Mechanical oil extraction of *Nicotiana tabacum* L. seeds: analysis of main extraction parameters on oil yield

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Abstract

The aim of this study is to find the best conditions of tobacco seed oil (TSO) press extraction, combining multiple extraction factors such as screw rotational speed, seeds preheating and extraction temperature, in order to have a higher oil yield. The extracted oil, having peculiar chemical properties, can be used for several purposes, also as edible oil. TSO was obtained using a mechanical screw press that has been assembled with a head press and with speed and temperature sensors mounted on the machine. Results show that the combination of high extraction temperature, slow rotational screw speed and seeds preheating has a significant effect on the oil yield. Extracting under such conditions, oil yield is 79.47±0.12 as % (w/w), which is 25% (w/w) more than the lowest yield among investigated condition.

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Introduction

Tobacco (*Nicotiana tabacum L.*) is a species of the genus *Nicotiana*, belonging to the Solanaceae family, cultivated throughout the world for its leaves, mainly for smoking purpose. Tobacco is also considered as an oil seed crop when untopped. At maturity, the inflorescence of the tobacco plant consist of a terminal panicles more or less expanded, which presents seed pods containing an extremely large quantity of oval-shaped seeds very small in size. The average mass of 1000 seeds is in the range of 0.08 to 0.09 g. Tobacco seeds are rich in oil and free of nicotine and can be preserved for a long time if stored in dry conditions (Rapp *et al*., 1946; Abdoh and Pirelhai, 1964).

Tobacco seed oil (TSO) content varies between 30 and 43% on weight basis, it is comparable to mustard, sunflower and safflower oil and is classified as semi-drying oil, which partially hardens when it is exposed to air (Paris, 1920; Salisbury, 1936; Brozzetti, 1948a; Chi and Tso, 1968; Giannelos *et al*., 2002; Modestia *et al*., 2013).

The main fatty acids (FA) in TSO are linoleic acid (65 to 75%), oleic acid (10 to 16%), palmitic acid (8 to 11%) and stearic acid (2 to 3%) (Riemenschneider *et al*., 1945; Crawford *et al*., 1950; Lotti *et al*., 1971; Sengupta and Mazumder, 1976; Frega *et al*., 1991; Giannelos *et al*., 2002; Abbas *et al*., 2008; Bucciarelli *et al*., 2012, 2013; del Piano *et al*., 2014a, 2014b).

In the first half of the last century, Italy and some Eastern European countries used the tobacco seeds, a by-product of tobacco leaf production, for the extraction of oil as raw material in the manufacturing of soap, paints, lubricants, fuel, or after refining, even as edible oil (Balbi, 1959).

The refined oil was subjected to rat feeding studies (Thakur *et al*., 1998) in comparison with groundnut oil and vanaspati ghee, a hydrogenated vegetable fat commonly used in India as a substitute for butter. Results showed that TSO had not caused any adverse effect on the growth and physiology of rats. The total lipid content of serum, heart, liver, brain and kidney were comparable with groundnut oil. In case of total phospholipid content of different organs, TSO was on par with groundnut oil and histopathological studies of the major organs had not showed any toxic effect.

In the last few years, due to the need to find renewable energy sources and reduce the environmental impact, the feasibility of using tobacco as a no-food crop source of vegetable oil in different industrial sectors is being explored (Giannelos *et al*., 2002; Bucciarelli *et al*., 2012, 2013). In the field of the oil industry TSO can be used as bio-fuel, after conversion into methyl ester. In fact the product of methyl esterification of this oil can be added to diesel fuel up to 25% without any engine modifications and without causing significant changes in its performance (Giannelos *et al*., 2002; Usta, 2005a, 2005b). Furthermore, recent studies have shown that the oil from the seeds of tobacco may be used for the production of high quality biodiesel (Usta *et al*., 2011; Srinivas *et al*., 2013). In the field of industrial coatings, TSO is an excellent raw material for the production of alkyd resins that do not turn yellow (Mukhtar *et al*., 2007; Ogunniyi *et al*., 2007; Patel *et al*., 2008), also contributing to reduce the environmental impact of this sector with the use of less polluting technologies, which use renewable raw materials such as vegetable oils.

TSO is comparable to other edible oils and it is free of nicotine. The low proportion of saturated FA is a positive factor to consider TSO as nutritionally appropriate, compared to vegetable oils with a high concentration of saturated fat. Moreover, the tobacco seeds (TS) by-product in oil extraction (TS cake) has a high concentration of nitrogen as amino acids (15.6%) making it usable at up to about 25% of the ratio into concentrate mixtures for dairy cattle feed (Stanisavljevic *et al*., 2009). The possible use of seed cake and stalks should also be taken into account, in the economic evolution of production from tobacco. Seed cake, rich in protein and fiber, but devoid of alkaloids, might be utilised for animal feed (Brozzetti, 1948a, 1948b; Maestri *et al*., 1993; Abbas *et al*., 2008) and stalks as biomass or for paper production (Shakhes *et al*., 2011).

According to all these characteristics, TSO could be a hope for a healthy use of tobacco and its agriculture. TSO can be extracted with pressing or with solvent extraction. Soxhlet extraction, using n-hexane or diethyl ether, is the most common method to extract TSO under laboratory conditions. Maceration has also been used, with petroleum ether and n-hexane. Recently, indirect ultrasonic extraction has been used for oil extraction from tobacco seed. Among all the extraction techniques, seed pressing seems to be more acceptable than the other methods for economic, environmental, health and safety reasons. Using this technique, Stanisavljevic *et al*. (2009) achieved an oil yield of 32.9/100 g. The aim of this study is to investigate and find the best combination of extraction factors, such as extraction temperature, seeds preheating and screw speed rotation, in order to have a higher oil yield.

Materials and methods

Tobacco seed material

In this study, dried tobacco seeds (CAT-V1 genotype) were procured from a prior PON-Enerbiochem experimental trial. The place of origin was characterised by annual precipitation of 351.2 mm and a temperature range of 16÷30°C recorded by a weather station placed on the field. The plantation was transplanted on May 10th 2013 and its yield was calculated to be around 1.51 t/ha. Seeds were harvested manually during August-October 2013 and were cleaned with a pneumatic separator to remove impurities (Galloway, 1976; Karaj and Muller, 2011) and then stored in jerry cans in a warehouse at temperatures between 15 and 25°C. The moisture content of seeds was 8% (w/w) and it was determined by the standard hot-air oven (BD115, BINDER GmbH, Tuttlingen, Germany) method at 105±1°C for 24 h to reach a final constant weight (Garnayak *et al*., 2008; Pradhan *et al*., 2011). Moisture content of seeds was not altered during oil extraction. The oil content from untreated samples was about 43.77±0.58% (w/w) and it was determined using solvent extraction with ASE® 200 - Accelerated Solvent Extraction (Dionex Corp., Sunnyvale, CA, USA).

Oil extraction by mechanical press

The literature reveals that mechanical screw press oil extraction is widely used in commercial and industrial seed oil extraction, thanks to safety and simplicity of the whole process. (Cappella *et al*., 1997; Singh and Bargale, 2000; Uquiche *et al*., 2008; Stanisavljevic *et al*., 2009; Bordoni *et al*., 2010).

In this study, tobacco oil was obtained with a mechanical screw press assembled with a head press provided by Bracco [Bracco s.r.l., Bagnatica (BG), Italy] powered by a 2.2 kW electric motor. Different sensors were installed for measuring temperatures and rotational speed of the screw and oil recovery (Karaj and Muller, 2011) (Figure 1). The temperature sensors were installed in two different positions (T1-T2). Compression zone temperature (T1), where the highest temperature was expected during the operation, was measured by a resistance temperature detectors (RTDs), *e.g*., Pt100. Temperature of extracted oil (T2) was measured by RTDs Pt100 just after passing out of the press cylinder holes. Screw speed was adjusted by a variable speed regulator (mechanical control) and it was measured with a rev counter.

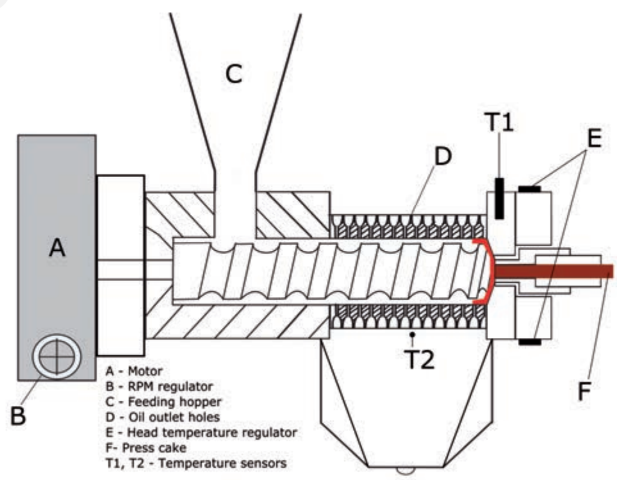

Figure 1. Mechanical screw press for tobacco oil extraction and installed sensors used in this study.

Table 1. Dependent variables for optimisation of tobacco oil extraction.

Mechanical variables	Unit	Symbol	Method
Oil yield	% w/w	O_y	Balance
Oil residual in press cake	% w/w	O_c	Solvent extraction
Temperature of extracted oil	°C	T_o	Pt100 RTDs
Time	mm:ss	t	Computer time
Throughput	kg/h	TP	Weight (kg) and pressing time (t) ratio

Independent and dependent variables

In this study, three parameters were considered in order to optimise oil extraction of tobacco using the mechanical screw press. These parameters are: i) two different initial temperatures of extraction: 50°C (T50) and 70°C (T70) of head press (Tambunan *et al*., 2012); ii) thermal pre-treatment: yes (H) or not (NH). The seeds were dried for 1 h at 50°C and processed (Galloway, 1976; Singh *et al*., 2000); iii) two different rotational speeds of the screw: 22 *rpm* (ω22) and 32 *rpm* (ω32) (Karaj and Muller, 2011).

According to Karaj and Muller (2011), seeds oil yield increases using smaller nozzle restriction size. Preliminary test has been done in order to choose the smaller nozzle size suitable for this study. The mechanical press used in this study has been provided with 4 nozzles, each one with a different restriction size (6; 8; 10; 12 mm). Five preliminary tests have been carried out using 6 and 8 mm nozzles. All 5 preliminary tests carried out with 6 mm nozzle failed, causing safety bolts breakage. All 5 preliminary tests, instead, carried out using 8 mm nozzle restriction size have been successful. According to preliminary tests, a nozzle with restriction size of 8 mm has been used for oil extraction in this study. The dependent variables such as oil yield (% w/w), oil residuals in press cake (% w/w), temperatures of extracted oil and the throughput (kg/h) were shown in Table 1. The sensors were connected to the National Instruments (Austin, TX, USA) data-logger (cDAQ-9171) and data were transferred *via* LabVIEW 2014 data logger software (National Instruments) to a laboratory computer.

Other studies show the influence of extracted seed oil temperature on oil quality (acid value, phosphorous, calcium, magnesium, oxidation stability and water content) (Canvin, 1965; Karaj and Muller, 2011; Tambunan *et al*., 2012). In this study, considering that extracted oil temperature affects oil quality, it has been taken into account as dependent variable in order to evaluate the potential influence of analysed factors among dependent variable.

Mechanical experimental procedure

Two different experimental Setups were carried out with combinations of three different parameters in order to determine the effect of independent variables on dependent variables (Table 2). In Setup 1, the temperature of the press was kept constant at 50°C, whereas the pre-treatment was varied in combination with speed of screw. The constant temperature for the Setup 2 was 70°C and the same combinations between pre-treatment and speed of screw of Setup 1 were applied. For each sample, 1000 g (Stanisavljevic *et al*., 2009) of seeds were used and six replications were done (Unquiche *et al*., 2008). Before each extraction there was a preparatory phase during which the press was cleaned, the temperature of the press head has been set with an electrical resistance (Ferchau, 2000; Beerens, 2007; Karaj and Muller, 2011) and the speed of screw was regulated. Lightproof glass containers were used to collect oil. Fine particles in the expressed oil were separated by filtration. This filtered crude oil was subsequently centrifuged in an ALC centrifuge (ALC T535 PK130R 4500 rpm) at 3500 rpm for 20 min to remove components that settled during storage (Unquiche *et al*., 2008). The press cake was collected in vacuum packages. Oil yield was calculated as percentage of extracted oil on the total oil extractable basis. According to Stanisavljevic *et al*. (2008) the extractable oil basis has been calculated as sum of extracted oil and oil content in press cake. The following Eq. 1 has been used in this study (Beerens, 2007; Unquiche *et al*., 2008; Karaj and Muller, 2011).

$$O_y = [O_e / O_e + O_c] * 100 \qquad (1)$$

where:
O_y is the oil extraction yield in % (w/w);
O_e is the extracted oil in g; and
O_c is the oil content in press cake in g as described in the *Determination of oil content in press cake* section.
In this study, the throughput has been calculated as ratio between the seed sample weight (kg) and the time (h) required for pressing the sample.

Chemical experimental procedure

Determination of oil content in press cake

The crude oil and press cake were weighed after each experiment and taken to the laboratory for additional analysis. The percentage of oil content in press cake was determined with ASE® 200 (Dionex Corp.), in the following conditions using hexane as a solvent: i) 4 g of tobacco press cake were ground in a mixer mill MM 400 (Retsch GmbH, Haan, Germany) for 2 min at 25 Hz, in presence of diatomaceous earth (ASE Prep DE; Dionex Corp.), as a dispersant and drying agent, in a 1:1 w/w ratio (Modestia *et al*., 2013); ii) oven temperature set at 105°C; iii) pressure about 1500 psi, static time 10 min, rinse volume 100%, purge time 90 s and 3 static cycles. After the extraction, the vials (previously weighted) containing the extract were placed in a TurboVap II (Zymark Corp., Horsham, PA, USA) to evaporate the solvent using dry nitrogen.

Table 2. Experimental setup: 8 mm nozzle size; T50 and T70 temperatures of extraction (°C); H and NH thermal pre-treatment (yes or not); ω32 and ω22 rotational speeds of screw (rpm).

Setup 1	Setup 2
T50_H_ω32	T70_H_ω32
T50_H_ω22	T70_H_ω22
T50_NH_ω32	T70_NH_ω32
T50_NH_ω22	T70_NH_ω22

Table 3. Results of dependent variables.

		O_y [% (w/w)]	O_c [% (w/w)]	TP [kg/h]	T_o [°C]
Setup 1	T50_H_ω32	69.78±0.84	16.75±0.21	18.40±0.47	50.3±0.58
	T50_H_ω22	76.85±0.27	13.64±0.42	13.2±0.10	53.5±1.77
	T50_NH_ω32	61.34±1.56	20.37±1.77	18.2±0.55	41.0±1.73
	T50_NH_ω22	75.79±1.08	14.19±0.27	12.6±0.65	46.9±1.50
Setup 2	T70_H_ω32	74.19±0.37	15.67±0.31	18.3±0.70	55.3±1.15
	T70_H_ω22	79.47±0.12	13.31±0.26	13.4±0.25	53.7±0.58
	T70_NH_ω32	72.21±1.22	16.83±0.18	18.3±0.79	51.3±1.15
	T70_NH_ω22	78.09±0.83	13.72±0.09	12.3±0.33	49.0±1.73

O_y, oil yield; O_c, oil residue; TP, throughput; T_o, extracted oil temperature.

The oil yield was measured by weighting and expressed as percentage on dry weight basis. Seed moisture content was determined after drying seed samples for 3 h at 105°C. The procedure accuracy was evaluated by determining oil yield of the Institute for Reference Materials and Measurements (IRMM) Certified Reference Material (CRM) BCR-447 (medium oil content rapeseed: https://ec.europa.eu/jrc/sites/jrcsh/files/rm_catalogue_0.pdf).

Statistical analysis

Data were analysed using Analysis of Variance with temperatures, thermal pre-treatment and rotational speeds of the screw as factors. Statistical significance of the differences observed among mean values was assessed using Tukey's multiple comparisons of means. A probability of P≤0.05 was considered significant. The data has been elaborated with Ordinary Least Squares regression following the model:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 \quad (2)$$

The y represents the dependent variable that is the phenomenon explained, the x represents all the factors used to explain the phenomenon, and the β represents the magnitude associated to each independent variable. According to Anania *et al*. (1995) data has been normalised in order to compare the results. The R Studio statistical software (version 3.1.2) was used to analyse the collected data.

Results and discussion

Oil yield from tobacco seeds samples at different processing conditions is shown in Table 3. The highest oil recovery of 79.47±0.12 %(w/w) was achieved by using Setup 2 (T70_H_ω22) and the lowest oil recovery efficiency of about 61.34±1.56 %(w/w) by using Setup 1 (T50_NH_ω32). Oil residue in press cake was higher in Setup 1 (T50_NH_ω32) and it was lower in Setup 2 (T70_H_ω22). Table 3 shows the mechanical press throughput of Setup 1 and Setup 2. The average throughput of 18.3±0.55 (kg/h) has been calculated for high rotational screw speed and an average throughput of 12.9±0.56 (kg/h) has been calculated for slow rotational screw speed. Extracted oil temperature is between 41.0±1.73 and 55.3±1.15°C. According to Karaj and Muller (2011) and Tambunan *et al* (2012), higher oil yield has been obtained with seed preheating, high press temperature and slow rotational screw speed. The results of Tukey's multiple comparison of means [Tukey's honest significant difference (HSD)] analysis on the experimental data is shown in Figure 2. The results show that the extraction temperature, the preheating and the speed rotation of screw respectively gave significant effect to the oil yield. In Setup 1 is possible to notice a significant difference (P<0.05) between preheated samples and not preheated samples only using a high rotational screw speed. Moreover, the rotational screw speed factor determines significant differences (P<0.05) between slow and fast samples both in preheated

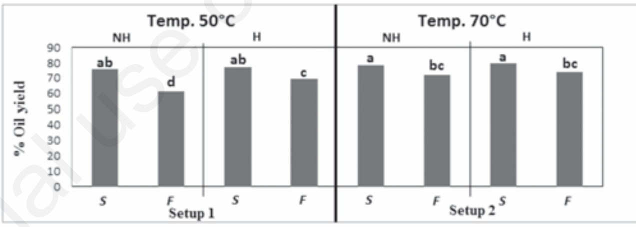

Figure 2. Results of Tukey's honest significant difference analysis on oil yield.

Table 4. Ordinary least squares regression results.

	Estimated value	t value	Pr(>|t|)	P
(Intercept)	72.8608	87.241	<2e-16	***
Temperature of extraction (β_1)	4.9696	5.95	3.99e-07	***
Pretreatment (β_2)	1.5138	1.813	0.044	*
Rotational speed of screw (β_3)	–8.1788	-9.793	1.27e-12	***

***P<0.001; *P<0.05.

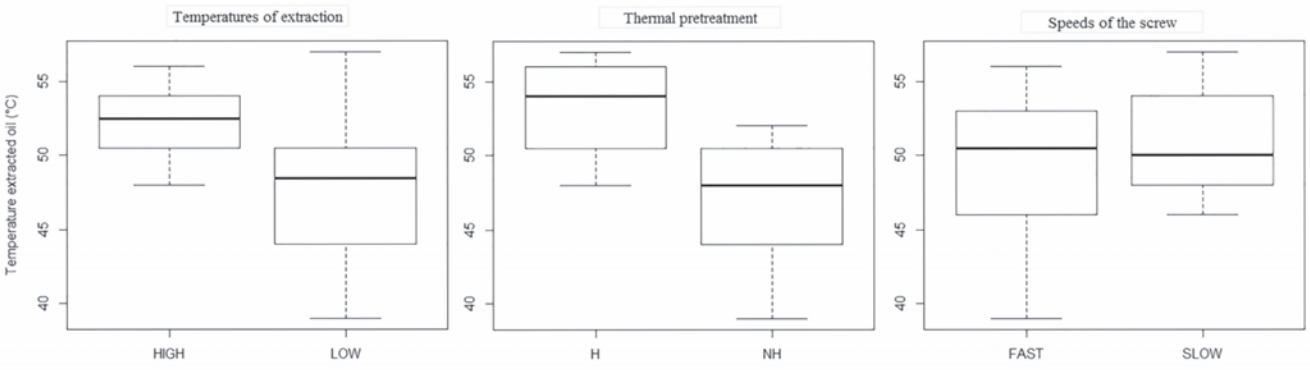

Figure 3. Statistical analyses for extracted oil temperatures; error bars indicate min and max values of each factor. Boxes depict median, mean values and interquartile range from 25th to 75th percentiles.

and not preheated seeds conditions. In Setup 2, as in Setup 1, the rotational screw speed has a significant influence ($P<0.05$) on oil yield both in preheated seeds and not preheated seeds conditions. Comparing Setup 1 and Setup 2 in not preheated seeds conditions and only using a high rotational screw speed, it is possible to notice a significant difference ($P<0.05$) in oil yield. The results show that the rotational screw speed determines a significant difference in oil yield ($P<0.05$) among slow and fast samples in both Setups. All the combinations in this study, setting the slow rotational screw speed, show no significant differences in oil yield.

Linear regression standardised coefficients are reported in Table 4.

Coefficient β_1, associated to extraction temperature predictor, is significant and positive. Accordingly, with a temperature rise there is an increase of oil yield. Coefficient β_2 associated to pre-treatment predictor, is significant. Coefficient β_3, associated to rotational screw speed predictor, is significant and negative. This leads to an increasing of oil yield decreasing the rotational screw speed.

The coefficient of determination (multiple R-squared) evaluates the effectiveness of the model that is the proportion of variability of y explained by the explanatory variables considered. In this study, R-squared is equal to 0.75, meaning that 75% of data is explained by the explanatory variables.

The results of Tukey's HSD test on the extracted oil temperature are shown in Figure 3. The results show that the rotational speed of screw has not significant effect on the extracted oil temperature, while the extraction temperature and the preheating respectively have significant effect on extracted oil temperature.

Conclusions

According to the results, the oil yield is higher with a high extraction temperature, seed preheating and setting the lowest screw rotational speed. In this study, results show that screw rotational speed has a higher influence on oil yield than extraction temperature, as demonstrated by regression values β_3 β_2 and β_1. The coefficient β_3 of screw rotational speed variable is higher in absolute value than coefficients β_1 and β_2 respectively of temperature and preheating variables. Extracted oil temperature is not affected by screw rotational speed, whereby changing this factor does not influence oil quality.

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