55.0 ± 1.00

Each data represents the mean of three replicates ± standard deviation

O_y oil yield, T_o extracted oil temperature

Table 2 Free fatty acid, peroxide value and UV indices of TSO extracted in different experimental setups

	FFA (as % oleic acid)	PV (meq kg ⁻¹)	K ₂₃₂	K ₂₇₀	ΔK
Step 1					
T50_H_ω32	1.04 ± 0.07	1.90 ± 0.09	1.56 ± 0.04	0.29 ± 0.02	0.02 ± 0.00
T50_H_ω22	1.11 ± 0.10	2.26 ± 0.12	1.56 ± 0.06	0.31 ± 0.02	0.02 ± 0.001
T50_NH_ω32	1.16 ± 0.12	1.94 ± 0.52	1.66 ± 0.04	0.34 ± 0.02	0.02 ± 0.000
T50_NH_ω22	1.21 ± 0.17	2.11 ± 0.41	1.70 ± 0.09	0.37 ± 0.04	0.02 ± 0.002
Step 2					
T70_H_ω32	2.11 ± 0.09	1.72 ± 0.60	2.00 ± 0.12	0.52 ± 0.03	0.03 ± 0.001
T70_H_ω22	1.82 ± 0.09	1.18 ± 0.09	1.89 ± 0.08	0.51 ± 0.04	0.03 ± 0.002
T70_NH_ω32	1.17 ± 0.10	2.04 ± 0.83	1.65 ± 0.12	0.33 ± 0.05	0.03 ± 0.001
T70_NH_ω22	1.19 ± 0.09	1.97 ± 0.23	1.69 ± 0.06	0.34 ± 0.03	0.03 ± 0.002

Each data represents the mean of three replicates ± standard deviation

The spectroscopic index K₂₃₂ was within the limits for an extra-virgin olive oil (K₂₃₂ ≤ 2.5) for all treatments, while K₂₇₀ and ΔK were out of the limits for an extra-virgin olive oil (K₂₇₀ ≤ 0.22 and ΔK ≤ 0.01), but within those for an olive oil (K₂₇₀ ≤ 0.90 and ΔK ≤ 0.16), according to the International Olive Council (2015).

The comparison of means analysis (Tukey HSD) on the experimental data are shown in Table 3. The temperature had a significant effect on FFA, K₂₃₂, K₂₇₀ and ΔK values but no significant effect on PV. The average values of these parameters, however, slightly risen with increasing temperature as reported in Table 3. According to Jabar et al. (2015) and Aladic et al. (2014), the rise of temperature increased FFA owing to a process of enzymatic hydrolysis of the glycerides with the release of fatty acids. According to Zhang et al. (2013), for flax seed oil, heating temperature did not affect PV, but had a great impact on the K₂₃₂ and K₂₇₀. They suggested that flax seed oil, rich of polyunsaturated acids, as well as TSO, can be rapidly oxidized to

hydroperoxide, but hydroperoxide are unstable at high temperature.

The preheating of seeds had a significant effect on FFA, K₂₃₂, K₂₇₀ values of extracted oil. When the preheating was used, higher mean values of FFA, K₂₃₂, K₂₇₀ were observed (Table 3). These results are in accordance with other studies that observed the increase of FFA, dienes and trienes measured respectively by K₂₃₂ and K₂₇₀ (Abbadi et al. 2014; Soetaredjo et al. 2008).

The speed of the screw had not significant effect on all dependent variables analyzed. Indeed, screw speed does not influence extracted oil temperature (Faugno et al. 2016).

The interaction between the extraction temperature and preheating had a significant effect on all parameters considered, except for PV and ΔK values. Indeed, high extraction temperature and pretreatment increased significantly FFA, K₂₃₂ and K₂₇₀ compared to the other combination of these extraction parameters.

Table 3 Comparison of FFA, PV and UV indices of oil for the extraction parameters tested

		FFA (% oleic acid)	PV (meqO ₂ kg ⁻¹ oil)	K ₂₃₂	K ₂₇₀	ΔK
Temperature	T70	1.57 ^a	1.73 ^a	1.807 ^a	0.426 ^a	0.028 ^a
	T50	1.13 ^b	2.05 ^a	1.619 ^b	0.329 ^b	0.021 ^b
Pretreatment	H	1.52 ^a	1.76 ^a	1.753 ^a	0.409 ^a	0.025 ^a
	NH	1.18 ^b	2.01 ^a	1.673 ^b	0.345 ^b	0.024 ^a
Rotation speed	ω32	1.37 ^a	1.90 ^a	1.715 ^a	0.372 ^a	0.024 ^a
	ω22	1.32 ^a	1.87 ^a	1.710 ^a	0.383 ^a	0.024 ^a
Temperature X pretreatment	T70, H	1.95 ^a	1.45 ^a	1.945 ^a	0.515 ^a	0.028 ^a
	T70, NH	1.18 ^b	2.01 ^a	1.670 ^b	0.336 ^b	0.028 ^a
	T50, H	1.08 ^b	2.08 ^a	1.561 ^b	0.303 ^b	0.021 ^a
	T50, NH	1.10 ^b	2.01 ^a	1.676 ^b	0.355 ^b	0.020 ^a

For each parameter, means in the same column followed by the same letter are not significantly different by Tukey test at the 5% level

Table 4 Fatty Acid composition (%) of press extracted oil in the different experimental conditions

	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)
Setup 1					
T50_H_ω32	10.03 ± 0.06	3.00 ± 0.10	11.6 ± 0.25	74.5 ± 0.47	0.77 ± 0.06
T50_H_ω22	9.90 ± 0.10	2.80 ± 0.10	11.1 ± 0.06	75.4 ± 0.15	0.70 ± 0.04
T50_NH_ω32	9.77 ± 0.31	2.97 ± 0.12	11.3 ± 0.40	75.2 ± 0.91	0.77 ± 0.06
T50_NH_ω22	9.97 ± 0.12	2.9 ± 0.10	11.5 ± 0.36	74.8 ± 0.55	0.73 ± 0.06
Setup 2					
T70_H_ω32	9.77 ± 0.23	2.80 ± 0.10	11.0 ± 0.35	75.7 ± 0.70	0.73 ± 0.06
T70_H_ω22	9.93 ± 0.15	2.87 ± 0.25	11.3 ± 0.78	75.2 ± 1.22	0.70 ± 0.10
T70_NH_ω32	9.70 ± 0.10	2.83 ± 0.06	10.9 ± 0.20	75.9 ± 0.26	0.70 ± 0.04
T70_NH_ω22	9.93 ± 0.15	2.97 ± 0.21	11.6 ± 0.36	74.7 ± 0.72	0.77 ± 0.06

Each data represents the mean of three replicates ± standard deviation

Table 4 shows the fatty acid composition of tobacco seeds oil extracted with different setups. The results showed that the main fatty acid of tobacco oil was linoleic acid, ranging from 74.5 to 75.9% followed by oleic acid, accounting about 11.3%, and palmitic acid with content of 9.9%. Figure 1 shows a representative chromatogram of

tobacco oil. There were no significant differences in the fatty acid composition of tobacco oil in the different experimental conditions. The results were comparable to other studies (Sena-Moreno et al. 2015).

Fig. 1 Chromatogram of fatty acids profile of tobacco seed oil obtained by GC–MS. RT 26.38 = Palmitic acid methyl ester; RT 31.20 = Stearic acid methyl ester; RT 31.66 = Oleic acid methyl

ester; RT 32.85 = Linoleic acid methyl ester; RT 34.50 = Linolenic acid methyl ester

Conclusion

The results showed that utilizing a mechanical screw press and a combination of 70 °C extraction temperature, 50 °C seeds pretreatment and 22 rpm screw rotation speed with an 8 mm nozzle, an oil yield of 80% corresponding to 35.2% (g/100 g seed d.w.) was obtained from tobacco seeds. Therefore, from 1 ton of tobacco seeds it is possible to extract 0.352 tons of oil, comparable to other oil crops. No significant changes in PV and fatty acid composition of oil were observed under the extraction conditions applied. Although, the average values of FFA, K_{232} , K_{270} and ΔK slightly increased rising the extraction temperature up to 70 °C, in presence of seeds pretreatment at 50 °C, they were in the Codex Alimentarius ranges of edible oil. The optimal mechanical extraction conditions for achieving high yield TSO did not affect substantially the major oil quality indices, so these can be advantageously utilized to produce TSO for different industrial uses.

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