

Blotting Method to Determine Oil Content in Mustard Seeds: A Solution to Asymmetries in the Mustard Seed Economy

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Abstract: Determining oil content in mustard seeds is a time consuming and expensive process, often inaccessible to the common farmer. The proposed oil blot methodology has the potential to be used in low income, remote settings as a first estimate of the oil content in the mustard seed crop. Oil blots were formed by pressing oil out of the seed onto an adsorptive surface using a repeatable force from a vise grip. The area of the blots was an indicator of oil content. While the oil blot methodology would not replace industry standard methods such as Soxhlet extraction, it serves as an accessible, non-specialized method to provide a first-estimate result. This was proved using a Pearson Correlation test ($r = 0.95$) and t-statistic comparison. The positive significance values show a strong, positive linear correlation between oil content and blot areas. It was determined that the ideal tool for the oil blot methodology was a vise grip due to its repeatability, portability, and low bulk cost. The total cost of the kit was \$0.90 with low recurring costs. The extreme simplicity, low capital, and operating cost, make the oil blotting method a valuable tool for small mustard seed producers for estimating the fair value of their crop, reducing their potential exploitation by middlemen.

Keywords: Mustard seed; Oil blot; Oil content; Soxhlet Extraction.

1. Introduction

Mustard is a plant that is widely cultivated by farmers, due to its mobility across agroclimatic conditions and its high yield. Mustard seeds have thus become a valuable source of many bioactive ingredients, such as polyunsaturated fatty acids and antioxidants (carotenoids, phenolic compounds, and tocopherols). The increasing popularity of natural ingredients in food creation and other industries has made mustard a material for creating new products [1].

Globally, many smallholder farmers sell their products through traders, or middlemen, who aggregate farm products at local levels to sell to wholesale markets. It has long been recognized that this dynamic leaves smallholders vulnerable to exploitation [2-4]. Rana et al. (2022), in an article summarizing a national meeting of Indian agriculture experts, identify the practice of selling to intermediaries as the root of agricultural marketing problems for smallholders [5].

One contributor to small-holder exploitation is information asymmetry between farmers and middlemen. Farmers often have limited or no information about wholesale and/or export market prices, enabling middlemen to buy and sell agricultural products at large profit margins [6]. Empirical and theoretical work has shown that increasing farmer access to information can reduce market distortions, increase prices paid to farmers, and improve farmer welfare [7-9]. Mitchell (2017) showed, using an experiment with farmers and middlemen in Gujarat, that providing farmers with price information would cause many middlemen to offer higher prices [10]. On the other hand, Mitra et al. (2018) predicted that increasing farmer access to information about wholesale potato prices in West Bengal, India, would have negligible impacts on farmer welfare unless farmers were provided with direct access to wholesale markets [11]. Overall, the literature supports the notion that reducing information asymmetries between farmers and middlemen can be part of a strategy for reducing smallholder exploitation.

This project was initiated by a research visit to Rajasthan, India, which accounts for approximately 40% of India's rapeseed production [12]. Many farmers expressed frustration that middlemen and wholesale buyers would cheat them of fair prices. In India, mustard seeds are graded and priced based on their oil content [13], but farmers generally have no way to test their seeds prior to taking them to market or selling to middlemen. Oilseed analysis equipment is expensive and requires specialized training to operate and calibrate [14-15]. This creates an

information and power asymmetry where the middleman or market buyer not only has better knowledge about wholesale prices, but also owns and operates the equipment used to test the product quality.

It was hypothesized that a simple, very low-cost device that could estimate the oil content of mustard seeds could help reduce the power imbalance between farmers and buyers. A farmer could analyse their seeds at home and calculate a fair price for their crop based on current wholesale market prices. The farmer could use this information to their advantage in bargaining with middlemen. They could also use the information to decide whether to incur the transportation expenses associated with selling directly to a wholesaler.

The oil blot methodology developed by the Food Engineering Research Group, University of Toronto, is a novel method based on the area of oil blots to determine the oil content in seeds.

2. Materials & Methods

Samples of mustard seed were procured from Bangladesh, India, Thailand, and Canada. The seed types obtained were black and yellow mustard seeds. Table 1 highlights the seed source location and the seed type. The samples were labelled as shown in the table. S3* seed samples were identical in source and quality as S3, however, S3* samples were not stored in airtight containers.

Table 1. Sources of Mustard Seed tested in this Study.

Sample	Region	Seed Type
S1	Canada	Yellow
S2	India	Black
S3	India	Black
S3*	India	Black
S4	Faridpur, Bangladesh	Black
S5	Sirazgong, Bangladesh	Black
S6	Mymensingh, Bangladesh	Black
S7	Sayeedpur, Bangladesh	Black
S8	Chennai, India	Black
S9	Thailand	Yellow

2.1 Industry Standard Method- Soxhlet Extraction

The oil content of the seed samples was determined by Soxhlet Extraction [16]. Briefly, high temperature hexane was used as the solvent in the extraction of oil from mustard seed samples. The process was run for 8 hours until the hexane ran clear. The recovered solvent samples were then transferred to a rotary evaporator to remove the hexane from the oil. The oil content in the sample was then determined gravimetrically. All reported values were averages of triplicate analyses.

2.2 Blotting Method

When seeds are crushed on an adsorptive surface, it was hypothesized that the area of the oil blot produced is proportional to the seed oil content. To determine the blot area for each seed sample a novel method was developed to crush the seeds. Briefly, the method would crush the seed with repeatable force onto an adsorptive surface. The blot size determined after a certain time would correlate to the oil content determined from Soxhlet extraction.

2.2.1 Selecting Hand Tools for Crushing Seeds

The criteria for the method were to use inexpensive commonly available hand tools that could effectively crush the seeds with a repeatable force. Many potential hand tools were explored including screw style c-clamps, adjustable wrenches, and calibrated weights. Through testing it was found that the most effective tools were bench vise, tablet press and vise grip. The vise grip used in the experiment was a wood clamp style tool that has flat surfaces at the clamping point. To ensure repeatability, the set screw in the vise grip was kept at a fixed depth to ensure that the tension in the grips was constant.

2.2.2 Use of Graph Paper for Blotting Area Measurements

One or five whole mustard seeds were placed in a folded piece of standard graph paper and placed in the one of three tools listed above. The graph paper was cut into 4 cm² pieces. The seeds were crushed, and the blots were measured using the grid lines on the graph paper. The blot areas were measured as soon as pressure was applied to the seeds (t=0 min) and again after 10 minutes (t=10 min).

For the single seed experiments, the blots were circular, and the lines were used to estimate the diameter of the blot. For the 5 seed experiments the blots were approximately rectangular in shape and the lines on the graph paper were used to determine the length and width of the blot.

3. Results and Discussion

It was hypothesized that oil content in mustard seeds can be approximated from the size of the blot area obtained from crushing the mustard seeds onto an adsorptive surface.

3.1 Soxhlet Extraction Results

The oil content of the samples is presented in Figure 1. below. The oil content results in Figure 1 are the average oil content obtained from triplicate analyses. The error bars indicate the sample standard deviation. These ordered results were used as the basis of the correlation between oil content and the blot sizes.

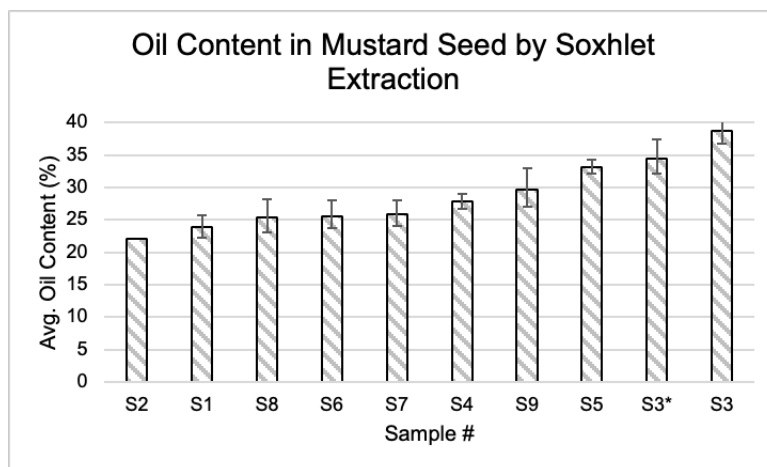


Figure 1. Oil Content of Mustard Seeds measured through Soxhlet Extraction

3.2 Trend Analysis

The blot area results were tested to determine if the blots could be used as a first estimate of the oil content. The oil content and blot area were plotted on independent y-axes to determine if the estimation using oil blot method was valid. Figures 2 and 3 show the trends in the one seed and five seed blot areas overlaid on the Soxhlet oil content. The blot areas were measured at two time intervals –immediately after crushing (t=0) and after ten minutes had elapsed (t=10). Ten replicates of the blot area were measured. The error bars indicate the sample standard deviations.

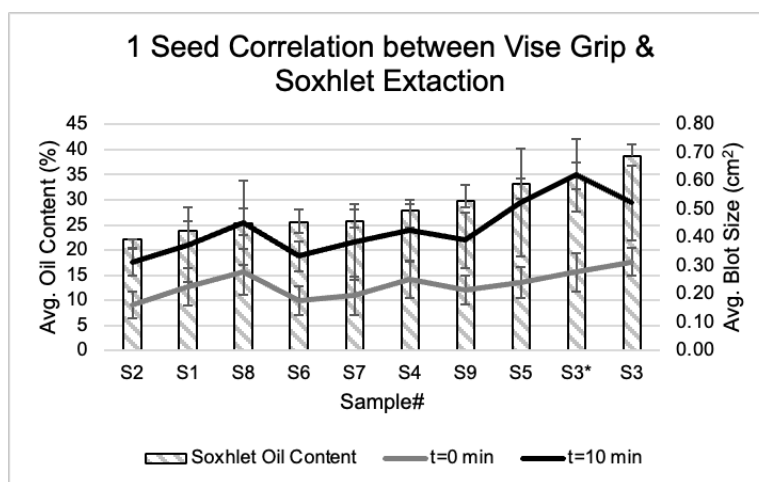


Figure 2. Trend comparison of oil content and blot areas for one seed trials

From Figures 2 and 3, it is evident that the blot area increases with time. It was expected that seeds with higher oil content should have larger areas. The expected trend was observed more clearly with the five seed data (Fig.2). The five seed blot areas measured after 10 minutes most closely followed the trend from the Soxhlet oil extraction results.

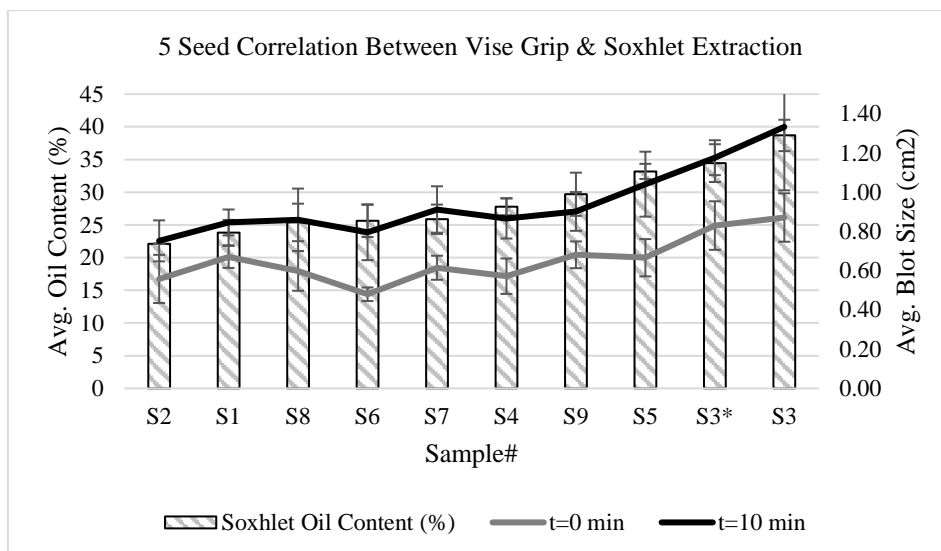


Figure 3. Trend comparison of oil content and blot areas for five seed trials

3.3 Correlation between Oil Blots and Blot Areas

To investigate the relationship between the blot areas and the oil content a Pearson Correlation analysis was performed. The Pearson correlation was calculated using the =CORREL function in Microsoft Excel using the data presented in Figures 2 and 3. It was expected that the results would show strong linear correlation.

A statistical significance was conducted using t-statistics. A calculated t-statistic was compared to critical t-value. The t-statistic was calculated based on ten trials performed on one or five randomly selected seeds. Therefore, the number of pairs (n) associated with the experiment was 10 for the one seed trial and 50 for the five seed trials. The degrees of freedom were 8 for the one seed trials and 48 for the 5 seed trials. The critical t-value was calculated using Microsoft Excel’s =TINV function. The confidence level chosen was 95% i.e. $\alpha=0.05$. The t-statistic was determined using the following equation.

$$t = \frac{r \sqrt{n-2}}{\sqrt{1-r^2}} \tag{1}$$

where r is the Pearson correlation coefficient and n is the number of ordered pairs. Table 2 shows the Pearson correlation coefficient and significance at 95% and 99% confidence levels.

Table 2. Pearson Correlation Values for Oil Content and Blot Areas

Trial	Pearson Correlation	Significance, 95%	Significance, 99%
1 Seed, t= 0	0.74	2.34	1.28
1 Seed, t= 10	0.83	5.23	4.18
5 Seed, t= 0	0.83	16.17	15.50
5 Seed, t= 10	0.95	72.37	71.70

The results in Table 2 show an increasing linear correlation between the oil content and blot area. Specifically, increasing the number of seeds and the time elapsed when the blot area is measured improves the correlation. It also validates that the ten-minute wait time is appropriate, as shown with the highest value of 0.95 for the 5 seed trial.

Additionally, significance values are greater than 1, indicating that there is a statistically significant linear relationship between the oil content and blot area. The positive significance value indicates that as one variable is increased, the other tends to increase as well.

3.4 Economic Analysis

Based on the results a field kit was designed. The kit consists of a set of C-clamp style vise grip, a set of tweezers and a graph paper pad. To determine if this approach would be affordable to a farmer, the cost of materials when supplied in bulk was determined. The items included in the kit and the price breakdown in U.S. dollars is shown in Table 3. The bulk cost for each item was estimated based on 2023 pricing, kit cost may vary slightly depending on order size [6].

Table 3. Bulk Cost Breakdown for Test Kit

Item	Cost in US Dollars (\$)
5" C-Clamp Vise Grip	0.50
Tweezer	0.10
Graph Paper Pad (100 Sheets)	0.20
Scissors (optional)	0.10
Total Cost	0.80
Total Cost (w/ Optional Item)	0.90

The average cost based on the above values is less than a dollar. Crucially, the recurring cost for the farmer is low and is subject to how quickly the graph paper is used. The inclusion of the \$0.10 scissor increases the number of tests that can be done per one sheet of graph paper. However, the same can be achieved by folding and tearing the paper therefore making it an optional addition to the estimated cost.

Costing less than a dollar for the equipment and less than 1¢ per test, the Oil Blot Methodology can be accessible to farmers in the global south. Unlike Soxhlet, ~\$10/test, and Near Infrared Analysis (NIRA) that have a high cost of setup and require expensive solvents, this method is a quick, inexpensive, semi-quantitative method for producers to estimate the oil content, and therefore the value of their crop.

4. Conclusions

Comparison with the standard Soxhlet extraction method demonstrated that the size of oil blots from crushed mustard seeds is well correlated with oil content. As expected, crushing five seeds, and allowing 10 minutes for the oil to seep into a graph paper gave more reproducible results.

Reproducibility of blot size required reproducible crushing force. Good results were obtained using a bench vise, a vise grip, and a tablet press; however, the initial cost of the vise grip makes it the obvious choice for the application of this methodology. Further, the high correlation and positive significance values justify the validity of this methodology.

A complete field kit, consisting of a 5" C-Clamp Vise Grip, tweezers, scissors, and graph paper for 100 tests can be procured for 90 US¢. The price per analysis for the first 100 tests is ~1US¢, with subsequent analyses costing ~2US¢. The method is applicable to oilseeds with high oil content, including mustard, canola, flax etc.

Due to its extreme simplicity, low capital and operating cost, the oil blotting method could be a valuable tool for small oilseed producers for estimating the fair value of their crop, reducing their potential exploitation by middlemen.

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6. Contributions

Naayaab Nagree was responsible for the experiments and co-wrote the manuscript. Sacha Ruzzante co-wrote the manuscript and was jointly responsible for the conceptualization of the project. Rahul Juneja conceptualized and performed preliminary testing of the concept. Dr Levente Diosady conceptualized and supervised the research and edited the manuscript. The authors have no conflicts to declare.

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