

THE UTILIZATION OF HEAT COAGULATED
BEER WORT PROTEIN (TRUB).

by

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B.Sc. (Agr), University of B.C., 1975

A thesis submitted in partial fulfilment of
the requirements for the degree of

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

DEPARTMENT OF FOOD SCIENCE

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

June, 1978



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Abstract

Trub obtained from the brewing process was extracted using isopropanol as solvent to yield a bitter hop resin fraction and an insoluble protein complex. The protein was subjected to an amino acid analysis; lysine was the limiting amino acid in trub protein, followed by isoleucine. Its essential amino acid index was approximately equivalent to that of barley protein. Functionally, it was found that trub protein had poor solubility, particularly at acid pH values, but that its water-holding capacity was good.

Quantitative evaluation of the trub resin was carried out by lead conductometry, the lead conductance value of 68% being used as a guide to potential brewing value. The extracted resin was also examined qualitatively for its bittering potential by TLC. Although many resin components were separated by means of TLC, difficulties with identification yielded inconclusive results in this area. Finally, the bittering capacity of the trub resin was organoleptically compared to fresh hops in prepared beers. Trub resin was capable of bittering beer but imparted a harsher character to beer than fresh hops. Moreover, the resin tended to inhibit the formation of a stable yeast head during the ale fermentation.

Finally, the actual resin extraction procedure was empirically examined to determine the combined effect of

agitation and solvent-ratio on the yield of resin and protein. Protein yield was unaffected by these 2 factors, but resin yield was found to be most efficient when employing maximum agitation and using the minimum amount of solvent. Using more solvent did not significantly increase resin yield and tended to depress the effect of agitation.

LIST OF CONTENTS

	Page
Introduction.....	1
Literature review.....	3
Materials and methods.....	8
Continuous extraction method.....	8
Batch extraction method.....	8
Recovery of protein flour, resin and the water-dispersible fraction.....	9
Moisture determination.....	10
Measurement of the weight of resin extracted.....	10
Lead conductance value of the trub resin.....	12
Thin layer chromatography of the hop and trub resins.....	12
Protein determination.....	14
Amino acid analyses of the trub protein flour.....	14
Nitrogen solubility profile of the trub protein flour.....	15
Emulsifying capacity of the trub protein flour.....	15
Water-holding capacity of the trub protein flour....	16
Color determination of the trub protein flour.....	17
The preparation of beer for taste evaluations.....	17
Taste evaluations in order to determine the difference in bitterness values.....	18

LIST OF CONTENTS continued

	Page
Results and discussion.....	20
Part A: The extraction.....	20
Part B: Evaluation of the protein fraction.....	39
Part C: Evaluation of the trub resin fraction.....	56
Appendix.....	70
References.....	77

LIST OF TABLES

Table	Page
1. The dry weight of resin-extracted trub.....	22
2. The percent yield of crude resin extracted from wet and dry trub by the continuous reflux method.....	24
3. Percent yield of crude resin batch-extracted from wet pressed trub with isopropanol.....	26
4. The yield of dried trub protein flour after 5 extractions at 3 different solvent ratios of volume of isopropanol: weight of wet trub.....	28
5. A summary of the two-way factorial analysis of variance with replication for (i) the total yield of resin after 5 consecutive extractions, (ii) yield of resin after one extraction.....	29
6. The number of theoretical extractions required to obtain 1% and 0.1% of the initial resin level (w/w) at a constant yield of 115 g (23%) of dried protein flour, extracted from 500 g wet pressed trub.....	33
7. The debittering equations derived from the linear regression of \log_{10} resin weights (Y) vs. the number of extractions (X).....	35
8. A recipe for the preparation of muffins which contain trub protein flour.....	36
9. The color evaluation of dried trub protein flour.....	37

LIST OF TABLES continued

Table	Page
10. General yield data showing variability of the various analytical estimates.....	41
11. The protein content (6.25 x N) of various trub fractions, soy protein isolate and soy flour.....	42
12. The amino acid composition of trub protein flour (g/16g N).....	44
13. The nitrogen solubility profile of trub protein flour, freeze-dried trub, and trub protein flour in 1 M NaCl solution (g soluble protein/100g protein).....	52
14. A comparison of the emulsifying capacity of trub protein flour, soy protein flour, soy protein isolate and the water-dispersible fraction (ml oil per gram sample).....	54
15. A comparison of the water holding capacity of trub protein flour, soy flour and soy isolate (g water/ g sample).....	55
16. Summary of chemical score calculation for trub protein flour by old and new method (I & II).....	46
17. Summary of the Essential Amino Acid Index calculation for trub protein flour (method III).....	47
18. Essential amino acids of various proteins expressed as a percentage of the corresponding amino acid of standard egg protein (method I).....	49

LIST OF TABLES continued

Table	Page
19. A comparison of the amino acid composition of trub protein, barley, wheat, soybean and rapeseed (33,34)..	50
20. T.L.C. comparison of fresh hop components stored at 10°C for different time periods.....	57
21. T.L.C. comparison of trub resin components stored at 10°C for zero and 6 months.....	58
22. To show the number of ml of lead acetate required to precipitate the 4 main trub resin fractions & to show the lead conductance value calculation.....	61
23. Summary of paired comparison results to determine if differences in bitterness is detectable between:	
1. Fresh hop samples and 100% trub resin samples,	
2. Fresh hop samples and 50% trub resin samples,	
3. Fresh hop samples and pooled trub resin samples...	65
24. Summary of Chi-square analysis with Yates' correction (47) applied to determine level of significance between 1, 2 and 3 (see table 23).....	66
25. Summary of the test (Chi-square analysis with Yates' continuity correction) to determine if the extent of differences (choice of which is more bitter) was due to chance or was significant.....	67
26. Comparative bitterness levels in local (B.C.) beers...	69

LIST OF FIGURES

Figure	Page
1. Batch extraction scheme for trub.....	11
2. Standard curve for estimation of soluble resin concentration in isopropanol.....	13
3. Effect of solvent ratio on resin yield in 5 minute extractions.....	30
4. The effect of agitation time on resin yield @ 1:1 solvent ratio.....	31
5. Approximate yields in weight % of the various fractions extracted from wet pressed trub.....	40
6. Nitrogen solubility profile of trub protein flour before extraction, after extraction and in 1 M NaCl....	53
7. Lead conductance plot for trub resin extract.....	62

ACKNOWLEDGEMENTS

I extend my deepest gratitude to Dr. P. Townsley for his guidance in the direction of the thesis and for his constant encouragement throughout the study. Furthermore, I thank Molson Brewery B. C. Ltd. for their cooperation and funding which made this study possible.

INTRODUCTION

In the fiscal year ending 31st March 1975, 459 million gallons of beer were produced in Canada (28). It has been estimated that as much as 500 mg of trub (dry weight) may be precipitated per liter of wort boiled (3). Thus, it may be crudely estimated that some 1150 tons of trub in dry weight were produced in Canada for the year 1974-75.

This unwanted material is disposed in various ways. Where hop jacks are used, the trub is trapped in the hop filter bed and disposed together with the spent hops. Where other methods for hop separation are used, the trub is collected separately from the spent hops, either by centrifugation or by the use of a "whirlpool" separator. In the latter case, the trub is disposed either by incorporation with other brewery waste materials such as spent grains and yeast or diverted into the brewery effluent stream.

Thus it may be seen that at the present time, trub is of negative economic value. Where it is incorporated into spent grains for use as feed, the bitter flavor of the trub generally lowers the feed quality of the grain. The diversion of trub into the effluent stream is an unwarranted solution as there is an increasing trend toward more stringent pollution controls affecting food plants by obligating them to install costly waste disposal systems to handle material of no economic value. Therefore it is logical to reassess the potential value of

waste in general whether for partial or complete waste reutilization, or in order to lower the long term cost for waste treatment.

A cursory examination of trub reveals that it certainly merits attention. It has been reported (1) that trub consists of 50% - 60% protein, 20 - 30% polyphenols, 16 - 20% hop resins and 2 - 3% ash. From a food source standpoint, the high protein content may be a bonus. Also since hops are the single most expensive item on the brewer's list of raw materials, the 16--20% resin content deserves some attention in terms of its potential bittering value.

Compared with other natural and novel protein sources such as cottonseed, rape and leaf protein, trub is relatively free of toxic components such as thioglucosides in rape or gossypol in cottonseed. Since trub protein is derived from barley that has been a staple meal for centuries, the possibility of toxic effect is remote. Furthermore, because of the prolonged boiling treatment, trub is likely to be free from pathogenic microorganisms.

This study was launched with the objective of waste reutilization and the results obtained suggested that eventually all waste materials will be incorporated in the production scheme.

LITERATURE REVIEW

There are no direct references in the literature on the reutilization of trub per se, not even for use as feed. The brewing text by Hough, Briggs and Stevens (1) refers to trub as an insoluble coagulum, occurring in particles about 30 - 80 microns in diameter, with a specific gravity of 1.2 - 1.25. It consists of 50 - 60% protein, 16 - 20% hop resins, 20 - 30% polyphenols and 2 - 3% ash. Although heat is the principal precipitating factor in trub flocculation, the presence of tannins and polyphenolic compounds derived from the added hops aid trub formation by complexing with the available protein.

Protein-polyphenol interactions have been studied mainly with regard to haze formation but brewing texts generally agree that similar protein-polyphenolic interactions occur during wort boiling. The chemical force responsible for the interactions is presumably hydrogen bonds, the strongest being that formed between N - substituted amides and phenols. Hop resins are classified as polyphenols and hence as well promote trub precipitation by a similar mechanism. Resins may also copolymerize with tannins.

The estimated production of trub varies. Briggs et al (1) reported 75 - 100 lb wet weight per 1630 hectoliters of beer; Luers (2) estimated 0.25 kg trub dry weight per 100 kg of malt and according to Royston (3) it was 500 mg dry weight trub per liter of wort boiled.

The protein portion of trub has been of little interest so far. No direct information on reutilization of this protein is available. The nitrogenous constituents of wort account for 3 - 5% of the mash extract and include small molecular weight compounds such as peptides and nucleic acids in addition to proteins. Guenther & Stutler (4) in 1965 showed the effect of boiling on the molecular weight distribution of the wort proteins (appendix 1). It is shown that most of the high molecular weight proteins were precipitated as trub after boiling for 95 minutes.

Compared with other cereal grains, barley has a high albumin or water soluble protein fraction (appendix 2). During germination, the soluble nitrogen, represented mainly by albumin, globulin and the non-protein nitrogen fraction, shows an overall increase with a concomitant decrease in hordein and glutelin (appendix 3). During mashing, the soluble nitrogen is extracted and becomes part of the wort. During the wort boiling, most of the higher molecular weight nitrogen compounds are precipitated (appendix 1) and together with the spent hops constitute the hot break or trub. As a result, trub nitrogen consists mainly of the (i) soluble, (ii) heat coagulable and (iii) high molecular weight compounds. Presumably then, more albumin and globulin proteins would be precipitated than hordein or glutelin.

It is generally recognized that the albumins and globulins function as enzymes and other biologically active compounds.

Munck (5) has mentioned that barley grain extracts possess the activities of phosphatase, beta-amylase and protease. Because of their role as functional enzymes, the cereal albumins and globulins generally demonstrate a more balanced amino acid profile than the insoluble proteins. For cereal proteins in general, Shukla (6) has noted that the soluble fraction is richer in tryptophan and ionizable residues such as arginine, lysine and glutamic acid. They also have more sulfur containing amino acids. A comparison of the amino acid profile between barley embryo, where the soluble proteins predominate, and barley endosperm, where the insoluble matrix protein locate, show that in general, soluble barley protein is richer than insoluble barley protein in the essential amino acids (appendix 4). All these findings suggest that possible use may exist for trub protein, especially from the nutritional aspect.

With regard to the spent bitter substances present in trub, some investigations have been conducted. Spetsig (7) examined the bitter substances of spent hops, trub and the yeast cover using reversed phase partition chromatography to separate them. Laws (8) also examined the oxidation products of the alpha and beta-acids and their significance in brewing. It appears that (i) although some of the transformations which take place during the wort boiling are to non-bitter end products, a significant portion of the bitter substances remain in the

form of oxidised products, (ii) that these oxidation products may have some brewing value.

Specifically, it is known that:

- (1) the beta-acids themselves contribute little bitterness to beer but their oxidised products (hulupone, its analogues and derivatives), which may form on boiling, are bitter, being approximately 50% as bitter as trans-iso-humulone (27).
- (2) humulinic acid, formed from the hydrolysis of alpha-acid (humulone) is non-bitter, and therefore undesirable.
- (3) the oxidised iso-alpha-acids, formed during wort boiling, have 10 - 20% the bittering effect of trans-iso-humulone.
- (4) the further oxidation products of hulupone contribute negligible bitterness to beer (27).
- (5) the iso-alpha-acids are extremely bitter and therefore desirable; on the other hand, the oxidation products of humulone are generally non-bitter.

In summary, it may be seen that little work has been done on trub utilization per se. Related work indicates that the resinous components may be bound by hydrogen bonding to the protein fraction or be copolymerized with other polyphenolic materials. If extractable, this resin material will most likely be oxidised. Since some oxidation products have been shown to be bitter, bittering potential may still be present in the resin extracted from trub. The protein fraction is most likely

derived from the soluble fraction of the barley grain. Thermal denaturation is certain to have taken place during the prolonged boiling treatment. Because it is most likely derived from the soluble protein fraction, trub protein may have some nutritional promise.

MATERIALS AND METHODS

Trub was supplied courtesy of Molson Brewery Ltd. of 1550 Burrard St., Vancouver. The chemicals used for extraction were of analytical grade (Fisher Scientific Co. Ltd.).

Continuous Extraction Method:

A Goldfish^c fat extractor (Labconco 35001) based on a reflux principle was used to continuously extract resin from solid trub samples. Both wet and dry samples were extracted at the lowest temperature setting for 5 days using three different solvents, pure isopropyl alcohol, an azeotropic mixture of isopropyl alcohol and water, and hexane. For the extraction of wet pressed trub containing 26.5 - 28.3% dry matter, a 5 g sample size was used. A 1.25 g sample was used for the extraction of freeze-dried samples. The dried solvent chambers were tared prior to the extraction. After extraction, the remaining solvent was evaporated off at 50°C and the cooled chambers reweighed to obtain total weight of extract plus chamber. Net weight of extract was obtained by difference.

Batch Extraction Method:

Prior to batch extraction, the excess water was removed from the wet trub by squeezing through cheese cloth. A sterilized mop strainer was employed to press out as much excess water as possible. Approximately 50 - 56% water was removed in this manner thus halving the original weight of trub. This wet pressed trub, containing 26.5 - 28.3% dry matter, was the raw material used in every batch extraction.

500 g of wet pressed trub was weighed into a 4000 ml Nal-gene beaker and a fixed volume of isopropyl alcohol added. The solid-liquid mixture was agitated by means of a top drive propeller turning at 700 rpm. The beaker was covered during agitation to prevent evaporation and spillage. The shaft was maintained at $\frac{1}{2}$ " from the bottom of the beaker to ensure as uniform an agitating motion as possible. After agitating for a specified time, the slurry was vacuum filtered through a #4 Whatman filter paper on a 18 cm diameter Buchner funnel. Vacuum was maintained until no more solvent filtered through. The extracted trub would then be re-extracted or air dried at room temperature depending on the number of extractions required in the experiment. By means of this basic procedure, 60 different extractions were performed using 5 sequential extractions, 4 different extraction times for $\frac{1}{2}$, 1, 2 and 5 minutes, and three solvent volumes of 500, 1000 and 1500 mls for 500 g of wet pressed trub. Each extraction was repeated using a different 500 g trub sample giving a total of 120 extractions. From each extraction, a 10 - 15 ml sample of resin-containing solvent was saved for later spectrophotometric measurement.

Recovery of protein flour, resin and the water-dispersible fraction:

After extraction, the debittered trub was dried in a stream of air for 24 hours without heat. On drying, it became a light whitish powder. This was called Trub Protein Flour.

The resin was recovered by vacuum evaporation of the solvent in a Buchler flash evaporator at 40°C. Upon complete

evaporation of the alcohol-water mixture, the residue, termed the Crude Resin Extract, was redissolved with a small quantity of pure isopropanol. Invariably a small part of the Crude Resin Extract was alcohol-insoluble. However, this light colored alcohol-insoluble fraction was easily dispersible in water and hence it was termed the Water-Dispersible Fraction. This Water-Dispersible Fraction was ultimately recovered in a crystal-like form by freeze drying.

Meanwhile the alcohol-soluble portion was filtered and vacuum evaporated again to remove the isopropanol. As the last traces of solvent were removed, the resinous dark brown residue became more and more viscous. Vacuum was maintained until no alcohol odour could be detected. This final residue was termed the Trub Resin. A schematic outline of the entire recovery operation is shown in figure 1.

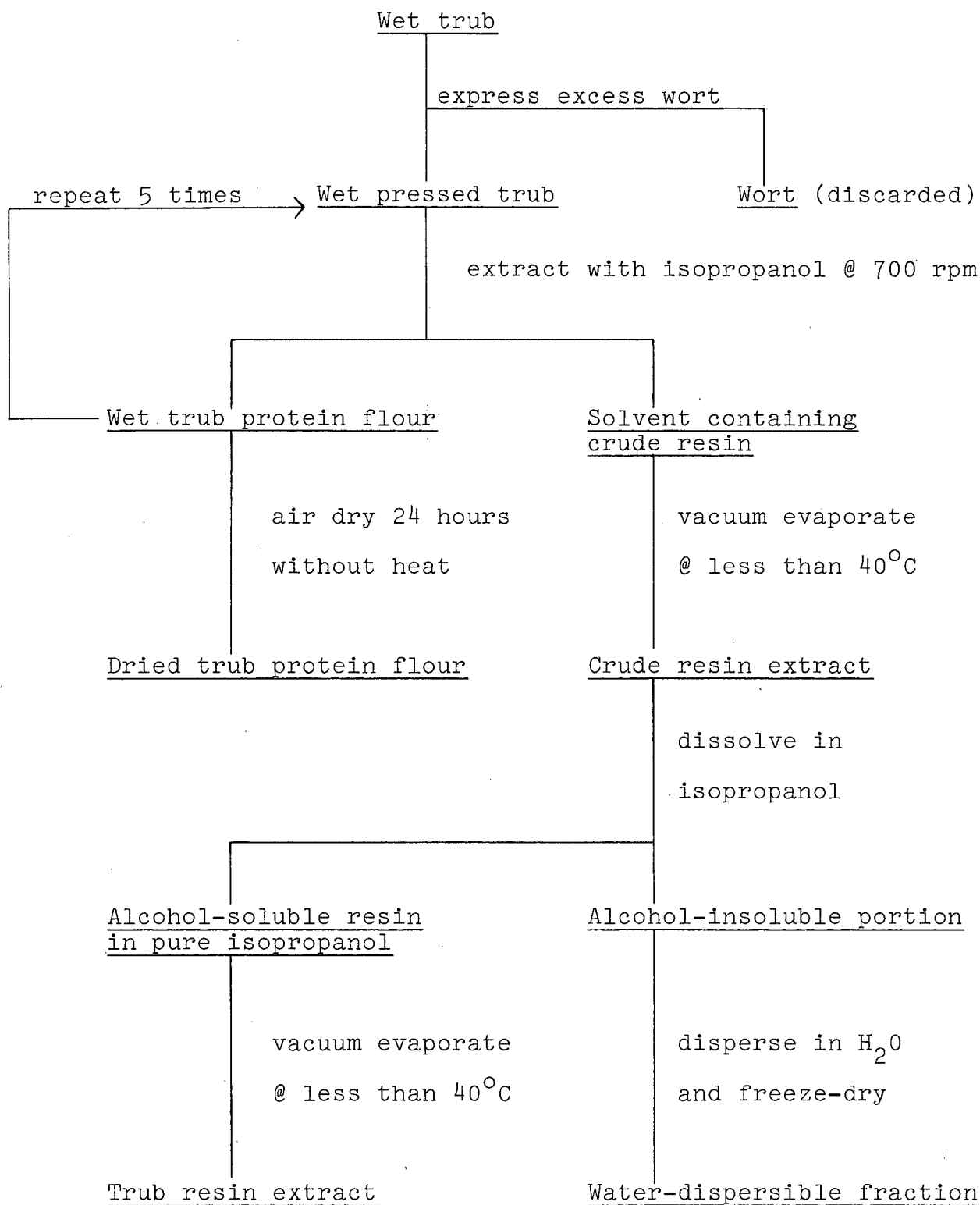
Moisture determination:

For trub and protein flour samples, moisture was determined by drying samples in a 110°C oven to constant weight, the time required being usually 48 hours.

Measurement of the weight of resin extracted:

The amount of resin dissolved in isopropyl alcohol was estimated by measuring resin absorbance at 275 nm against pure isopropyl alcohol as reference. Prior to taking absorbance readings, samples were filtered through Whatman #1 paper and quantitatively diluted to measureable range with pure isopropyl alcohol. Readings were taken as soon as possible

FIGURE 1: Batch Extraction Scheme For Trub



after the extraction, usually within 2 hours or less. It was found that a direct correlation exists between the weight of soluble resin and its absorbance at 275 nm in isopropyl alcohol (figure 2). By linear regression, it was calculated that:

$A = 4525 B - 82$ where A = absorbance

B = concentration of soluble resin in g/ml.

The value A was obtained by dissolving a known weight of resin in a fixed volume of isopropyl alcohol, measuring the absorbance and multiplying this value by the dilution factor required to achieve range.

Lead conductance value of the trub resin:

The procedure recommended by the J.I.B. Analysis Committee was followed (9). Both the BIRF variant and the Wöllmer variant were tried as methods of extracting the precipitable resin. It was found that the Wöllmer variant using ether was more satisfactory because the toluene employed in the BIRF variant was a poor solvent for trub resin.

Thin layer chromatography of the hop and trub resins:

The procedure outlined by Franiau & Mussche 1974 (11) was followed. A uv spectrum between 200 and 400 nm was measured for each separated trub resin component, using alkaline methanol as the extracting solvent. However, conclusive identification of the trub bitter components was not carried out since pure standards were commercially unavailable. Comparisons were made between freshly extracted resins and 6-month-old resin extracts stored at 4°C. Comparisons were also made between

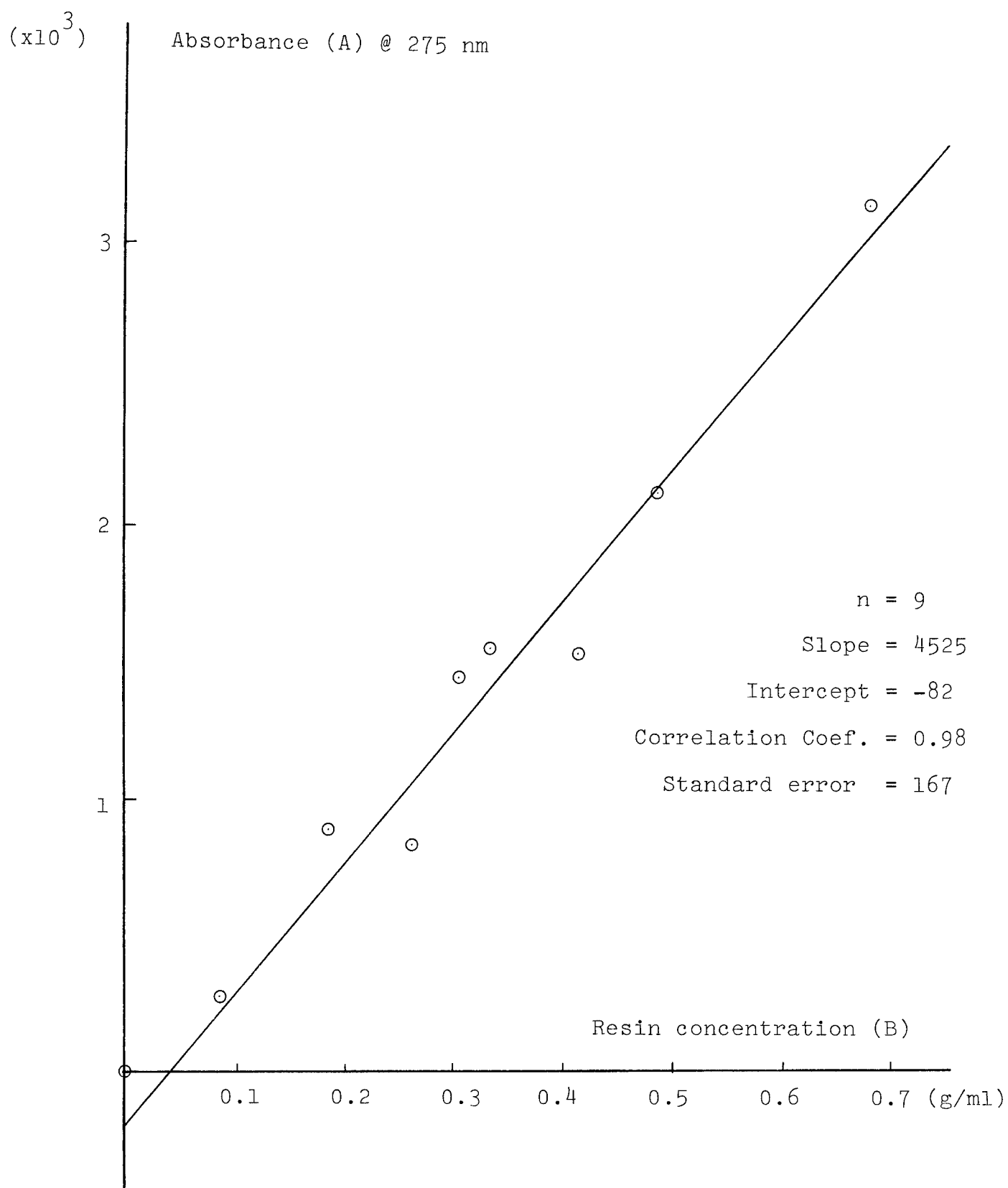


FIGURE 2: Standard curve for estimation of soluble resin concentration in isopropanol.

hop resin components and trub resin components. The hops used were of Brewer's Gold variety and were purchased from the local Wine Art store.

Protein determination:

The amounts of protein in freeze-dried trub, trub protein flour and the freeze-dried water-dispersible fraction were estimated by applying a factor of 6.25 to the Kjeldahl total nitrogen value. The rapid microkjeldahl procedure of Concon & Soltess 1973 (12) was used for digestion. The digest was then diluted to 25 ml with distilled water and the total nitrogen determined in the Technicon Nitrogen Auto Analyser. For trub and the protein flour, 20 - 30 mg of sample containing 2 - 100 ppm nitrogen was required. A larger amount, 100 mg of the water-dispersible fraction was digested because of its lower nitrogen content.

Amino acid analyses of the trub protein flour:

Cysteine was first protected by pyridylethylation according to the method of Cavins et al (13). The protein sample was then hydrolysed with para-toluenesulfonic acid for 24 hours according to the method of Liu & Chang (14). After hydrolysis, the amino acids were assayed using a Phoenix M6800 amino acid analyser, equipped with the Durrum single column system. The area of each amino acid peak was calculated by the HW (height-weight) method where the HW value = peak height x dot count of each peak. The amount of each amino acid calculated from the HW value was expressed as grams of amino acid per 100 g of sample.

Nitrogen solubility profile of the trub protein flour:

The standard method proposed by Balmaceda et al (15) was applied. For trub protein flour, the nitrogen solubility was determined as follows:

200 mg of flour weighed into a centrifuge tube was dispersed in 10 ml distilled water to make approximately a 1% protein solution. With 0.1 N HCl or 0.1N NaOH, the pH of the solution was adjusted to the desired values (pH 2, 3, 4.5, 7, 8.5, 10, 11 and 12). After weight equalization with distilled water, the tubes were centrifuged at 28000 g for 30 minutes. The supernatant was decanted or filtered into Kjeldahl flasks for total nitrogen determination. The result was expressed as:

$$\text{Percent solubility} = \frac{\text{grams of N in supernatant} \times 100}{\text{grams of N in 200 mg flour}}$$

Solubility profiles were also evaluated for freeze-dried unextracted trub and for trub protein flour in 1 M NaCl. The average of duplicate assays was reported in this thesis.

Emulsifying capacity of the trub protein flour:

The standard procedure in the General Foods Methodology (15) was modified as follows:

- (i) the pH of the dispersion was not adjusted to 7
- (ii) pure corn oil (Mazola) was used rather than Fisher oil
- (iii) the result was expressed as mls of oil emulsified per gram of crude flour rather than per gram of protein. Thus the emulsifying capacity of 1 gram of trub protein flour was compared with 1 gram of soy protein flour and 1 gram of soy isolate

on an "as is" weight basis rather than a protein basis. The procedure was as follows:

One gram of flour was dispersed in 100 ml of water. The oil flow rate was maintained throughout the experiment. 70 ml of the protein dispersion was then added to the mason jar equipped with:

- (i) an ohm-meter for measuring the resistance of the dispersion
- (ii) a top-drive blending unit set for 10,000 rpm
- (iii) oil intake with an on/off clamp

After 30 seconds of mixing, oil titration began and was timed with a stopwatch. End point was indicated at infinite resistance, denoting a change in the continuous phase. This measurement was repeated at least 4 times. Emulsifying capacity of the untreated or unextracted trub and of the water-dispersible fraction was also determined in a similar manner.

Water-holding capacity of the trub protein flour:

Again the standard method proposed by Balmaceda et al (15) was adopted. One gram of protein flour was weighed into a previously tared polycarbonate centrifuge tube and 30 ml of distilled water was added and shaken to disperse the flour. The tube was then heated for 30 minutes at 60°C and subsequently cooled for the same period in tap water. Following weight equalization with distilled water, the sample was centrifuged at 18000 g for 10 minutes at 25°C. After decanting the supernatant, the tube was reweighed. For loose packing samples, a filter paper was used to recover loose particles which were

returned to the tube. To compensate for the amount of soluble protein lost in the supernatant, the supernatant was quantitatively collected and its dry weight subtracted from the original 1 gram of dry protein. Thus:

$$\text{Water holding capacity} = \text{Wt of wet sample} - \text{Wt of dry protein} - \text{Wt of soluble protein}$$

per gram of sample tube tube

Color determination of the trub protein flour:

The Unicam Spectrophotometer 800B equipped with the specular reflectance accessory was used to plot the reflectance spectrum of finely ground samples from 400 - 700 nm. Magnesium oxide was employed as the standard for 100% reflectance. Readings at every 10 nm were used to calculate the CIE tristimulus values X, Y & Z by the weighted ordinate method based on illuminant C (16).

Measurement of the bitterness of beer:

The method favored by the Institute of Brewing Analysis Committee was followed (9). A modification of the original method of Rigby & Bethune (17) for estimating iso- α -acids in beer, this procedure extracts acidified beer using iso-octane and measures its absorbance at 275 nm. The result is expressed as European Bitterness Units (E.B.U.) which is 50 x absorbance.

The preparation of beer for taste evaluations:

Wort of specific gravity 1.040 was prepared by dissolving 55 g of dark John Bull malt extract and 55 g of invert sugar per liter of water. The wort was then divided into 3 batches. 3 g/liter of Northern Brewer hops were added to the first, 0.57 g/liter of trub resin extract to the second, and none to the

third. All three batches were boiled for exactly 1 hour to incorporate the bitterness. Losses due to evaporation (approx. 50%) were compensated for by adding water at the end of the boiling. Spent hops were discarded. After the wort had cooled to 30°C, ale yeast was pitched in and allowed to ferment to completion (3 days). The fermenting brew was vigorously stirred twice daily. After fermentation, the beer was filtered and the bitterness assayed. The bitterness level in the first 2 batches were adjusted to 20 E.B.U. (European Bitterness Units) by blending in the unhopped beer. Then equal volumes of the adjusted batches were blended together to form the mixed batch. At the end, there were 3 batches of beer @ 20 E.B.U. each, viz., the beer brewed with fresh hops, beer brewed with trub resin only and beer consisting of a 1:1 blend of the two.

Taste evaluations in order to determine the difference in bitterness values:

The test procedure adopted was a paired comparison (19). Two samples were presented simultaneously and the panelist was asked to decide whether they were the same or different in bitterness. If the panelist decided that the samples were different, then the more bitter sample was to be identified on the form supplied. The chosen panel consisted of 18 persons, 2 women and 16 men. Sample containers were labelled with random 3 digit numbers. The beer samples contained no carbonation. Crackers and water were provided as palate refreshment.

Six pairs were presented to each panelist for judgement. Each pair always contained a sample of beer made from fresh hops as the reference sample. The other sample in the pair would always be beer made from either 100% trub resin or 50% trub resin adjusted to the same level of analytical bitterness as the reference sample. The data obtained was analysed using Chi-square analysis with the Yates' continuity correction applied for degrees of freedom = 1 (47).

RESULTS AND DISCUSSION

The discussion of this project may be divided into three parts viz.,

- A. The extraction procedure
- B. Evaluation of the protein fraction
- C. The examination and evaluation of the resin fraction.

Part A: The extraction:

From the start it was reasoned that if any brewing concern decided to extract resin from trub, the process would entail a batch rather than a continuous extraction because of the prohibitive capital cost involved in setting up the continuous mode of extraction. Also, because of the large energy demand required in any type of drying operation, it was decided that all extraction work be conducted on wet rather than dried trub. Once it was settled that the type of extraction be conducted on wet trub using batch extraction, the question on the choice of solvent arose.

Three resin solvents were closely examined, viz., hexane, methylene chloride and isopropyl alcohol, on the basis of their ability to debitter trub. The debittering effect of each solvent was tested simply by tasting the dried protein residue after sequential extraction involving agitation in a Waring blender followed by vacuum filtration. A 4:1 (v/w) batch extraction performed sequentially 5 times failed to debitter the wet pressed trub when either methylene chloride or hexane was used. On the other hand, isopropanol debittered the trub under these conditions.

The choice of isopropanol was based mainly on its debittering effect on the protein residue. However, the low toxicity of this solvent as well as its profound dehydrating effect on the wet trub (see table 1) further supported the choice of isopropanol.

The superior performance of isopropanol over the non-polar solvents hexane and methylene chloride was partly explained by the presence of a large amount of water (71.7 - 73.5%) in the wet pressed trub. The excessive water present may act as a physical barrier to methylene chloride and hexane extractions because of their strong non-polarity. On the other hand, isopropanol is completely miscible with water and forms a stable binary azeotrope with water (see appendix 5 for the distillation constants).

The altered nature of the resins present in trub may also explain the effectiveness of the alcohol solvent. During the wort boiling, various hop components undergo chemical transformations to more polar derivatives. For instance, the iso- α -acids are more soluble than the α -acids. Similarly many studies (7, 22, 23, 24) have shown that oxidised hop components are more water-soluble than their unoxidised derivatives. Ashurst (25) has shown that the mechanism responsible for the increased solubility of humulone during oxidation is the production of an α -glycol from an olefinic bond. He extended this mechanism to the oxidation of other hop resins as well. Spetsig (7) has reported that trub resin contains many such polar oxidised components.

Furthermore, it has been stated that the bonds between the resin and protein fraction of trub are most likely hydrogen bonds (1).

Table 1: The dry weight of resin-extracted trub protein.*

Trub protein flour	% Dry Weight
Unextracted	26.85
Extracted once	85.70
Extracted twice	92.50
Extracted four times	92.60
Extracted five times	93.20

*Extracted with isopropanol @ 4:1 v/w for 2 minutes

Loomis & Battaile (20) alluded to the hydrogen-bond-breaking capability of isopropanol. Hence it is almost certain that the extracting power of isopropanol, a polar solvent, be greater than that of hexane and methylene chloride in the trub system. On the same grounds, the extracting power of the more polar azeotropic mixture of 88% isopropanol and 12% water may be even greater.

Indeed, our results from the continuous extraction appears to confirm this premise (table 2). The azeotrope extracts approximately 24% more crude resin than isopropanol (11.11% vs. 8.93%). Verzele (27) in his work (appendix 6) pointed out that with increasing solvent polarity, more and more unspecified material is extracted from dried hops. If this trend occurs in the trub system, then it is likely that the use of the azeotrope will extract more polyphenolic and tannin material from the trub than would the use of isopropanol as extractant. Such extraneous material may not be desirable in terms of reutilising the resin in beer but may be desirable from the standpoint of debittering the protein residue.

For this study, pure isopropanol was chosen as extractant because it lent itself for the resin quantification procedure which involved spectrophotometry. However, in practice where solvent recycling is a standard procedure, the azeotropic extraction would be preferable.

Before beginning the quantitative assessment of extraction characteristics, a brief explanation on the water-dispersible

Table 2: The percent yeild of crude resin extracted from wet and dry trub by the continuous reflux method.

Reflux solvent	Hexane	Isopropanol	Isopropanol-water azeotrope
	Wet pressed trub		
% Yield (wet basis)	0.64	8.93	11.11
n	6	6	6
Std deviation	0.10	1.67	2.28
Std error	0.04	0.68	0.93
Coeff. of variation	14.90%	18.70%	20.54%
% Yield (dry Basis*)	2.39	33.08	41.19
	Freeze-dried trub		
% Yield (dry basis)	1.12	23.98	39.04
n	2	2	2
Std deviation	0	2.57	0.85
Std error	0	1.82	0.60
Coeff of variation	0%	10.73%	2.17%

* Based on 27% dry matter in wet pressed trub.

fraction and its effect on the extraction data may be helpful. As outlined in figure 1, the water-dispersible fraction would precipitate out as an alcohol-insoluble residue when the crude resin extract was redissolved in pure isopropanol. Most of this hydrophilic fraction would be found in the first extract because most of the water present in the trub was removed in the first extraction. When an aliquot of this filtered first extract was dissolved in pure isopropanol for resin estimation on the spectrophotometer, a slight turbidity invariably developed, indicating the presence of the alcohol-insoluble, water-dispersible fraction. Subsequent comparisons of resin yields estimated gravimetrically (table 2) and spectrophotometrically (table 3) showed that the latter tended to be generally higher. The discrepancy was attributed firstly to the effect of turbidity on the spectrophotometric readings and secondly to the incomplete extraction of the resin extracted for gravimetric estimation. This probably occurred because no agitation was used in the continuous reflux resin extraction. Consequently, because of the unexpected presence of the water-dispersible fraction, the spectrophotometric method, originally devised to measure only alcohol-soluble resin, was extended to estimate the total crude resin content, which includes both the water-dispersible fraction and the alcohol-soluble resin.

From an economic aspect, the important considerations in the extraction step are:

- (i) to maximise the yield of trub protein flour
- (ii) to maximise the yield of trub resin
- (iii) to minimise solvent utilization
- (iv) to maximally debitter the trub protein flour.

Table 3: Percent yield of crude resin batch-extracted from wet pressed trub with isopropanol.

No. of extracts		1	2	3	4	5
Solvent	Agitation					
Ratio**	time (min)					
(v/w)						
1:1	½	4.87*	6.49	7.25	7.72	8.00
1:1	1	6.39	7.72	8.41	8.81	9.05
1:1	2	7.31	8.81	9.51	9.96	10.22
1:1	5	8.63	10.26	11.01	11.40	11.64
2:1	½	6.42	8.59	9.67	10.18	10.45
2:1	1	6.30	8.47	9.54	9.96	10.17
2:1	2	7.39	9.22	10.09	10.42	10.56
2:1	5	9.18	11.13	11.93	12.24	12.40
3:1	½	6.67	9.36	10.59	11.19	11.46
3:1	1	8.29	11.02	12.05	12.48	12.69
3:1	2	7.66	10.52	11.53	11.68	11.82
3:1	5	8.63	11.05	11.79	12.02	12.13

* % Yield based on absorption at 275 nm expressed on basis of wet trub. Each value is the average % yield of 2 independent extractions.

**Solvent ratio = volume of isopropanol/wt of wet trub

With respect to the first consideration (i): since native barley protein is known to have a large content of hordein, the alcohol-soluble protein, it was originally speculated that some significant protein losses would occur by solubilization in the isopropanol-water mixture. However upon analysis, little protein was found either in the resin (< 5% protein) or in the water-dispersible fraction (2.36% protein). Moreover, the yields of protein flour after 5 sequential extractions were found to be approximately the same regardless of the amount of solvent used or the agitation time involved (table 4). Consequently, the yield of true protein flour was considered as being approximately constant (23%) and not subject to maximization.

The batch extraction experiment was performed to optimise the other three considerations (ii, iii & iv). The experiment empirically examines the effect of two factors viz., the isopropanol-trub ratio (v/w) and the agitation time on the yield of resin. An analysis of variance (table 5) on the resin yield data (table 3) showed that both solvent ratio and agitation time were significant to the 5% level in affecting resin yield. The general trend is for the yield of resin to increase with more solvent and longer agitation (figures 3 & 4).

The resin yield data also reveals the negative logarithmic character of the extraction. Figure 3 depicts this trend in comparing the resin yield of the three solvent ratios at 5 minutes extraction time. Most of the resin is extracted in the first 2 extractions. A quick calculation from table 3 shows that in all 12 cases, 81 - 91% of the resin has been extracted after 2 extract-

Table 4: The yield of dried trub protein flour after 5 extractions at 3 different solvent ratios of volume of isopropanol: weight of wet trub.

Solvent ratio (v/w)	1:1	2:1	3:1
Extraction			
time (min)	Yield of dried trub (%)*		
$\frac{1}{2}$	21.22	20.55	23.03
1	24.53	21.35	21.35
2	25.72	24.12	22.12
5	23.60	21.86	22.26

* Yield expressed as % of wet pressed trub.

Table 5: A summary of the two-way factorial analysis of variance with replication for:

- (i) the total yield of resin after 5 consecutive extractions;
- (ii) yield of resin after one extraction

	DF	SS	MS	F	P
<hr/>					
5 extractions					
Total	23	49.89			
Replicate	11	43.65			
Solvent	2	21.12	10.56	20.31**	P 0.0005
Time	3	13.64	4.55	8.74**	P 0.0025
Solvent					
x time	6	8.90	1.48	2.85	P 0.10
Error	12	6.24	0.52		
<hr/>					
1 extraction					
Total	23	40.03			
Replicate	11	34.17			
Solvent	2	4.11	2.06	4.22**	P 0.05
Time	3	24.78	8.26	16.94**	P 0.0005
Solvent					
x time	6	5.29	0.88	1.81	P 0.25
Error	12	5.85	0.49		

DF = Degrees of freedom; SS = Sum of squares; MS = Mean Square

F = F value; P = Probability

* Significant at the 5% level.

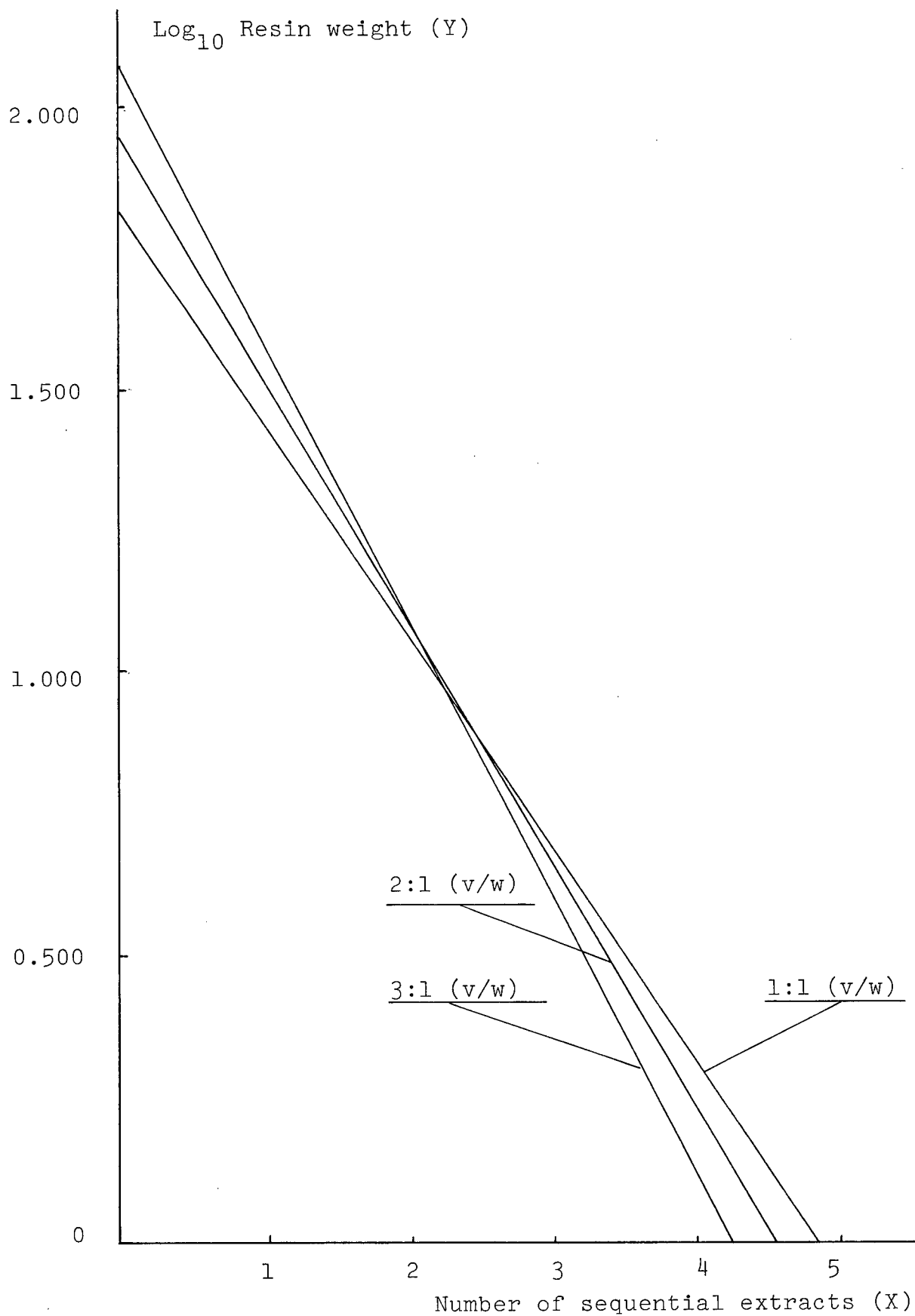


FIGURE 3: Effect of solvent ratio on resin yield in 5 minute extractions.

Weight of
resin (g)

11

10

9

8

7

6

5

4

5 minutes

2 minutes

1 minute

 $\frac{1}{2}$ minute

Number of sequential extractions.

1

2

3

4

5

FIGURE 4: The effect of agitation time on resin yield @ 1:1 solvent ratio.

ions. Thus, from the standpoint of solvent economy, 2 extractions may be considered to be the arbitrary point at which resin recovery terminates. After 2 extractions, the concern shifts away from resin recovery toward the debittering of the protein residue.

At the point of two extractions, the effect of different solvent ratios on resin yield is not marked, particularly if 5 minute agitation times are employed (table 3). Thus for the sake of solvent economy, it is logical to adopt the lowest of the solvent ratios. The use of the lowest solvent ratio also improves the efficiency of agitation, due to the interaction effect between solvent ratio and agitation time (table 5). In practice, the wet trub-isopropanol mixture was difficult to handle at the 1:1 solvent ratio. There was no free solvent present and uniform agitation and solvent expulsion was difficult. These difficulties may justify the use of a higher solvent ratio in actual practice.

The final consideration (iv) is the debittering of the protein flour. The residual resin level at which the protein flour may be considered "debittered" is unknown. Tannins and polyphenolic compounds may so contribute to the bitterness of trub protein flour that even at very low resin levels bitterness may be detectable. On the other hand, the presence of other food components in a particular formulation employing "debittered" trub may mask the bitterness of the trub protein flour so much that it may not be necessary to extract the flour residue to extremely low resin levels.

Table 6 shows the number of theoretical extractions necessary to reach 1% and 0.1% resin level (w/w) in the dried protein flour.

Table 6: The number of theoretical extractions* required to obtain 1% and 0.1% of the initial resin level (w/w) at a constant yield of 115 g (23%) of dried protein flour, extracted from 500 g wet pressed trub.

Solvent

ratio	Time	1% Resin level	0.1% Resin level
(v/w)	(min)	Number of extractions required	
1:1	$\frac{1}{2}$	5.04 (6)**	8.35 (9)
1:1	1	4.65 (5)	7.60 (8)
1:1	2	4.83 (5)	7.74 (8)
1:1	5	4.70 (5)	7.37 (8)
2:1	$\frac{1}{2}$	5.07 (6)	8.03 (9)
2:1	1	4.80 (5)	7.52 (8)
2:1	2	4.39 (5)	6.77 (7)
2:1	5	4.43 (5)	6.76 (7)
3:1	$\frac{1}{2}$	5.18 (6)	8.09 (9)
3:1	1	4.74 (5)	7.23 (8)
3:1	2	4.40 (5)	6.66 (7)
3:1	5	4.14 (5)	6.20 (7)

* Calculated from the debittering equations in table 7.

** Numbers in parenthesis indicates the number of complete extractions necessary to debitter the protein flour to less than 1% and 0.1% resin levels respectively.

The debittering equations (table 7) used to calculate the theoretical number of extractions were derived from linear regression of the \log_{10} resin weight vs. number of extractions. Good correlation in these equations justifies the overall assumption that debittering or resin extraction follows a negative logarithmic path. Thus the protein flour debittering process is also hampered by the increasingly slower resin removal rate, which necessitates the use of much solvent at this debittering stage of the process. Even at 1:1 solvent ratio, as much as 4000 mls of solvent are required to debitter 500 grams of wet trub to less than one part per thousand resin. The problem may be alleviated by (a) the use of counter current extraction, where fresh solvent is used to extract, say, the last 2 extracts before being used on heavier extractions, (b) using an alternative method of debittering the protein flour such as an alkaline wash where the flour is debittered by destruction of the resin to non-bitter components.

This latter method was tentatively tried at 8:1 (v/w) alkaline water: flour ratio at pH 9.2 and pH 10. Subsequent informal taste evaluations on muffins (see table 8 for formulation) indicated that the alkaline-extracted-flour muffins had barely detectable bitterness levels compared to the 5 times-isopropanol-extracted muffin. The predominant bitter flavor was noted as an aftertaste rather than an immediate sensation. After drying, the alkaline-extracted flour appeared darker (see table 9 for comparative luminance data), coarser and heavier than the

Table 7: The debittering equations derived from the linear regression of \log_{10} resin weights (Y) vs. the number of extractions (X).

Solvent ratio	Agitation time(min)	Debittering equation*	Correlation coefficient	Std error of estimate
1:1	$\frac{1}{2}$	$Y = 1.584 - 0.302X$	-0.983	0.079
1:1	1	$Y = 1.633 - 0.338X$	-0.966	0.128
1:1	2	$Y = 1.719 - 0.343X$	-0.963	0.136
1:1	5	$Y = 1.821 - 0.375X$	-0.969	0.136
2:1	$\frac{1}{2}$	$Y = 1.778 - 0.339X$	-0.995	0.048
2:1	1	$Y = 1.822 - 0.367X$	-0.998	0.036
2:1	2	$Y = 1.907 - 0.421X$	-0.995	0.060
2:1	5	$Y = 1.959 - 0.429X$	-0.987	0.097
3:1	$\frac{1}{2}$	$Y = 1.841 - 0.344X$	-0.999	0.019
3:1	1	$Y = 1.969 - 0.402X$	-0.997	0.042
3:1	2	$Y = 2.012 - 0.443X$	-0.998	0.036
3:1	5	$Y = 2.069 - 0.485X$	-0.997	0.052

* Debittering equation: $Y = \log_{10}$ weight of resin extracted

X = number of extractions

Table 8: A recipe for the preparation of muffins which contain trub protein flour.

$\frac{1}{4}$	cup shortening
$\frac{1}{2}$	cup brown sugar
$\frac{1}{4}$	cup molasses
2	eggs, unbeaten
1	cup of milk
1	cup of all-purpose flour
$1\frac{1}{2}$	tsp. baking powder
$\frac{1}{2}$	tsp. baking soda
$\frac{1}{2}$	tsp. salt
$\frac{3}{4}$	cup Quaker bran
$\frac{3}{4}$	cup TRUB PROTEIN FLOUR
$\frac{1}{2}$	cup raisins or dates

Bake at 400°F for 15 - 20 minutes.

Table 9: The color evaluation of dried trub protein flour

Solvent	Time	No. of	Chromaticity coordinates			Luminance
ratio		extracts				
(v/w)	(min)		x	y	z	Y (%)
1:1	$\frac{1}{2}$	5	0.353	0.351	0.296	49
1:1	1	5	0.351	0.348	0.301	57
1:1	2	5	0.334	0.327	0.338	44
1:1	5	5	0.351	0.349	0.301	56
2:1	$\frac{1}{2}$	5	0.348	0.346	0.306	57
2:1	1	5	0.349	0.348	0.303	58
2:1	2	5	0.350	0.347	0.303	58
2:1	5	5	0.350	0.347	0.303	57
3:1	$\frac{1}{2}$	5	0.347	0.345	0.308	59
3:1	1	5	0.347	0.345	0.308	60
3:1	2	5	0.346	0.345	0.310	60
3:1	5	5	0.348	0.346	0.306	59
8:1 @ pH 9.2		1	0.351	0.345	0.305	25
8:1 @ pH 10		1	0.352	0.342	0.307	21
4:1 No agitation		2	0.361	0.355	0.284	30
4:1 No agitation		3	0.365	0.357	0.278	29
4:1 No agitation		4	0.362	0.355	0.283	32
4:1 No agitation		5	0.362	0.355	0.283	31
4:1 No agitation		7	0.354	0.349	0.297	44

isopropanol-extracted samples. The alkaline debittering procedure would likely entail extra steps in the flour preparation viz., a subsequent water-washing step followed by drying and grinding. Also, some protein loss from alkali solubilization is expected.

The concluding summary of trub extraction is as follows:

1. Isopropanol debittered better than hexane or methylene chloride.
2. The isopropanol-water azeotrope extracted more crude resin than isopropanol (table 2).
3. The protein flour yield was not greatly affected by changes in the solvent ratio from 1:1 to 3:1 and in the agitation time from 0.5 to 5 minutes (table 4).
4. Both solvent ratio and agitation time affected the resin yield as shown by analysis of variance (table 5).
5. As much as 8 - 9 extractions were required to debitter the protein flour to less than 1 part per thousand (table 6).
6. The extractions were in a characteristic negative logarithmic relation (table 7).
7. Increasing solvent ratios did not substantially increase resin yeild when the agitation was longer than 2 minutes so that the 1:1 solvent ratio was the most efficient method of extraction in terms of solvent economy.
8. Solvent conservation and protein flour debittering were the conflicting factors.
9. Alkaline extraction was moderately effective for debittering but may entail additional finishing treatments such as washing and drying as well as protein losses.

An overall scheme showing the yields of the various fractions is presented in figure 5. Variability of the individual yield estimates including dry matter estimates is shown in table 10.

Part B: Evaluation of the protein fraction:

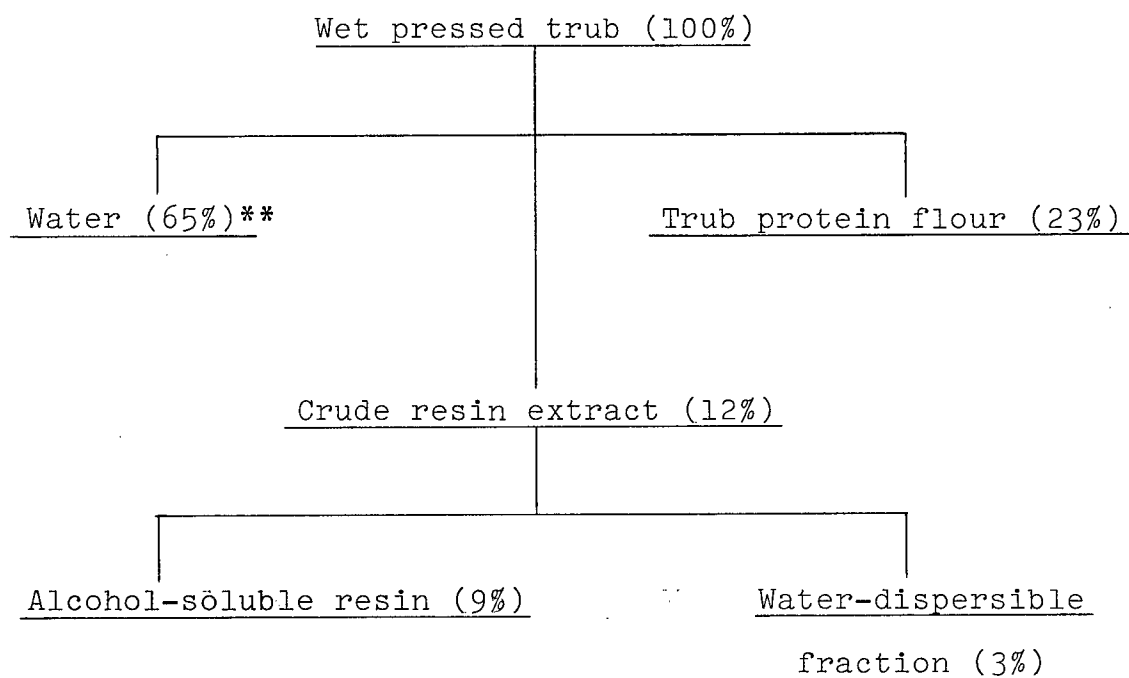
Prior to extraction, trub was a dark greenish-brown solid with a texture similar to cottage cheese. It had a slightly sweet wort-like odour and an extremely bitter taste. After extraction, the solid was transformed to a fluffy bland-tasting, whitish powder, with no odour. Unlike the original trub, which dried in brittle clumps, the protein flour, when dried under the same conditions was easily crushed, with a friability resembling that of caked sand.

After its extraction, the trub protein flour was evaluated for its protein level (table 11), color (table 9), amino acid profile (table 12), nitrogen solubility (table 13), emulsifying capacity (table 14) and water-holding capacity (table 15).

The protein level of the extracted trub protein flour appeared relatively constant, regardless of the amount of extracting solvent used (table 11), with an overall average of 49.65% protein. Conversion to a dry weight basis (89.65% dry weight), gives a value of 55.38% protein that indicates a slight increase from that of the original unextracted trub (52.99%). This increase is expected since extraction of the resin component may alter the protein/non-protein ratio.

It appears that isopropanol-soluble material is responsible for the dark greenish-brown color of trub. After extraction,

FIGURE 5: Approximate yields* in weight % of the various fractions extracted from wet pressed trub.



* To the nearest 1%

** Estimated by difference

Table 10: General yield data showing variability of the various analytical estimates.

	<u>Wet pressed trub</u>	<u>Dried protein flour</u>
Mean dry weight (%)	26.85	89.65
n	9	9
Std deviation	2.81	1.48
Std error	0.09	0.49
Coeff. of variation	10.46%	1.65%

	<u>Protein flour</u>	<u>Crude resin</u>	<u>Water-dispersible</u>
		(@ 5 min.)	fraction
Mean yield (%)	23.35	12.05	2.89
n	30	6	7
Std deviation	1.81	0.74	0.68
Std error	0.33	0.30	0.29
Coeff. of variation	7.75%	6.12%	26.49%

NB. All data expressed on basis of wet pressed trub.

Table 11: The protein content (6.25 x N) of various trub fractions, soy protein isolate and soy flour.

	Protein (%)	n	S.D.	S.E.	C.V.* (%)
Dried trub	52.99	3	1.36	0.79	2.6
Trub protein** Flour @ 1:1, 5 min	49.95	3	0.26	0.15	0.5
Trub protein flour @ 2:1, 5 min	51.37	3	2.53	1.46	4.9
Trub protein flour @ 3:1, 5 min	47.63	3	1.22	0.71	2.6
Water-dispersible fraction	2.36	3	0.03	0.02	1.3
Soy protein isolate	90.21	3	2.26	1.30	2.5
Soy protein flour	60.87	3	0.35	0.20	0.6

* Coefficient of variation.

** All trub protein flour samples were extracted 5 times with isopropanol.

the extracting solvent demonstrated the similar greenish hue. With increasing resin concentrations, as during vacuum evaporation, the solvent liquid progressively became dark green, light brown, cloudy light brown, dark brown, and finally turned to a very dark, almost black color. Thus it is not surprising that decolorization of the trub occurred simultaneously with resin extraction and depended on the same extracting conditions, viz., agitation and solvent ratio. The last column of table 9 indicates that the whitening effect (Y%) was generally enhanced by intensifying either condition. All the batch extractions achieved a luminance ≥ 44 which was equivalent to a mild greyish color, quite suitable for incorporation into food systems. Further decolorization above luminance 44 may not be necessary. The only color problem which may arise is from the alkali debittering step, which darkens the flour (luminance 21 - 25), presumably due to the formation of darker colored degradation products.

The amino acid analysis (table 12) showed up high variability in the analysis for many of the amino acids; especially Proline, Tryptophan and Arginine. This suggests that perhaps because trub was prepared by thermal precipitation, the amino acid composition of trub protein flour may be more variable than that of naturally occurring proteins.

The essential amino acids of trub protein flour were evaluated in three different ways as shown in tables 16 & 17. The classic chemical score method (I) which expresses each of the essential amino acids of the sample protein as a percentage

Table 12: The amino acid composition of trub protein flour (g/16gN)

	Mean	Std dev.	n	Std error	C.V.(%)*
Aspartic acid & asparagine	7.75	1.94	9	0.65	25
Threonine	3.55	0.69	9	0.23	19
Serine	4.45	0.51	9	0.17	11
Glutamic acid & glutamine	17.73	2.25	9	0.75	13
Proline	6.46	6.54	9	2.18	101
Glycine	4.49	0.33	9	0.11	7
Alanine	7.68	1.37	9	0.46	18
Valine	5.31	1.30	9	0.43	24
Methionine	2.07	0.47	9	0.16	23
Isoleucine	3.45	0.40	9	0.13	12
Leucine	7.94	1.47	9	0.49	19
Tyrosine	4.19	0.90	9	0.30	21
Phenylalanine	3.65	0.58	9	0.19	16
Lysine	3.39	0.80	8	0.28	24
Histidine	1.96	0.33	9	0.11	17
Tryptophan	1.41	1.26	9	0.42	89
Arginine	8.04	2.84	8	1.00	35
Cystine & Cysteine	2.41	0.69	9	0.23	29

* Coefficient of variation to the nearest percent.

of the corresponding amino acid in whole egg protein as reference, has been criticized because it does not take into account the total essential amino acid figure. Thus, evaluation by method (II), proposed by the FAO/WHO in 1965 (37), and which accounts for the total essential amino acids was also included. This method (II) gives a higher chemical score than method (I) (66.61% vs. 48.60%). However, neither method correlates well with the Net Protein Utilization (N.P.U.) value obtained from animal feed trials or with the pepsin digestibility value. Hence method (III) which correlates better with the N.P.U. value was also included. This method, developed by Oser (38), involves taking the log of the fractions obtained in method (I), summing them and dividing by the number of essential amino acids, and taking the antilog to obtain a new chemical score value subsequently renamed the Essential Amino Acid Index (EAAI). The EAAI was calculated using the 8 amino acids considered essential for adults (Ile, Leu, Lys, Met, Phe, Thr, Trp & Val). For infants, Arg and His are included in the above list of amino acids (table 17). This latter evaluation for infants gave a higher index than for adults (0.74 vs. 0.68) when applied to trub protein.

Based on the amino acid values in table 12 and the calculations in table 16, it appears that the first limiting amino acid in trub protein flour is lysine, followed by isoleucine then methionine. The limiting effect of methionine is mitigated by the relatively high cysteine content. Tyrosine and arginine also occur at comparatively high levels. A comparison of the limiting amino acids in trub protein flour, wheat, barley, soy-

Table 16: Summary of chemical score calculation for trub protein flour by old and new method (I & II)***

	Std egg protein (SEP)	Trub protein flour (TPF)	Method I TPF/SEP x 100	Method II TPF/34.28 x 51.21/SEP
	(g/16g N)	(g/16g N)	(%)	(%)
Thr	5.120	3.55	69.34	95.03
Val	6.848	5.31	77.54	106.27
Met	3.360	2.07	61.61	84.44
Ile	6.288	3.45	54.87**	75.20**
Leu	8.816	7.94	90.06	123.43
Tyr	4.160	4.19	100.72	138.04
Phe	5.728	3.65	63.72	87.33
Lys	6.976	3.39	48.60*	66.61*
Trp	1.488	1.41	94.76	129.87
Cys	<u>2.432</u>	<u>2.41</u>	<u>99.10</u>	<u>135.82</u>
Total essential a.acids	<u>51.216</u>	<u>34.28</u>		

* 1st limiting amino acid and chemical score.

** 2nd limiting amino acid.

*** Reference #36 and 37 respectively.

Table 17: Summary of the Essential Amino Acid Index
calculation for trub protein flour (method III).**

	TPF/SEP*	Log ₁₀ TPF/SEP	
Thr	0.6934	-0.1590	
Val	0.7754	-0.1105	
Met	0.6161	-0.2103	
Ile	0.5487	-0.2607	
Leu	0.9006	-0.0455	
Phe	0.6372	-0.1957	
Lys	0.4860	-0.3134	
Trp	0.9476	-0.0234	
Total (Adult)		-1.3185	Mean (Adult) = -0.1648 Antilog = 0.6842 EAAI (Adult) = 0.68
His	0.8059	-0.0937	
Arg	1.3189	0.1202	
Total (Child)		-1.2920	Mean (Child) = -0.1292 Antilog = 0.7427 EAAI (Child) = 0.74

* Grams of essential amino acid in trub protein flour divided
by grams of essential amino acid in standard egg protein.

** Reference #38.

bean and rapeseed is given in table 18. Trub protein scores better than wheat and is nearly equivalent to barley protein according to method (I). This result is supported by a comparison of their EAAI values (lower half of table 18) which places trub protein flour almost on the same level as barley protein.

A comparison of trub protein flour with other common protein sources (table 19) indicates that:

- (i) overall, trub protein bears all the characteristics of a typical cereal protein with high Glu, and low Lys and Met.
- (ii) trub protein bears a general resemblance to barley protein in most of the amino acids, except that trub protein is notably richer in Arg, Ala and Tyr, and more deficient in Pro and Phe than barley protein.

It is interesting to note that the embryo protein of barley is also richer than the whole kernel barley protein in Arg, Ala and Tyr and more deficient in Pro and Phe (appendix 4). Thus one is led to conclude that trub protein appears to be a composite of barley protein and its embryo protein, a fact which points to its origin as a thermal precipitant.

It is generally agreed (29, 30, 31, 32) that any examination of protein functionality should begin with the nitrogen solubility profile, as solubility is probably the single most useful index in judging other functional potentialities and in assessing the severity of prior treatment given to the protein. In this case, the effect of using isopropanol, a known denaturant in the preparation of fish protein concentrate (29), was tested

Table 18: Essential amino acids of various proteins expressed as a percentage of the corresponding amino acid of standard egg protein (method I).

	Trub protein	Wheat	Barley	Soybean	Rapeseed
Thr	69.34	54.10	68.75	69.14	84.96
Val	77.54	65.13	79.44	77.83	79.15
Met	61.61	50.00	47.62	38.39*	62.80
Ile	54.87	60.75	60.75	75.38	69.02
Leu	90.06	76.23	78.72	79.85	76.79
Tyr	100.72	64.18	60.82	73.08	62.26
Phe	63.72	83.62	88.51	80.48	68.09
Lys	48.60	33.26	49.74	87.87	66.47*
Trp	94.76	102.82	95.43	119.62	111.56
Cys	99.10	106.50	101.97	84.70	112.66
His	80.59	90.87	87.58	111.84	109.79
Arg	131.89	65.29	71.69	110.73	101.05

* Limiting amino acid

Essential amino acid index:

Trub protein	0.68
Wheat	0.63
Barley	0.69
Soybean	0.75
Rapeseed	0.76

Table 19: A comparison of the amino acid composition of trub protein, barley, wheat, soybean and rapeseed (33,34).

	Trub Protein	Selkirk wheat	Barley 6-row	Soybean	Rapeseed
Asp	7.75*	4.67	6.11	11.33	7.34
Thr	3.55	2.77	3.52	3.54	4.35
Ser	4.45	5.01	4.24	4.64	4.42
Glu	17.73	33.12	24.48	18.08	18.08
Pro	6.46	11.14	10.94	5.14	6.13
Gly	4.49	3.76	4.19	4.21	5.02
Ala	7.68	3.26	4.06	4.18	4.34
Val	5.31	4.46	5.44	5.33	5.42
Met	2.07	1.68	1.60	1.29	2.11
Ile	3.45	3.82	3.82	4.74	4.34
Leu	7.94	6.72	6.94	7.04	6.77
Tyr	4.19	2.67	2.53	3.04	2.59
Phe	3.65	4.79	5.07	4.61	3.90
Lys	3.39	2.32	3.47	6.13	5.86
His	1.96	2.21	2.13	2.72	2.67
Trp	1.41	1.53	1.42	1.78	1.66
Arg	8.04	3.98	4.37	6.75	6.16
Cys	2.41	2.59	2.48	2.06	2.74

* All values in grams of amino acid/16g N

from the solubility profiles before and after extraction. Table 13 and figure 6 prove that the extraction with isopropanol decreased the solubility slightly, but that the overall solubility of the original trub was already so low that any decrease in solubility brought about by isopropanol was insignificant. Increasing the ionic strength with 1M NaCl resulted in slightly increased solubility at acid pH but depressed it at alkaline pH. Again the effect was almost negligible. Removal of the resin appears to enhance solubility at pH 12, probably because of the neutralizing effect of the bitter resin acids in the unextracted trub. The extremely low overall solubility of all the samples suggests their thermal history and appears to be symptomatic of extensive and irreversible denaturation. The effect of heat on protein solubility has been thoroughly examined in the literature (32, 39, 40, 41, 42, 43).

The above findings explain the results of the emulsification experiment (table 14), which indicated that, on a weight to weight basis, trub protein flour was able to emulsify 2/3 as much oil as soy protein flour under identical conditions. This rather fair performance was, however, marred by the great instability of the emulsion formed, which usually lasted less than 15 seconds before separating.

The water-holding capacity of trub protein flour appears to be good, being 2.3 times greater than soy flour and 0.7 times that of the isolate on a weight basis (table 15). It is thought that proteins sorb water by binding molecules to specific hydrophilic sites (43). Moreover, denaturation appears to slightly

Table 13: The nitrogen solubility profile of trub protein flour, freeze-dried trub, and trub protein flour in 1 M NaCl solution (g soluble protein/100g protein).

	Trub protein flour(49.65% protein)	Freeze-dried trub (52.99% protein)	Trub protein flour in 1 M NaCl(49.65% protein)
pH of the protein-water mixture	% protein (6.25 x N) in solution (g soluble protein/100g protein)		
pH 2	2.52*	2.87	2.21
pH 3	1.93	2.47	2.14
pH 4.5	1.93	2.76	2.36
pH 7	2.56	2.83	2.69
pH 8.5	2.28	2.99	2.21
pH 10	2.87	3.65	2.53
pH 11	4.09	4.53	3.13
pH 12	16.64	7.11	11.46

* Average of duplicate runs.

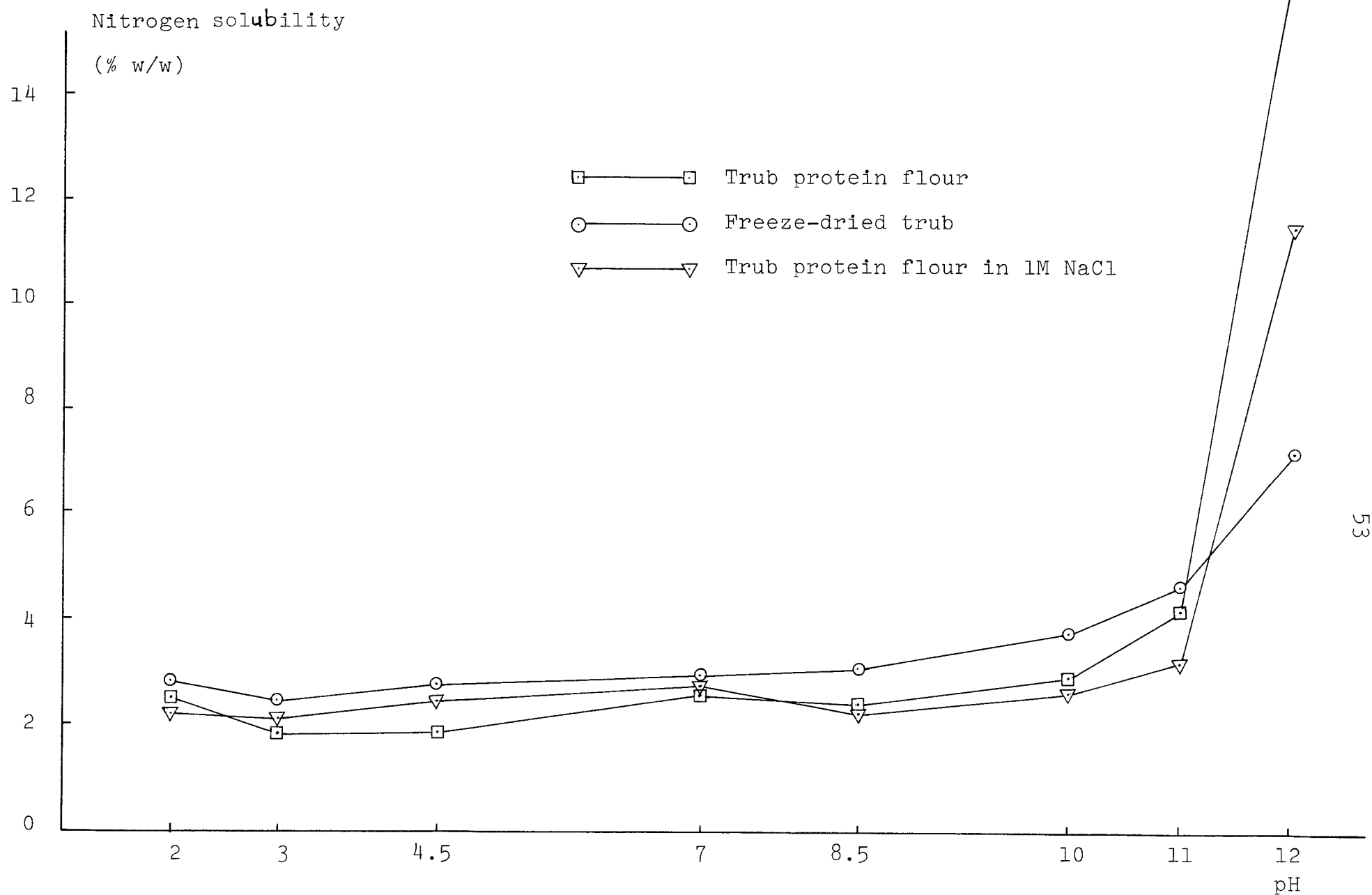


FIGURE 6: Nitrogen solubility profile of trub protein flour before extraction, after extraction and in 1 M NaCl.

Table 14: A comparison of the emulsifying capacity of trub protein flour, soy protein flour, soy protein isolate and the water-dispersible fraction (ml oil per gram sample)

	Trub protein flour (49.65% prot)	Soy protein flour (defatted) (60.87% prot)	Soy protein isolate (90.21% prot)	Water- dispersible fraction (2.36% prot)
Mean (ml of corn oil emulsified)	106.0	152.9	247.8	97.3
Std dev.	1.6	3.2	21.2	2.6
n	7	4	9	4
Std error	0.6	1.6	7.1	1.3
Coefficient of variation (%)	1.6	2.1	8.6	2.6

Table 15: A comparison of the water holding capacity of trub protein flour, soy flour and soy isolate (g water/g sample)

	Trub protein flour	Soy protein flour (defatted)	Soy protein isolate
Mean			
(g H ₂ O/g sample)	5.88	2.55	8.25
Std dev.	0.18	0.04	1.09
n	3	3	2
Std error	0.10	0.02	0.77
Coefficient of variation (%)	3.03	1.42	13.20

enhance water-holding capacity (44, 45). This is thought to be due to an increase in the number of water-binding or hydration sites, arising from the unfolding of the polypeptide chain during denaturation.

An overall observation of trub protein flour leads to the following conclusion:

1. Trub protein has a nutritional potential almost equivalent to that of barley, judging from its essential amino acid index. Nutritional quality may be conclusively established in actual feed trials.
2. The good water-holding capacity of the trub protein flour should be confirmed with other related functional tests such as swelling before testing in actual products.

Kinsella (31) suggests incorporation into meat systems as an excellent way to test this particular property. Since the water binding capacity is also closely related to viscosity (30, 46), its performance in viscous foods such as soups, purees and even doughs may be enlightening.

Part C: Evaluation of the trub resin fraction:

Trub resin was separated by TLC according to the method of Franiau & Mussche (11). The resin components of trub were separated on a silica gel G/cellulose plate at pH 5. Under uv light each component exhibited a distinctive fluorescent color. These however, did not correlate well with R_f value, and an attempt to match trub resin components with those from fresh and aged hops was unsuccessful (table 20 and 21). Thus whenever possible, uv scans

Table 20: T.L.C. comparison of fresh hop components stored at 10°C for different time periods.

# uv color	Rf value			λ_{\max} (nm)
	1 day	20 days	6 mths	
1. Black			> 0.37	230,373b,254s*
2. Light blue			0.37	
3. Broad red			0.44	
4. Black (trace)			> 0.44	
5. Fluorescent yellow			0.46	283b
6. Dark purple	0.51	0.49		
7. Light yellow			0.50	
8. Black			> 0.50	
9. Orange-red		0.55	0.55	233,274b
10. Blue-white		0.58		
11. Pink			0.58	231,320b
12. Black			0.61	234,322b
13. Blue-white		0.61		
14. Purple-pink		> 0.61		
15. Yellow	0.64			
16. Blue	0.69	0.68	0.67	
17. Pink	0.72	0.70		
18. Blue		0.76		
19. Green-yellow	0.80	0.92	0.87	
20. Violet	0.85			

*b = broad, s = shoulder

Table 21: T.L.C. comparison of trub resin components stored at 10°C for zero and six months.

#	uv color	Rf value		λ max. (nm)
		0 months	6 months	
1.	Light blue		0.23	
2.	Black		0.31	232 s*
3.	Broad grey		0.37	255
4.	Black		0.43	
5.	Orange-red		0.44	282 b, s
6.	Purple	0.47		
7.	White		0.48	281
8.	Broad pink		0.51	266 b
9.	Light purple	0.57		
10.	Purple-pink	0.62		
11.	Dark green		0.62	253
12.	Light blue-purple	0.64	0.65	354
13.	I ₂ only **	> 0.64		
14.	I ₂ only	0.68		
15.	Pink	0.72		
16.	Light blue	0.82		
17.	Green-yellow	0.91	0.81	273
18.	I ₂ only	0.98		
Blank scan of the silica gel				207, 220

* s = shoulder, b = broad peak

** Could be detected only after saturation with I₂ vapor.

were made from the separated components to determine their absorption maxima for possible identification. Maxima at wavelengths less than 220 nm were ignored because of gel absorption at 220 and 207 nm.

Some difficulty was encountered in obtaining conclusive scans because of (i) the instability of the separated components to air and light, and (ii) interference from tailing, particularly in the fresh hop separation. However, the maximum wavelengths obtained, when compared to published values (appendix 6 and 7), tentatively indicated the presence of:

- ((i) the iso-alpha-acids (#11, table 21)
- (ii) the beta-acids (#12, table 21)
- (iii) humulinic acid (#8, table 21)

No alpha-acid was detected in trub resin, possibly because of oxidation losses during the extraction. Hulupone, the bitter first oxidation product of the beta-acids (lupulones) may be present at #3, table 21; the second maximum at 325 nm was masked probably because of the small quantity of hulupone present.

An overall uv scan of the full mixture of trub resin components indicates a maximum ranging from 226 - 243 nm, with a shoulder at 275 nm. An overall visible scan shows a small peak at 658 nm, with a rapid increase as one approaches the uv range. A quantitative estimation of the separated trub resin components was not attempted since the pure bitter components were not available to use as references.

Any evaluation of the bittering capacity of the trub resin components would be premature at this point and must await the

results of the bittering trials. However, as mentioned in the literature review section, the contribution to bitterness of the reboiled trub would be derived mainly from the oxidised beta-acids, the oxidised iso-alpha-acids, and any remaining iso-alpha-acids.

Lead conductometry (9) was carried out to obtain a quantitative value for the bittering potential of the trub resin. It is known that fresh hop analysis by lead conductometry yields a lead conductance value (LCV) closely approximating its alpha-acid content, and therefore provides a good estimate of its bittering potential during the boiling. In the case of old extracts or old hops, the LCV is less reliable as a guide to bittering potential, since other fractions interfere with the analysis by also precipitating the lead. Nevertheless, the LCV is still used as a guide to brewing value in these cases, since the fractions responsible for bitterness in old hops also precipitate lead. The LCV of old hops has been statistically correlated with its bittering power in bitterness taste trials (10).

The LCV obtained for trub resin extract was 68.4% (table 22). This value of 68.4% represents the LCV of the highest possible concentration of the trub resin, after as much isopropanol and water as possible were removed by vacuum evaporation at 40°C. Since no alpha-acid was detected in the TLC experiment, it appears that the LCV of 68.4% is due mainly to the precipitating action of other bitter fractions, presumably the oxidised resin fractions. In contrast, a solvent extract of fresh hops, concentrated to an LCV of 68.4% contains approx. 68.4% alpha-acid and little or no oxidised material.

The conductivity plot (fig. 7) indicates that the trub

Table 22: To show the number of ml * of lead acetate required to precipitate the 4 main trub resin fractions and to show the lead conductance value calculation.

Sample #	Run #	ml of lead acetate precipitated (3.778% w/v)		
1	1	3.493 (1)**	9.608 (2)	
1	2	3.735 (1)	7.871 (2)	22.918 (4)
1	3	4.010 (1)	8.164 (2)	14.707 (3)
2	4	4.784 (1)	9.467 (2)	14.550 (3)
2	5	4.384 (1)	12.277 (3)	
2	6	3.523 (1)	8.795 (2)	20.617 (4)
3	7	3.585 (1)	13.880 (3)	27.803 (4)
3	8	3.931 (1)	10.893 (2)	21.951 (4)
3	9	4.056 (1)	7.861 (2)	19.651 (4)

Mean of fraction (1) = 3.945 mls, with n = 9

Mean of fraction (2) = 8.951 mls, with n = 7

Mean of fraction (3) = 13.854 mls, with n = 4

Mean of fraction (4) = 22.588 mls, with n = 5

Calculation for lead conductance value (LCV):

$$\begin{aligned}
 \text{Formula: Percent LCV} &= \frac{\text{ml of 4\% (w/v) PbAc} \times 76.98^{***}}{\text{g of extract}} \\
 &= \frac{3.9 \times (3.778/4.000) \times 76.98}{4.1298} \\
 &= \underline{68.41\%}
 \end{aligned}$$

* The number of ml of lead acetate are the abscissae of the intersection points of the 4 lines obtained by linear regression of the conductivity plot.

** Numbers in brackets signify the resin fraction eg. (1) = first resin fraction.

*** 76.98 = constant for LCV formula when using Wöllmer variant.

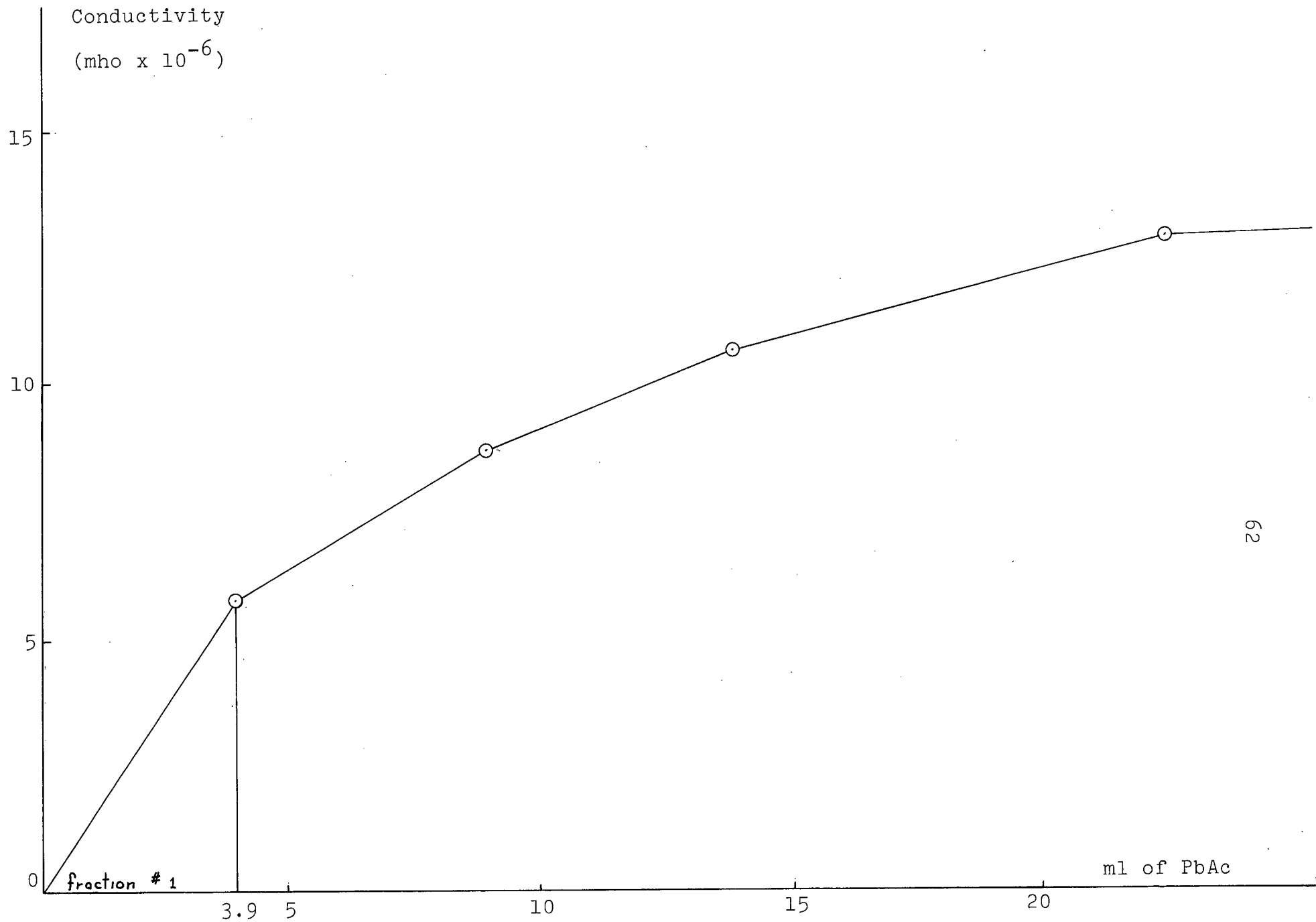


FIGURE 7: Lead conductance plot for trub resin extract.

resin components tended to precipitate in 4 main fractions, viz., at 3.9, 9.0, 13.9 and 22.6 mls of lead acetate. As in standard lead conductance estimations, only the 3.9 ml value was used to derive the LCV of 68.4%, the assumption being that the contributions to bitterness of the other fractions are negligible.

It was felt that some kind of preliminary brewing test was necessary in order to determine

- (i) if the bitter substances in trub resin are capable of imparting bitterness to sweet wort,
- (ii) whether the imparted bitterness is significantly different than that derived from fresh hops.

Dark malt extract was quantitatively hopped in a model system with fresh hops and trub resin extract (see method section) and fermented with ale yeast. Dark malt extract was used to minimize the color difference between paired samples in the subsequent taste trial, thereby eliminating color bias. Ale yeast was chosen partly because an aerobic fermentation was simpler to control and partly because of the practice by lager brewers of not allowing their spent hops to stand for any time in the wort because it is believed to affect flavor. Moreover, ale character appears more compatible with stronger hop additions than lager. During fermentation, the trub resin batch failed to form a stable yeast head, making regular skimming impossible. Nevertheless, after 3 days, fermentation completed as the wort was no longer sweet.

The overall results of the taste trials indicate that there is a detectable difference between the ales brewed with trub resin and those brewed with fresh hop. Specifically however, the difference appears to be slight, since with 54 opinions, no difference could be detected (table 23, 24). Moreover, the judgments as to which of the "different" samples were more bitter were not at all significant (table 25) indicating that the bitterness levels in the fresh hop and trub resin samples were very close together. Furthermore, the taste trials have shown that firstly, trub resin can be reincorporated into wort by reboiling, and secondly, that trub resin does definitely contribute to wort bitterness.

An estimate of the amount of trub resin utilized in these trials may be interesting at this point. A random survey of local beer has revealed that most lagers lie in the range of 12 EBU (table 26). Toby Charrington, which is quite bitter to the Canadian palate, registers at 23 EBU. The trub resin ale was brewed at 20 EBU. It requires roughly 1 g of trub resin per liter of wort boiled to achieve 20 EBU. If the yield of resin is conservatively placed at 10% of wet pressed trub, then 1 kg of trub yields 100 g of resin which is capable of bittering 100 liters of ale.

Possible problems from reutilizing trub resin may arise from 3 areas:

1. Yeast malfunction: top fermenting yeasts may cease to be top fermentors, thereby creating skimming problems, and

Table 23: Summary of paired comparison results to determine if difference in bitterness is detectable between:

1. Fresh hop samples and 100% trub resin samples
2. Fresh hop samples and 50% trub resin samples
3. Fresh hop samples and pooled* trub resin samples

Difference judgement	# of judgements	Extent of differences (more bitter)	
		Fresh hop	Trub resin
1. Fresh hop vs. 100% trub resin			
A difference exists	33	15	18
No difference exists.....	<u>21</u>		
Total	<u>54</u>		
2. Fresh hop vs. 50% trub resin			
A difference exists.....	34	15	19
No difference exists.....	<u>20</u>		
Total	<u>54</u>		
3. Fresh hop vs. pooled trub resin			
A difference exists.....	67	30	37
No difference exists.....	<u>41</u>		
Total	<u>108</u>		

* Data was subjected to heterogeneity test (47), found to be homogeneous and therefore pooled.

Table 24: Summary of Chi-square analysis with Yates' correction (47) applied to determine level of significance between 1, 2 and 3.

1. Fresh hops vs. 100% trub resin (54 opinions).

Chi-square value = 2.2407

Probably is between 10% and 25%

Therefore not significant at the 5% level.

This implies there is no difference in bitterness between fresh hops and 100% trub resin.

2. Fresh hops vs. 50% trub resin (54 opinions).

Chi-square value = 3.1296

Probability is between 5% and 10%

Therefore not significant at the 5% level.

This implies there is no difference in bitterness between fresh hops and 50% trub resin.

3. Fresh hops vs. pooled* trub resin (108 opinions).

Pooled Chi-square value = 5.7870

Probability is between 1% and 2.5%

Therefore significant at the 5% level.

This implies there is a difference between fresh hops and trub resin (50% and 100% batches) with respect to bitterness.

* Data was subjected to heterogeneity test (47), found to be homogeneous and therefore pooled.

Table 25: Summary of the test (Chi-square analysis with Yates' continuity correction) to determine if the extent of differences (choice of which is more bitter) was due to chance or was significant.

1. Fresh hop vs. 100% trub resin (34 opinions)

Chi-square value = 0.0606

Probability between 90% and 75%

Therefore not significant at the 5% level.

This implies that the bitterness choice was due to chance.

2. Fresh hop vs. 50% trub resin (34 opinions)

Chi-square value = 0.2650

Probability between 75% and 50%

Therefore not significant at the 5% level.

This implies that the bitterness choice was due to chance.

3. Fresh hop vs. pooled* trub resin (67 opinions)

Chi-square value = 0.5373

Probability between 50% and 25%

Therefore not significant at the 5% level.

This implies that the bitterness choice was due to chance.

* Data was subjected to heterogeneity test (47) and found to be homogeneous before pooling.

associated flavor problems. Ashurst (26) mentions that the addition of a small amount of hops or its water extract may remedy this tendency, which apparently also occurs with the use of isomerized hop extracts.

2. The polyphenolic constituents of the trub resin may act as haze precursors, leading to chill or permanent haze at the finishing stages of production. On the other hand, the polyphenolics may act beneficially as protein precipitants during the boiling of the wort.
3. The literature generally alludes to the harsher flavor notes associated with aged or oxidised resins; thus over-sparging of the spent hops or a pro-alkaline wort boil is generally avoided in the production of the finer tasting beers, although the harsher character is desirable in the manufacture of the more bitter stouts and ales.

Table 26: Comparative bitterness levels in local (B.C.) beers.

European bitterness units (EBU)	
Labatt's blue (lager)	12.3*
Cream Stout (Labatt)	15.7
Toby Charrington (Carling O'Keefe)	22.7
Guinness (Labatt)	41.0

* Average of duplicate readings

N.B. E.B.U. = 50 x absorbance @ 275 nm.

Appendix 1: The effect of boiling on the molecular weight
distribution of wort proteins (4).

M W	<5000	5-10,000	10-50,000	50-100,000	>1,000,000
Boiled*	0.0175	0.0125	0.0040	0.0010	0
Not Boiled	0.0336	0.0185	0.0101	0.0023	0.0028

* 95 minutes

Appendix 2: The protein composition of various grain
seed proteins (6).

	% Total Protein				Total Protein (% dry wt)
	Albumin	Globulin	Prolamine	Glutelin	
Rice	5.0	10.0	5.0	80.0	8 - 10
Oat	1.0	78.0	16.0	5.0	8 - 14
Barley	13.0	12.0	52.0	23.0	
(normal)			(Hordein)		
Barley	18.0	14.0	46.0	22.0	10 - 16
(Hiproly)					
Wheat	3 - 5	10.0	69.0	16.0	
Rye	5 - 10	5 - 10	30 - 50	30 - 50	9 - 14
Pearl					
Millet	13.2	9.4	40.0	28.0	12 - 18
Maize	4.0	2.0	55.0	39.0	7 - 13
Sorghum	8.0	8.0	52.0	32.0	9 - 13

Appendix 3: The nitrogen content of the 'crude protein'
fractions of 'Garton improved' barley and malt (48).

	Nitrogen, g/100g dry weight		
	Barley	Malt & Roots	Kiln malt without roots
Total N	1.703	1.684	1.597
Salt soluble N	0.563	1.048	0.935
Hordein	0.631	0.261	0.269
Glutelin	0.509	0.375	0.393
Globulin	0.159	0.210	-
Albumin	0.186	0.183	0.184
Proteose	0.093	0.198	-
Non-protein N	0.130	0.546	0.507

Appendix 4: The amino acid composition of barley (5).

BARLEY (g/100g protein)

Amino acid	Whole kernel	Endosperm	Embryo
Lys	3.90	2.80	6.20
His	2.20	2.00	3.30
Arg	4.40	3.90	9.80
Asp	6.80	4.80	10.30
Thr	3.40	2.80	4.10
Ser	3.70	3.20	4.40
Glu	26.10	29.50	15.80
Pro	11.40	14.00	4.90
Gly	4.20	2.90	6.40
Ala	4.40	3.20	6.40
Cys	1.25	1.20	0.70
Val	5.30	5.00	5.70
Met	2.60	2.90	2.10
Ile	3.80	3.70	3.50
Leu	7.10	6.70	6.50
Tyr	1.90	2.60	2.70
P - ala	5.40	5.40	4.20

Appendix 5: The distillation constants for methylene chloride, hexane and isopropanol (21).

	Methylene chloride	Hexane	Isopropanol
B.P. of solvent ($^{\circ}\text{C}$)	40.0	61.0	82.4
% Composition (v/v) of water azeotrope			
% H_2O	1	5.8	12.2
% Solvent	99	94.4	87.8
B.P. of azeotrope ($^{\circ}\text{C}$)	38.8	61.6	80.4
# of layers	2	2.	miscible
% Solvent in each layer			
Upper layer	2.	96.2	
Lower layer	99.9	3.8	

Appendix 6: Wavelength of hop bitter substances in alkaline methanol (11).

Component	1st maximum	2nd max.
Beta-acids	355	
Alpha-acids	324	360
1st grade degradation products of the iso-alpha-acids	255	271
Iso-alpha-acids	255	271
Humulinic acids	257	266
Allo-iso-alpha-acids	248	271
Hydrated iso-alpha-acids	255	271
Hulupones	255	325
Humulinone	259	273

Appendix 7: Bitter substances of hot trub isolated by reversed phase chromatography (7).

pH of eluate	Name of compound	Absorption max. (nm)
5.1	cohulupone	255, 325
5.4	hulupone	255, 325
5.6	adhulupone	255, 325
5.7	isocohumulone	255
6.0	isohumulone	255
6.4	isoadhumulone	255
8.9		272, 335
9.1		272
9.2		272
9.5		272
9.5	xanthohumol	380
9.6		272, 375
9.6		325, 370
9.8		320, 375
10.0	colupulone	355
10.0		272
10.2	lupulone	355
10.3	adlupulone	355

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