

Pulping

Rapid estimation of seasoning in logs and wood chips by enzymatic colorimetric determination of glycerides

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Abstract

A simple and fast procedure for estimating the elapsed time since tree felling (seasoning time) has been developed. The procedure is suitable for logs or wood chips. The method involves squeezing wood chips at high pressure and analyzing the resultant exudate for its glycerides content by an enzymatic colorimetric test. Fully seasoned chips yield a yellow coloration whereas fresh or partially seasoned chips yield a pink coloration the intensity of which is a function of the seasoning time. Results can be obtained in about 20 min. The procedure can distinguish between fresh and seasoned wood; it was used to monitor laboratory seasoning of several wood species.

Application: Mills could use this quick test to sort wood chips and logs for seasoning or pulping and reduce pitch deposition problems.

It is often possible for pulp manufacturers that cut and transport their own wood to determine the age (seasoning) of the logs arriving at the mill through records they keep of the felling dates. This is usually not the case, however, for mills that use wood chips from saw mills. These chips are made from sawmill residue wood that is not suitable for lumber manufacture. Batches of chips from trees with different felling dates and from many woodland sites are mixed together; moreover, a single truckload of chips may originate from more than one sawmill. This makes it impossible for mill personnel to know the average age of the chips arriving at the mill. A similar situation occurs in mills that obtain “seasoned” wood from farmers who do not always season the wood adequately.

Knowing the age of wood is important in many mills for several reasons. In sulfite and mechanical pulp manufacture, seasoning of wood is a significant means of reducing the amount of deposition of the resinous materials (pitch) from the wood during paper manufacture [1–3]. In kraft pulping of birch or aspen, seasoning is also important for pitch control [4–6]. In addition, the age of wood often can be correlated with loss of brightness and moisture, parameters that are especially important in the manufacture of mechanical pulps.

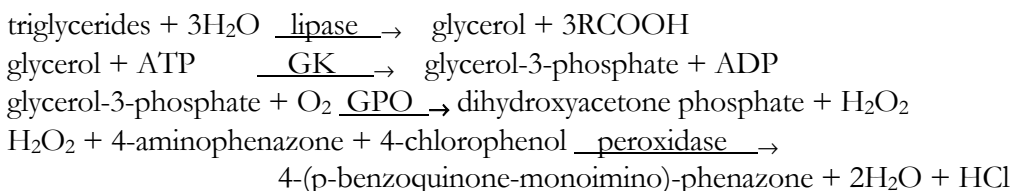
Glycerides, which are fatty acid esters of glycerol, are a significant portion of the resin in both hardwoods and softwoods. They are classified into monoglycerides, diglycerides, and triglycerides, depending on the number of fatty acids present (**Fig. 1**). Several studies have demonstrated changes in the extractives composition during wood seasoning [3,7–11]. The studies showed that glycerides decreased during seasoning (triglycerides, the predominant class of glycerides, experienced a 70% decrease over a 33-day seasoning period, in some cases [7]). This indicates that fresh wood has higher amounts of glycerides than seasoned wood. These results led us to postulate that one could estimate the age of wood through measurement of its glyceride content. Chips that have high levels of glycerides might be sent for storage, while those with low levels would be suitable for pulping. Ideally, the method should give mill personnel the result right on the spot, as soon as a truckload arrives at the mill.

At present, no technique available to mill personnel can do this. Current analytical methods for glycerides in wood extracts involve solvent extraction of the extractives from the wood chips, followed by evaporation, derivatization, and analysis of the individual glycerides by column chromatography with cation exchange resins, thin layer chromatography, high pressure liquid chromatography, or gas chromatography [10,12,13]. These procedures are time consuming and require sophisticated analytical instruments, which are not available in most mills. It may require three days to obtain results.

The method we've developed can be used to estimate the age of wood or wood chips by monitoring their total amounts of glycerides. The method is simple and easy to use, and it yields results in about 20 min.

Principle of the method

The method is a modification of the enzymatic colorimetric test for the determination of triglycerides in human serum, which is commonly used by clinical chemists in hospital laboratories [14, 15]. It involves enzymatic hydrolysis of glycerides, with subsequent enzymatic determination of the liberated glycerol by colorimetry. The test principle is as follows [16]:



(GK = glycerol kinase; GPO = glycerol phosphate oxidase; ATP = adenosine triphosphate; ADP = adenosine diphosphate)

The pink coloration observed is due to the 4-(p-benzoquinone-monoimino)-phenazone compound. The procedure for rapidly obtaining glyceride extracts from wood samples will be outlined later in the methods section.

METHODS

Reagents for enzymatic colorimetric analysis

We used the PeridochromR Triglycerides GPO-PAP test kit (Boehringer Mannheim Canada, Dorval, Quebec) for our study. The kit consists of a buffer solution and reagent test strips (**Fig. 2**).

The reagent concentrations in the buffer solution are as follows:

tris buffer	0.15 mol/L, pH 7.6
magnesium sulfate	17.5 mmol/L
EDTA, disodium salt	10 mmol/L
4-chlorophenol	3.5 mmol/L
sodium cholate	0.15%
potassium hexacyanoferrate (II)	6 μ mol/L
detergent	0.12%

The lyophilizate concentrations in solution from the reagent strips are as follows:

ATP	> 0.5 mmol/L
4-aminophenazone	0.35 mmol/L
lipase	> 3 U/mL
glycerol phosphate oxidase	> 2.5 U/mL
glycerol kinase	> 0.2 U/mL
peroxidase	> 0.15 U/mL

Preparation and stability of reagent solution

To prepare the solution, immerse one reagent strip with the lyophilizate concoction in one bottle of buffer solution and use it to stir the contents for approximately 10 s. Allow the strip to stand in the buffer solution for 5 min, stir once again for about 10 s, and then discard the reagent strip. The solution is stable for 2 weeks at 2°C to 8°C and for 2 days at 15°C to 25°C. To avoid contamination, do not touch the reagent patches or surrounding area.

STANDARDS

- Triglyceride stock standard (5000 mg/L). Dissolve 500 mg triolein (Supelco, Bellefonte, PA) in chloroform in a 100 mL glass-stoppered volumetric flask. Store tightly sealed at 4°C. This is stable for 3 months.
- Triglyceride working standard (50 mg/L). Dilute 1 mL of stock standard to 100 mL with chloroform. This is stable for 1 week at room temperature.

Although glycerides are actually a mixture of mono-, di-, and triglycerides they are conventionally calculated as amounts equivalent to triolein [17].

APPARATUS

- Spectrophotometer. Hewlett Packard diode array spectrophotometer (model HP8452A) or Bausch and Lomb Spectronic 88 with matched 10-mm cells.
- Hydraulic press. Model 75H hand-operated 75-ton DAKE Hydraulic Press with 15 cm ram (Testing Machines International, Montreal), or equivalent.
- Stainless steel adapter to hold wood chips in the press. This homemade device is illustrated in Fig. 3.

Laboratory seasoning of wood chips — initial phase

Fresh aspen and black spruce logs were obtained from a farm in eastern Ontario, chipped as reported previously [6], and stored in a room (average temperature of 26°C) in closed drums lined with plastic bags and then sampled over a 3 month period. The aspen chips were segregated into

top, middle and butt portions of the trees to enable profiling of extractives (glycerides) with tree height.

Laboratory seasoning of wood chips — second phase

The seasoning studies were repeated a year later with fresh logs of aspen, black spruce, maple, and white birch obtained from southwestern Quebec.

Testing on mill logs

Logs of known seasoning time were obtained from a mill in western Canada. The seasoning involved initial storage under water for some months followed by storage on dry land for a few more months. The logs obtained included balsam fir, jack pine, spruce, and beech. Upon arrival at the laboratory, they were chipped and their pressates collected for analysis of glyceride content by the enzymatic test. “Fresh” logs (3.5 months after felling) were also collected from the mill, shipped to our laboratory, chipped, and their glyceride contents monitored under laboratory seasoning conditions.

Fresh and 1-year seasoned aspen logs were also obtained from a mill in northern Ontario and analyzed for their glyceride content.

The moisture content of the wood chips was determined by drying representative samples overnight to constant weight at 125°C.

Extraction of wood chips for the enzymatic test

Our aim was to develop a rapid procedure for estimating glyceride content in wood chips. To achieve that, we needed to find a rapid way of obtaining glyceride extracts from the wood chips. Squeezing the juice out of the chips seemed a logical approach.

We observed that squeezing moist chips at high pressure forces liquid (pressate) from the chips. The pressate should contain water, extractives (including glycerides), and many other compounds. The method that we used was as follows:

Place a known weight of chips (~300 g) in the press adapter and apply a load of 60,000 kg (equivalent to 8000 psi on a 15 cm diameter ram) and hold for 1 min. Release the pressure, measure the volume of pressate exuded, and analyze for glycerides content.

Enzymatic colorimetric test analysis procedure

- Pipette an aliquot (50–400 μL) of sample with glycerides (pressate) into a test tube.
- Add 2.00 mL of reagent solution. For the triolein standard, evaporate the solvent with nitrogen before adding the reagent solution.
- Mix and incubate at room temperature for 10 min. The solution turns to a salmon pink color if the sample contains glycerides.
- Prepare a reagent blank sample by repeating the above-mentioned steps, replacing the pressate sample with 50 μL of distilled water. The blank normally gives a pale yellow color. For unknown samples, prepare a blank by substituting 2.0 mL of distilled water in step 2.
- Read absorbance of sample at 500 nm against a reagent blank within 60 min.
- Prepare a calibration curve using either triolein or glycerol equivalent.
- Calculate the glycerides concentration (C) by comparison with the calibration curve or use the formula (for the 50 $\mu\text{g}/\text{mL}$ standard):

$$C (\mu\text{g/mL}) = \frac{50 \times \text{Absorbance of sample}}{\text{Absorbance of standard}}$$

- Calculate the pressate glyceride concentration, C, as grams triolein/metric ton of chips using the formula:

$$C' = \frac{C \cdot \text{volume of pressate (mL)}}{\text{weight of chips used (g)}}$$

- If the glyceride concentration exceeds 10 mg/mL, dilute sample 1:5 with 0.9% NaCl solution and repeat the assay (we have never come across samples that needed dilution).

Safety measures: The buffer solution contains hazardous chemicals (4-chlorophenol and sodium azide). Therefore, one should avoid swallowing the liquid or inhaling vapors. Avoid contacting skin or mucous membranes with the chemicals. If the solution comes into skin contact, flush the skin immediately with plenty of water.

Solvent extraction

The amount of wood extractives obtained by solvent extraction can be used as a crude measure of wood seasoning, based on the premise that fresh wood has more wood resin than seasoned wood. However, this measurement process is not very sensitive to wood seasoning changes and is prone to contamination from non-resinous matter that may dissolve in the solvent used. Nevertheless, we used the solvent extractives values to compare with the results of the enzymatic test procedure. Acetone extractives contents of the chips were measured via Soxtec extraction, as previously described [18].

Analysis by gas chromatographic procedures

Acetone extracts were derivatized with diazomethane and analyzed by capillary gas chromatography [19].

RESULTS AND DISCUSSION

Enzymatic colorimetric test

With triolein as the standard, calibration curves were linear from 0 to 80 μg (**Fig. 4**). The color development was stable after 10 min. Analysis of the samples using either glass cells or disposable plastic cells in the spectrophotometer gave identical results. Thus, one can opt to use the disposable cells to avoid the inconvenience of cleaning the glass cells and to increase sample throughput. The simpler Bausch and Lomb spectrophotometer gave results that agreed with those of the more sophisticated diode array spectrophotometer, demonstrating that it is not necessary to use sophisticated instrumentation to obtain reliable results. **Figure 5**, which compares absorption spectra of a standard and a pressate sample, shows that the spectra were similar in the absorbance region of interest. Similar absorption curves were observed with other wood species.

Triolein is not easy to handle as a standard. It requires dissolution in chloroform, which has to be removed before addition of the enzymatic lyophilizates to prepare a calibration curve or a working standard. We decided to use glycerol as working standard instead because it is soluble in water. Comparison of absorbances of standards prepared from triolein and glycerol showed that for

solutions at equal concentrations, glycerol required 8 times less volume than triolein to yield the same absorbance. For example, for 60-ppm solutions, an absorbance reading of 0.26 was obtained with 25 μL of glycerol versus 200 μL of triolein. Thus, we recommend using glycerol as the standard.

Table I shows the volume of pressates collected at various pressures (1 min pressing) and the amounts of glycerides measured by the enzymatic test from seasoned aspen. Although variation in the pressure of squeezing had no effect on the volume collected, there was a small increase in the amount of glycerides measured at higher pressures. However, the time of squeezing did affect the volume of pressate collected. For example, further 5 min pressing of one sample, previously pressed for 1 min, resulted in a 10 mL increase in the pressate volume. This implied that all samples should be pressed for equal times. For our studies, we decided on pressing for 1 min at a load of 60,000 kg, which is equivalent to 55,200 kPa on a 15 cm diameter ram press. However, the amounts of glycerides measured were the same for two pressing times, which suggests that there was no preferential removal of glycerides during pressing. The color of the pressates ranged from light to dark brown. Wood chips from fresh logs gave higher pressate volumes than chips from aged logs, because of their higher moisture content. Pressing gave good reproducibility in pressate volumes from four sub-samples of a set (**Table II**).

Table I. Effects of variation of squeezing pressure on pressate volume and glycerides content of seasoned (1 year, as logs) aspen wood chips.

Squeezing pressure		Weight of chips, g	Volume of pressate, mL	Pressate glycerides content, g/metric ton chips
MPa	psi			
36.8	5.33 x 10 ⁻³	276	30	103
46.0	6.67 x 10 ⁻³	275	30	105
55.2	8.00 x 10 ⁻³	275	30	109

Table II. Reproducibility of pressate volumes and moisture contents of fresh and seasoned (1 year as logs) aspen logs from northern Ontario.

Seasoned chips			Fresh chips		
Weight of chips, g	Moisture content, % FD wood*		Weight of chips, g	Moisture content, % FD wood*	
		Volume of pressate, mL			Volume of pressate, mL
312	33	34	301	48	65
311	33	34	301	48	65
311	33	34	301	48	65
312	33	34	301	48	65

* FD = freeze-dried.

Initial laboratory seasoning studies

Aspen chips

Table III shows glyceride amounts obtained from fresh chips. It also shows acetone extractives and moisture contents of the chips before and after pressing. The results show that acetone extractives and moisture levels were higher in the top part of the tree than in the middle and butt sections (which showed similar amounts). This phenomenon has been observed before [20]. The amounts of glycerides, as determined by the enzymatic procedure, follow a similar trend. The higher amounts of

extractives in the top part of the tree may be due to the proximity of the tree portion to the tree leaves where photosynthesis takes place. After pressing, the acetone extractives and moisture contents are the same in all portions of the tree. Pressing at 55,200 kPa (8000 psi) for 1 min removes only a small portion of the total extractives in the chips. Previous, unpublished work at Paprican on deresination found that a plug screw feeder to a mechanical pulp refiner typically squeezes out about a quarter of the wood resin.

Table III. Acetone extractives, moisture contents, and total pressate glycerides in fresh aspen.¹

Tree section	Acetone extractives, % FD wood	Moisture content, % FD wood	Color of pressate	Pressate glycerides content, g/metric ton FD wood
BEFORE SQUEEZING				
Top	2.31	53.0		
Mid	1.73	47.0		
Butt	1.86	47.0		
AFTER SQUEEZING				
Top	1.76	29.3	dark brown	200
Mid	1.74	30.7	light brown	153
Butt	1.74	30.6	khaki	148

¹Average of three results.

Figure 6 shows acetone extractives and glyceride contents of fresh and seasoned wood chips as functions of seasoning time. The results show, as expected, that the glyceride amounts decreased with increasing time of seasoning. Higher volumes of pressates from seasoned samples were needed to obtain positive color changes (400 μ L instead of 50 μ L). The decrease in the glyceride amounts was confirmed by the decrease in extractives contents and by the gas chromatograms of the methylated extracts (**Fig. 7**). However, the flatness of the acetone extractives curves shows that they are less sensitive than are glyceride values in estimating wood seasoning. The gas chromatograms show that glyceride peaks (which elute in the 25–33 min retention time zone) were predominant in fresh chips and very much decreased in seasoned chips. The glycerides content decreased much more rapidly than the total acetone extractives content with seasoning time. This is because acetone extractives include compounds such as alcohols, weak acids, waxes, terpenes, etc., which do not contribute to the results of the enzymatic test. Actually, in wood seasoning, the major part of the decrease in wood extractives is due to the loss of glycerides [3,7–9,19]. These results clearly demonstrate the applicability of the enzymatic test to the estimation of wood seasoning.

Black spruce chips

The results for black spruce, shown in **Table IV**, demonstrate that the acetone extractives content and glycerides content were much lower than those of aspen. Seasoning decreased the glyceride amounts as in the case of aspen. Our results correlate well with literature reports on the seasoning of aspen and black spruce [3]. Thus, the enzymatic test procedure seemed to be applicable to both hardwoods and softwoods.

Table IV. Acetone extractives, moisture contents, and glyceride contents in fresh and seasoned black spruce.

Seasoning time, weeks	Acetone extractives, % FD chips	Moisture content, % FD chips	Color of pressate	Pressate glycerides content, g/metric ton wood
0	0.82	41.9	lime	8
6	0.62	35.8	lime	0.7
12	0.61	24.9	lime	0.7

Second set of laboratory seasoning studies

The results summarized in **Fig. 8** further confirm the applicability of the test procedure. Fresh aspen had such large amounts of glycerides that a positive reaction (pink coloration) was observed when the enzymatic mixture was applied directly to the wood chips.

Analysis of mill samples

Table V shows acetone extractives and glyceride contents of fresh and seasoned aspen logs from northern Ontario. The results show that the glyceride amounts and acetone extractives were about 42% lower in the seasoned wood than in fresh wood.

Table V. Acetone extractives and glyceride contents of fresh and seasoned (1 year, as logs) aspen wood from northern Ontario.

	Fresh chips	Seasoned chips
Acetone extractives, % FD chips	2.05	1.19
Pressate glycerides content, g/metric ton chips	176	104

Figure 9 shows glyceride contents of logs from a mill in western Canada. The results show that seasoning in log form was much slower than seasoning in chip form, and longer storage under water slowed down the seasoning process. These observations are well recognized in the literature of wood seasoning [7–9].

The results can be used to prepare calibration curves for seasoning under mill conditions. A central depository of calibration curves for individual wood species can be prepared and kept in a central laboratory like ours to facilitate access by mill personnel. However, a disadvantage with this scheme is that the seasoning data will be gathered under conditions different from those found in mill sites. Calibration curves prepared under individual mill conditions would be preferable.

Figure 10 shows laboratory seasoning of logs cut for a mill in central British Columbia. The studies terminated after 14 days because the machine shop press used for squeezing the chips broke down. Nevertheless, the data show that both acetone extractives and glyceride contents of the chips decreased with seasoning time over the 14 days.

From the calibration curves we expect good standard deviations on the steep parts of the curves and poor ones on the flat parts. However, this is not important since most of the seasoning action takes part in the first steep part of the curve.

CONCLUSIONS

A simple and fast procedure for estimating the age or seasoning time of logs or wood chips has been developed. The method is applicable to both hardwoods and softwoods with the exception of white birch. The only limitation is that the moisture content of the chips has to be 20% or greater in order

to obtain sufficient pressate for testing. A suggestion has been made that dry chips could be wetted first before pressing. We plan to explore this aspect in the near future.

Instead of investing in a spectrophotometer, a template with color variation of the calibration curve may be used. An unknown sample could then be compared with this color chart to estimate the concentration of the glycerides in the sample. Such templates are commonly found in commercial reagent kits for spot test analyses.

To summarize, the enzymatic colorimetric test can be used to differentiate between fresh and seasoned wood or wood chips. The procedure is as follows:

- Take a known weight of chips and press for 1 min at 55,200 kPa on a 15-cm diameter ram press. Representative samples of chips in truck-load can be obtained by using an auger chip sampler [21,22].
- Measure the volume of the pressate, transfer an aliquot (50 to 400 μ L) to a test tube and add 2 mL of the enzymatic test reagent. Mix well and incubate for 10 min at room temperature.
- Measure the absorbance at 500 nm and compare with the absorbance of a 50 μ g/mL solution of triolein.
- Correct for blanks and calculate the glycerides content in g/metric ton of chips.

Pressates from well-seasoned samples yield pale yellow colorations, whereas pressates from unseasoned or partially seasoned samples yield pink colorations. The intensity of the pink coloration decreases with decrease in glyceride content and reflects higher seasoning in wood or wood chips.

With the results in hand, a shipment of chips showing high glyceride amounts could be rejected or sent for seasoning whereas a shipment showing low amounts could be sent for immediate pulping.

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INSIGHTS FROM THE AUTHORS

Knowing the age of wood used for pulp is important because wood seasoning is a significant means of reducing pitch deposition problems in sulfite, mechanical, and kraft pulping of certain hardwoods. Current analytical methods for ascertaining the age of wood chips entail very long analytical procedures that can take up to 3 days to obtain results. The method reported here yields results in less than 15 min.

The most difficult aspect of this study was obtaining a sample from the chips for rapid analysis. The idea of pressing the chips to obtain a pressate for analysis is an innovative aspect of the work.

An interesting aspect of this research derived from the fact that trees have similar types of fats that we humans have. Consequently, the tests used in hospitals to measure fat content in blood could be adapted to measure fat content of trees!

Mills can use this information to rapidly monitor the age of wood chips on truckloads arriving at mill sites. With this information, the chips can then be sent either for immediate pulping (if aged enough) or to chip piles for further seasoning.

The reported methodology requires monitoring of the reaction products by a spectrophotometer. Pharmaceutical companies now have test kits that can be used to measure fats with a hand-held device. This technology can be adapted for use in our test procedure.

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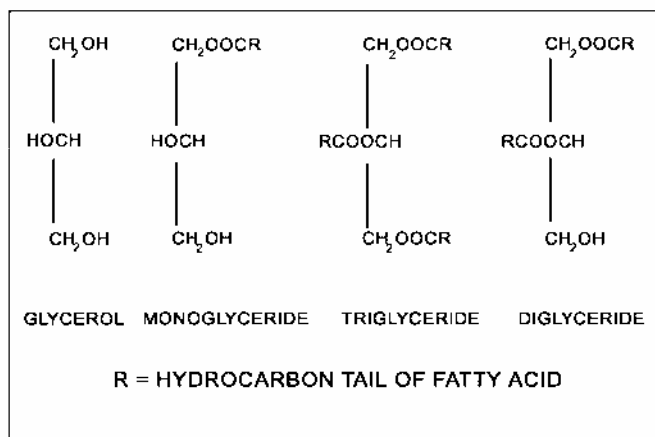


Figure 1. Chemical structures of glycerol and glycerides.

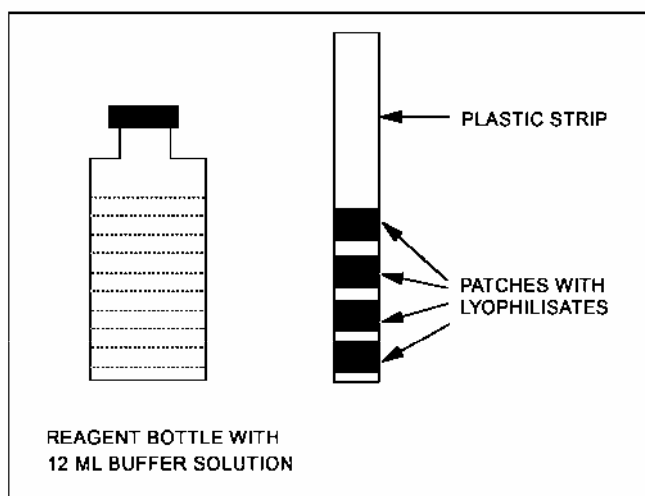


Figure 2. Schematic of the enzymatic colorimetric test kit.

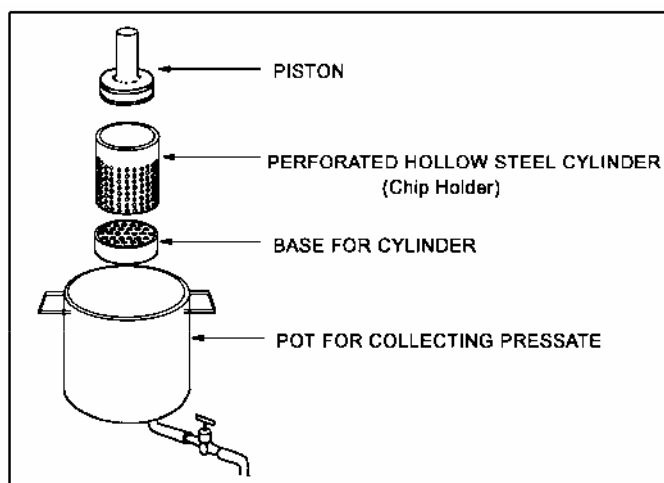


Figure 3. Schematic drawing of the chip press and collecting pan.

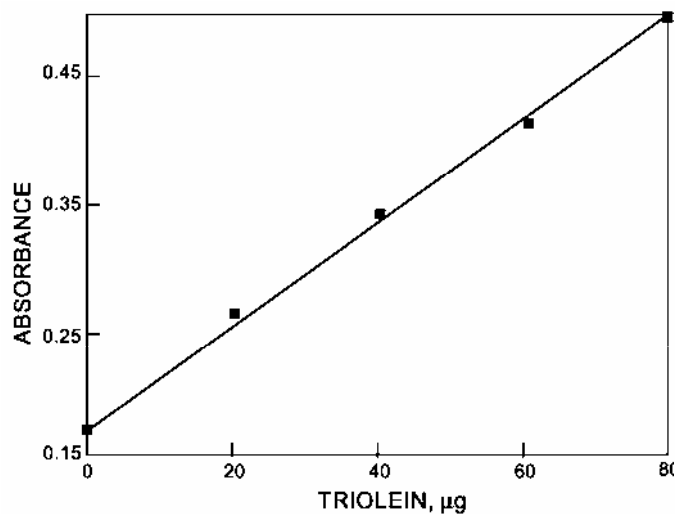


Figure 4. Calibration curve for triolein analyzed by the enzymatic colorimetric test.

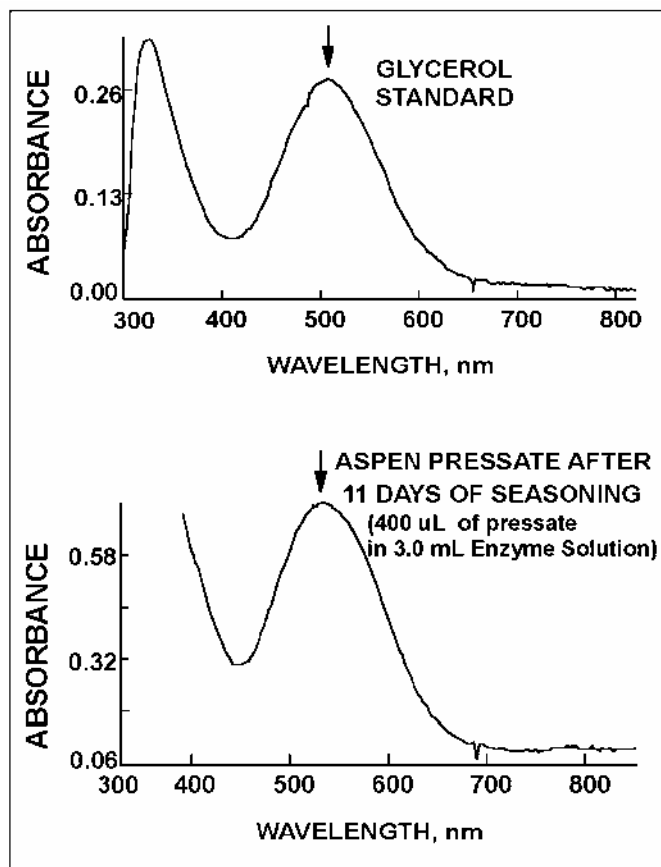


Figure 5. Comparison of absorption spectra of pink colorations of triolein and a balsam fir pressate sample after reaction with enzymatic reagents.

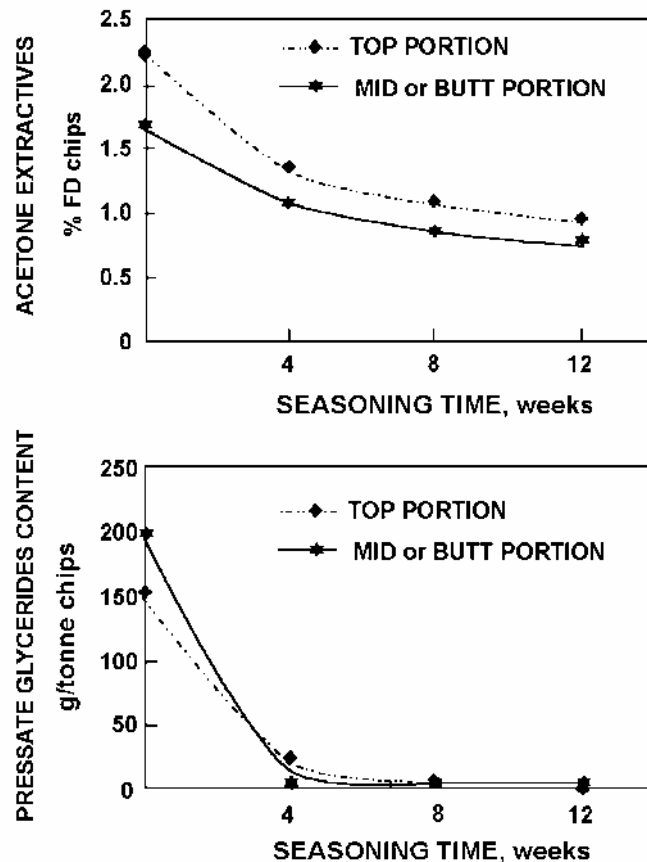


Figure 6. Effects of seasoning on the acetone extractives content and pressate glycerides contents of aspen. The extractives content were determined by Soxtec extraction while the glycerides contents were determined by the enzymatic colorimetric test.

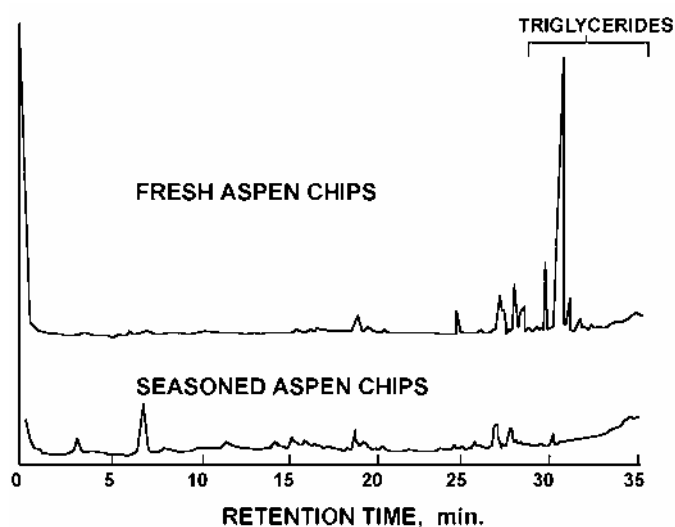


Figure 7. Gas chromatograms of methylated extracts of fresh and seasoned (4 weeks) aspen wood chips.

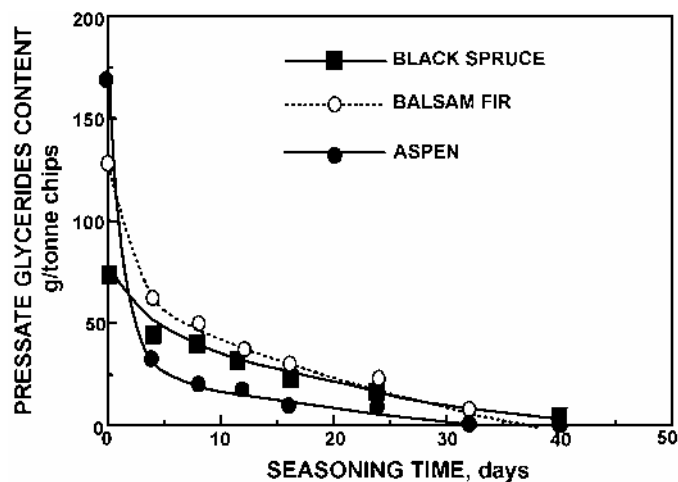


Figure 8. Results of a second set of laboratory seasoning studies on aspen, black spruce and balsam fir chips.

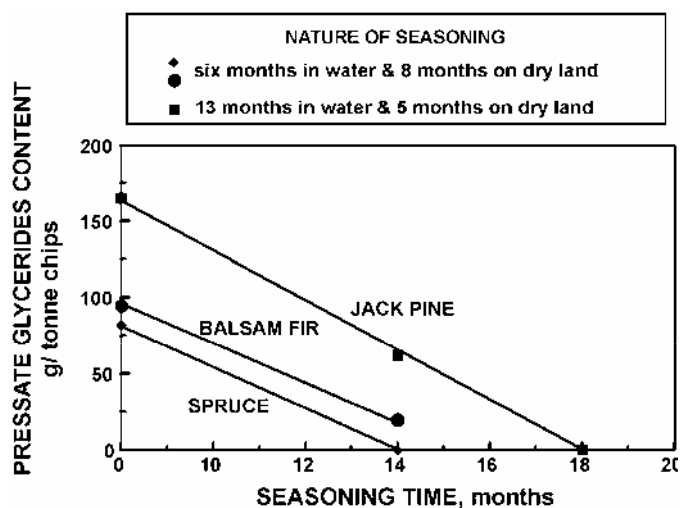


Figure 9. Pressate glycerides content of logs seasoned under mill conditions in western Canada.

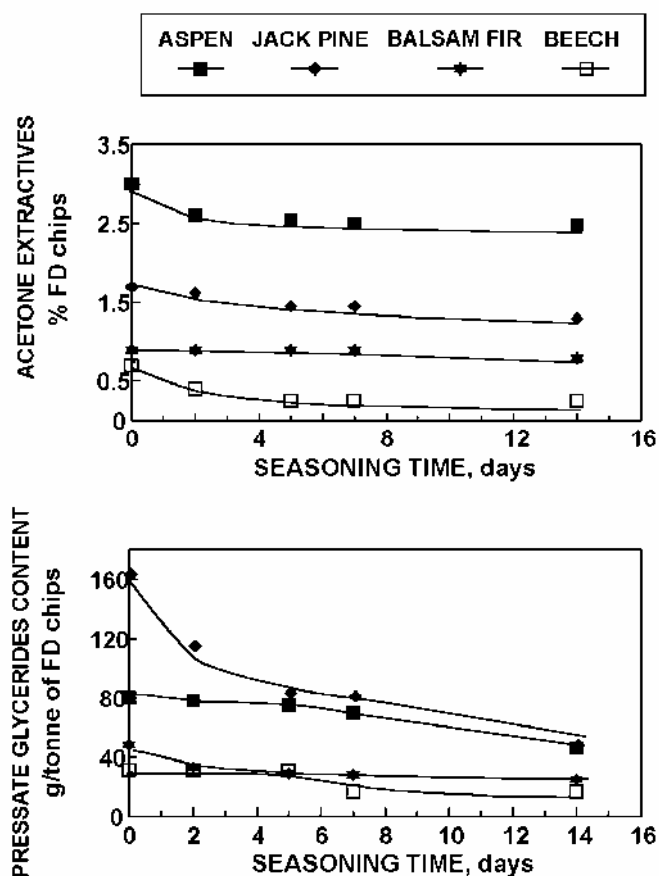


Figure 10. Laboratory seasoning of logs collected from a western Canadian mill.