

Seduced by Yeast

Graham G. Stewart,^{1,2} *G. G. Stewart Associates, Rhiwbina, Cardiff, Wales, United Kingdom, CF14 6RP; and International Centre for Brewing and Distilling, Heriot-Watt University, Edinburgh, Scotland, United Kingdom, EH14 4AS*

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When I completed the ordinary (O) level examinations at my Cardiff, Wales, grammar school in 1958, I wanted to study advanced (A) level subjects (history, etc.) that would enable me to study history (particularly modern history) at university. My father's comment was, "Obviously it is up to you, but do you want to become a school teacher? Because with a degree in history that is probably what will happen!" The answer to his question was that I did not

want to be a school teacher, although my interest in modern history does prevail to this day.

Instead of taking the Arts route, I successfully studied A level chemistry, botany, zoology, and physics. My father wanted me to study medicine—I was much less enthusiastic at this prospect! Up to this point, my best science subject had been chemistry. However, I knew that my inability to comprehend mathematics beyond its basic concepts would be a major impediment to studying chemistry at the honors degree level and beyond. Then, during a botany practical session, my fellow six-form students (equivalent to grade 12) and I added dried baker's yeast to a cane sugar solution in a conical flask and incubated it overnight on a radiator. Next morning, the flask's contents were foaming and smelled of alcoholic yeast extract—"marmite." A simple experiment but it influenced the rest of my life! For some reason, I was (and still am) fascinated with this microorganism and the process of alcoholic fermentation.

It confirmed to me that I wanted to study microbiology and biochemistry at university. I completed a double honors degree in these disciplines at what was then the University College of South Wales at Monmouthshire (now Cardiff University). For my honors research project, I investigated phenotypic effects on the extracellular production of invertase by baker's yeast.

Upon graduation, I wanted to conduct research for a Ph.D., preferably on yeast. During the last term of my undergraduate

studies, I came across an advertisement for a research assistant to pursue research on "the sugar uptake by fluorinated compounds in microbial systems." There was no mention of the type of microbial system to be employed. Indeed, the advertisement was rather vague. The position was at the Bristol College of Science and Technology (one of the colleges of advanced technology). Also, if the college became a university in the near future (as was expected because of the 1963 Robbins Report [51]) there would be the opportunity to register for a Ph.D. degree. I applied for this position and was invited to Bristol for an interview with Rod Brunt (who would be the project's supervisor) and also with two of his colleagues in the Department of Chemistry and Biology. The interview was held in the administrative building of the college, and the core point of the information that delighted me was that the microorganism to be employed in the research would probably be yeast because, as Rod Brunt put it, "yeast is cheaper than rats!" Also, during the interview, it was revealed that "all being well," Bristol College of Science and Technology would become Bath University within the next two years and gradually move onto a new campus to be built on the Claverton Down playing fields, which were located on the outskirts of the city. I said to myself during the interview, "If I am offered this position, I am taking it." A few days after the interview a letter arrived offering me the position, and I accepted it. I was to begin employment in Bristol during September 1964 on a three-year contract and would probably move to the Claverton Down Campus in the summer of 1965 with the rest of the new School of Biological Sciences. It would become the first academic school located in Bath.

RESEARCH WITH FLUOROACETIC ACID

Upon my arrival in Bristol in September 1964, Rod Brunt made it clear that although I was a research assistant on a salary, not a grant, I would be regarded effectively as a research student with minimal teaching duties (only laboratory demonstrating). My primary objective would be to study for a Ph.D. He, in association with his colleague, Norman Taylor, was interested in the synthesis and metabolic effects of fluorinated hexoses, pentoses, purines, and pyrimidines (108). After considerable discussion, it was decided I would initially study the effect of monofluoroacetic acid (HFA) upon glucose metabolism in resting (nongrowing) suspensions of baker's yeast cells. A systematic study of HFA on mammalian systems (rats, dogs, and monkeys) had been conducted during, and immediately after, the Second World War as a potential warfare agent (44). The major points of HFA attack were the central nervous system and the heart, with respiratory arrest following severe convulsions being the principal effects (37).

We obtained a regular supply of fresh yeast from the Distillers Co. Ltd. (DCL) Bristol Yeast Factory. Initially, we paid four shillings (20 new pence) per pound for this material. Subsequently, the factory kindly donated a fresh pound block every week for the rest of my time at Bristol and Bath. Even when we moved to

¹ Corresponding author. E-mail: profggstewart@aol.com and g.g.stewart@hw.ac.uk; Phone: +44 (0) 2920 611983; Fax: +44 (0) 2920 611954; Website: www.ggstewartassociates.co.uk.

² A great deal of the material in this biographical review was presented (and subsequently published) in lectures given as part of the following awards, for which the author is very grateful. Permission has been received to reproduce appropriate material here. "Schwartz Award Lecture" of the Master Brewers Association of the Americas – 1976 (67). "Charles Thom Award" of the Society of Industrial Microbiology and Biotechnology – 1987 (70). "Horace Brown Medal Award" of the Institute of Brewing and Distilling – 2008 (1,71). "Award of Distinction" of the American Society of Brewing Chemists – 2008 (73). "Award of Merit" of the Master Brewers Association of the Americas – 2010 (72).

Bath, a fresh delivery of yeast was received at Claverton Down every Monday morning!

In living cells, HFA undergoes lethal conversion to fluorocitric acid, which inhibits the tricarboxylic acid cycle (TCA) enzyme aconitase, resulting in the accumulation of intracellular citric acid. It is an inhibitor of respiration, ethanol production, polysaccharide synthesis, and glucose uptake. The degree of inhibition of these parameters has been compared in pH 2.2 and pH 6.8 buffers, and greater inhibition at pH 2.2 than at pH 6.8 was observed (9).

In addition, respiration with acetaldehyde as an exogenous substrate is greatly and equally inhibited at both pH 2.2 and 6.8. However, the respiration rates with ethanol or lactate substrates are greatly inhibited at pH 2.2 and less so at pH 6.8. It appeared that intracellular citrate accumulation, due to aconitase inhibition by fluorocitrate (produced as a result of lethal synthesis of HFA), has a different effect on glucose and ethanol metabolism depending on the prevailing intracellular pH (79). However, determination of yeast intracellular pH 45 years ago was difficult and unreliable. Today, the use of flow cytometry to measure intercellular yeast pH is a much more reliable procedure (14).

The School of Biological Sciences, myself included, moved to its new facilities in the South Building at Claverton Down, Bath, in July 1965. It was envisaged that the South Building would be a temporary facility and be demolished by 1970. However, it is still there! Indeed, an extension has been built onto the building. As it happened, I was the first person to begin laboratory work in this facility and consequently on the Claverton Down campus as a whole. The next two years were very busy with experimental work, and eventually I began to write my Ph.D. thesis.

As 1967 dawned, I started to think about future employment because my contract with Rod Brunt, and the college, would terminate at the end of August 1967. Then I found another advertisement in the careers section of a science magazine. This time it was for a lecturer in biochemistry in the Department of Pharmacology of the School of Pharmacy at Portsmouth College of Science and Technology. It was a case of “nothing ventured, nothing gained,” and I applied for the position. I was very surprised to be invited for an interview in Portsmouth in February 1967 and even more surprised when I was offered the position, which I accepted.

I moved to Portsmouth in September 1969 (Olga and I were married in Cardiff the same month), and I was given three months' respite at the college until I began lecturing “basic” biochemistry to pharmacy students in January 1968. In the meantime, I had completed my Ph.D. thesis, submitted it, and successfully defended it (64). One of the conditions of employment at Portsmouth was that I immediately begin a research project, and publications were very important. Then came confirmation of what I really already knew—the senior academics in the Department of Pharmacology (Ed Abbs and David Roberts) were not interested in research on yeast. Research on small mammals should be the “order of the day.” The School of Pharmacy possessed a very good animal house (at least very good by the standards of the day), and I was expected to enroll in an appropriate course in order that I could obtain a license from the Government's Home Office so that I could conduct experiments on rats, mice, rabbits, and guinea pigs. I embarked upon this course with some trepidation and, although I successfully completed it, and obtained the appropriate “piece of paper,” I have never enjoyed conducting experiments on laboratory animals. I am not an antivivisectionist as long as someone else is conducting the animal experiments. Nevertheless, I endeavored to make the best of it.

The question was “What should I do?” I had mentioned to Ed Abbs that HFA resulted in convulsions in rats and mice (44). He suggested that I verify this fact and at the same time gain experience in handling mice and rats. Consequently, I started on this

project with his invaluable help and that of a research technician. (One of the areas that surprised me in the School of Pharmacy at Portsmouth was the large number of trained and experienced laboratory technicians that were available and eager to be of assistance, many of whom were retired from the Royal Navy.) It did not take long to confirm that HFA, when injected interperitoneally, did cause convulsions in both rats and mice. The next question regarded the metabolic changes that were occurring in the rat brain, heart, and blood. HFA treatment resulted in two phases of behavior in rats: 1) a sedated phase followed by 2) a tonic extension convulsive phase. The levels of metabolites such as citric acid, lactic acid, ammonia, free glucose, and glycogen were determined in these two behavior phases in the heart, blood, and brain. In the heart, the citric acid level rose in both phases, whereas in the brain this metabolite fell during the convulsion following an initial citrate rise during the sedative phase. This alteration in brain citrate level would have a profound effect on the TCA cycle, which would affect overall brain function (44).

We then studied the impact of another convulsant, pentamethylenetetrazole, on rat function. We hoped to identify common metabolic changes in rat tissues (particularly in the brain) between the two convulsants. Preliminary results (39,78) found an increase in glycolytic flux and depletion of energy reserves in both convulsants. These early results were encouraging, offering the prospect of obtaining a number of publications. The pharmacology research laboratory was a very stimulating and lively environment, but the work increasingly confirmed to me that this type of research was not for me. I wanted to work on yeast. Also, I was working in a School of Pharmacy but I was not a pharmacist—I was not registered with the Royal Pharmaceutical Society of Great Britain. This meant that my long-term career prospects there were limited. Last, lecturing biochemistry to pharmacy students was not very exciting. They wanted to qualify in order to work in a pharmacy (hospital or retail) and biochemistry was, to them, a necessary evil (the perennial problem of service teaching). Consequently, I decided that sooner rather than later I would have to seek pastures new. However, I did not expect the opportunity to present itself quite so soon.

O, CANADA

In November 1968, yet another position was advertised in the career section of a science magazine that caught my eye. The advertisement was from a Canadian brewing company, The Labatt Brewing Company. Labatt was seeking a research microbiologist to establish a research group that would study brewer's yeast in the Labatt Technical Center based in London, Ontario. I had never heard of Labatt, but it had been founded in London, ON, in 1847. I was aware of London, ON, because the University of Western Ontario is located there. Western had (and still has) an international reputation for microbiological and biochemical research. Also, a cousin (my mother's brother's son) and his family lived there. I soon ascertained that Labatt had that year become Canada's largest brewing company, with eight breweries located from Vancouver Island in British Columbia to St. John's in Newfoundland. It was also embarking on a diversification program into the food manufacturing sector in both Canada and the United States (flour milling, malting, wheat starch upgrade, wine production, milk processing [including the manufacture of magnificent Canadian cheddar cheese and ice cream], and candy production and the retail sale of it). This diversification would eventually contribute to Labatt's demise because the brewing executives (with a few notable exceptions) really knew only about the brewing business (details later).

If I had written an advertisement for a position that was ideal for me at that time it would have been this one! The successful

candidate should develop his/her own research program, would be able to employ two or three technicians, and “adequate” funds would be available to conduct the research project(s) in a reconstructed, well-equipped laboratory with the flexibility to purchase further equipment as required. Also, developing relationships with Canadian universities and government research institutes was a primary requirement. I had always wanted to visit and live for a time in Canada, and Olga shared this aspiration.

For the next two days, Olga and I talked and thought of little else. We decided that I should apply for this position. Consequently, I sent a resumé and reprints of my yeast publications (9,79) to the Labatt Research Manager, Bert Shelton. Within two weeks (no e-mail facilities in those days), I received a reply from Bert stating that before the end of the year he would visit Britain and could we meet and discuss the research microbiologist’s position further? Shortly thereafter he wrote again saying that he was not coming to Britain, but in the New Year, at Labatt’s expense, would I travel to Canada for a three-day interview? I willingly agreed to go, and at the end of March 1969, I traveled to Ontario.

INTERVIEW AND MOVING TO CANADA

My knowledge of the brewing process was basic, although, like most students, I had been an enthusiastic beer drinker since my late teens. Indeed my involvement with the brewing industry stemmed from birth because my maternal grandparents and subsequently their daughter (my mother’s sister) were tenants of a public house in the docks area of Cardiff, and my mother and I lived there for most of the last three years of WWII while my father was in the RAF. Before I went to Canada for the interview, I undertook a crash reading course in basic brewing. I should have also visited a brewery but did not think of this!

In late March 1969, I flew to Toronto and on to London, ON. This was only the second time I had flown. The first time was five years previously when I flew from Southend to Ostend in a DC3 en route to a vacation in Dubrovnik, which was then in Yugoslavia (now in Croatia). I arrived in London, ON, late on a Sunday evening. It was snowing (hard by my standards—I was soon to learn that by local standards not hard at all!). I was met at the London airport by Bert Shelton, and he presented me with my itinerary for the next three days, which were going to be very busy!

My three days in the Labatt brewing research department and the London, ON, brewery were very intensive. They consisted of a series of interviews, tours of the facilities, lunches, and dinners. The hospitality I received was very generous (particularly during lunches and dinners). Although London, ON, at that time, did not contain any world-class restaurants (times have changed), everyone was very welcoming. On my last evening in the city, I was taken to a restaurant that also functioned as a night club. One of our party, a brewer in the London brewery (who, I am afraid, is no longer with us), had a reputation of being able (sometimes) to remove the cloth from the table without disturbing the crockery and cutlery. After considerable discussion, Bert Shelton persuaded him not to attempt his party piece that evening. A few years later, at the same restaurant when we were entertaining visitors from the U.K.’s Allied Breweries, he could be restrained no longer—with disastrous results!

I learned that the long-term objective of the position was to develop a “world class” research group studying brewer’s yeast. Also, where appropriate, it was expected that the findings would be published at brewing and related conferences and in peer-reviewed journals. A major project within the company at that time was the development of a continuous fermentation process for beer production. They were encountering problems with the yeast; it could have been some form of mutation but no one was sure. Also, if I was offered (and accepted) this position, Shelton

wanted me to visit a number of research laboratories (industrial and academic), in the U.K. and continental Europe that were interested in yeast research. The objective was to make contacts and develop background knowledge of ongoing relevant yeast research.

At the end of my three-day visit, during the concluding discussion with Bert Shelton, he presented me with a letter that contained a job offer. It was to be Labatt’s research microbiologist with the amazing salary (by my standards at the time) of \$CA13,000 (£6,500 or US\$11,500 p.a.). This was over three times more than I was currently being paid in Portsmouth. Also, travel expenses for my wife and me together with transport costs for our effects (the little we had in those days) would be paid. He asked me to let him have my decision within seven days by telegraph.

I returned to Britain with only one thought in mind: “What should we do?” Olga was very supportive, although we knew we would be embarking on a complete change of life. All of my family (and my wife’s) had reservations, although I could not say that they were negative, only apprehensive. The only exception was my father. His advice was, “If you do not accept this offer, you will regret it for the rest of your life.” He was right! Olga agreed that I should accept the position. We also thought we would move to Canada for about five years, but this was certainly not “cast in stone” and proved to be very different from what actually occurred (read on!). Indeed, we became Canadian citizens in 1979. The only “condition” that she established was that we would travel together to Canada and we would fly there. We had an opportunity to cross the Atlantic by sea, but (quite rightly) she did not want the time during the crossing to think about matters relevant to leaving the U.K., especially family.

I accepted the position, and it was agreed that we would move to Canada at the end of August 1969. Before this, I had to resign my position in the School of Pharmacy (not well received by some) and sell a house that we had only purchased eight months previously. We also had to obtain immigrant status to move to Canada. This involved a visit to and interviews at Canada House in London, England, medicals, and the completion of several forms by myself and Labatt. I embarked on visits to a number of research centers in the U.K. but only had time for one visit in Sweden (details of the visits and resulting discussions come later).

I left Portsmouth with some reluctance. The college had been a very interesting place, with increasing teaching and research aspirations (unusual in those days because it really was a further-education college funded by the local authority). The college and local dramatic societies (essentially the same people) introduced me to Gilbert and Sullivan operettas. This enjoyment of Gilbert and Sullivan continued when we lived in Canada, and we attended many G&S professional performances at the Stratford Ontario Festival Theatre.

In September 1968, I was joined by Steve Nahorski as a research student. That summer he had graduated from the University of Southampton with a pharmacology honors degree. When I left Portsmouth, Ed Abbs assumed responsibility for supervision of the research that considered neurochemical effects of convulsants in rats. In the short time that we were together, we three had been able to coauthor a peer-reviewed paper (39). Steve completed and defended his Ph.D. degree within three years and went to the States for three years as a post-doc. Upon his return to the U.K., Steve was appointed to an academic position at the University of Leicester and became professor of pharmacology there when he was only in his late thirties. He held this position until he retired a few years ago.

LABATT—MY FIRST FIVE YEARS

My first day working at Labatt brought me some surprising news. Bert Shelton informed me that it had been decided for proc-

ess cost and product-matching reasons to postpone (probably cancel) the development of continuous brewing processes, including fermentation. Consequently, at that time, research on yeast mutation in continuous systems would be unnecessary. However, I was quickly placated. The position of research microbiologist was still required, and for the next few weeks my objectives were to prepare a report on my visits to yeast research facilities in Europe and to develop, following discussions with appropriate Labatt colleagues, a listing of potential yeast research projects that I regarded appropriate.

Consequently, I spent the first month of my employment at Labatt writing this report (63) and talking to a large number of appropriate Labatt people. During the U.K. and Swedish tours, I had visited the following organizations: Courage, Barclay and Simmonds Ltd., London; A. Guinness & Son Ltd., Park Royal, London; Whitbread & Co. Ltd., Chiswell Street, London; Truman Brewery, Burton-on-Trent; Bass Brewers, Burton-on-Trent; Allied Brewers, Burton-on-Trent; Pripp-Bryggerierne AB, Stockholm, Sweden; British School of Malting and Brewing, Birmingham University; Bath University; University of Strathclyde; Queen Mary College, University of London; The Brewing Industry Research Foundation, Nutfield; The Distillers Co. (Yeast) Ltd. Surrey; Epsom, Surrey; Corn Products Ltd., Manchester; and Heriot-Watt University, Edinburgh.

I had received a cordial reception from all these organizations, and they were open with me in that they seemed to freely share information. At the time, in my naivety regarding brewing and associated organizations, I found this attitude very surprising! This open attitude has been confirmed on numerous occasions over the years, and I am proud that Labatt has been able to reciprocate to many brewing (and other) colleagues during subsequent years. However, as a result of the mergers and consolidations that have occurred between brewing companies subsequently, the industry is not nearly as open and welcoming as it was during my first two decades or so of employment by Labatt.

I developed a list of possible yeast research topics:

- Flocculation
 - Instability
 - Environmental conditions
 - Cell wall structure
 - Fermentations—cytoplasmic components
- Metabolic differences between lager and ale yeast strains
- Strain composition of the Labatt ale yeast culture
- Establish and begin to assemble a Labatt Yeast Culture Collection

At a meeting of senior technical and production staff to discuss my report, it was decided that the initial laboratory priorities were to study the strain composition of the Labatt ale yeast culture and to begin assembling a Labatt Yeast Culture Collection.

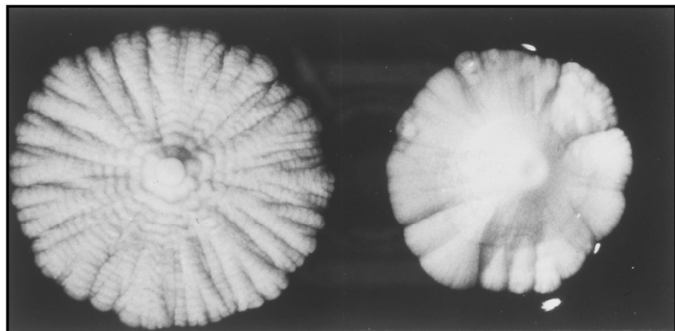


Fig. 1. Giant colony morphologies of Labatt ale yeast strains. Strain LAB A/69 (right colony). (Reproduced, by permission, from Stewart, 2009.) (71)

I could not conduct this agreed research program single handed! Consequently, during my first 10 months at Labatt, I was able to employ two technicians, Ivan Garrison (a biochemistry graduate from the University of Western Ontario) and Inge Russell (a medical laboratory technologist).

We focused on the top-cropping ale yeast culture because, in the 1960s and 1970s, ale consumption was popular in Canada; in 1970 it represented 60% of the beer consumed in Ontario and 80% in Quebec. However, similarly to the situation in Britain, the ale yeast cultures employed were largely uncharacterized. (This was not true of the Labatt lager yeast.) Also, no Labatt employee could tell me much about the history of this ale culture. The Labatt ale culture possessed classical top-cropping properties. It also exhibited intermittent premature flocculation characteristics, resulting in under-fermented worts containing residual sugars (mainly maltotriose). This was a problem in Canada because a $\pm 0.2\%$ (v/v) alcohol specification was the legal variation, and the beer's alcohol composition was on the label of the bottle or can. This problem was exacerbated during the high-gravity brewing trials that were ongoing when I joined Labatt (details later). It was of interest to enumerate the number of strains in this ale culture and to characterize them.

Coflocculation

One of the most suitable methods to examine culture composition available in the late 1960s for this purpose was the giant colony morphology method (50). This method involved inoculating the yeast culture onto wort solid media and examining the colonial morphology that developed after incubating under standard conditions for at least 3 weeks at 18°C. It had been found that gelatin, as the solidifying matrix, tends to enhance the distinctive features of the colonial morphology to a greater extent than agar and that wort, instead of a synthetic medium, gave distinctive and reproducible results.

Analysis of the Labatt ale culture's strain composition showed that two morphologically different colony types were present (Fig. 1) (66). On isolation, both colony types proved to be stable respiratory-sufficient separate strains of the species *Saccharomyces cerevisiae*, and they were coded LAB A/69 and LAB B/69, with the former strain being ~75% of the ale culture and the latter ~25%. When the London Brewery head brewer (Ernie Cowman) and the quality control manager (Harvey Hurlbert) were informed that their ale culture was a mixture of two distinct strains, they wanted to conduct brewing trials with the strain that was 75% of the culture (LAB A/69). I thought this trial was a little premature, but they would not take no for an answer! Consequently, a fermentation trial was conducted in a 200-hL open wood fermenter with a 12°P wort and ale yeast strain LAB A/69. Although the fermentation was under-pitched, it proceeded rapidly, and all the fermentable sugars were metabolized in 96 h. Then the problems began: a yeast head failed to develop on top of the fermentation. We waited and waited but to no avail; all the yeast remained in suspension. Ernie Cowman and Harvey Hurlbert were not pleased but were forgiving. As the brewery (at that time) did not possess a centrifuge, it was not possible to remove the yeast in suspension, and the fermentation had to be discarded into the sewer at the brewery's expense. We resumed the laboratory-scale characterization of both ale yeast strains.

When the two strains were cultured alone in wort, both were non-flocculent during all phases of growth. However, when cultured together in wort in a 1:1 ratio, the culture was flocculent in the later stages of fermentation and sedimented out of suspension (Fig. 2). This type of behavior, where two yeast strains are nonflocculent alone but flocculent when mixed together (65,83), has been termed *coflocculation* (64,82). Coflocculation has also been termed *mutual aggregation* and *mutual flocculation* (22,106,108).

The giant colony morphologies (Fig. 1) of the two Labatt ale yeast strains have played a very important part in my professional life. Also, in my and my wife's opinion, the colonies, side by side, are very pleasing on the eye! Consequently, a photograph of the two colonies has been enlarged, framed, and now hangs on a wall in the living room of our home.

To date, coflocculation has been observed only with ale strains, and there are no reports of coflocculation between nonflocculent lager yeast strains. Another type of coflocculation reaction that has been described is that in which an ale yeast strain has the ability to aggregate and co-sediment with contaminating bacteria such as *Hafnia protea* (106), *Lactobacillus brevis*, *Pediococcus* sp., and *Lactobacillus* sp. (111) (Fig. 3). The *Lactobacillus* sp. strain was isolated from a fuel alcohol fermentation in Brazil and its coflocculation characterized in the Labatt laboratories (111).

It was decided that the two-strain composition of the Labatt production ale culture was undesirable, particularly because of its tendency for premature flocculation and wort under-attenuation, resulting in failure to meet the beer's alcohol specification (this was before the introduction of high-gravity brewing on a production basis, when the problem was exacerbated). Production trials with LAB A/69 strain were conducted in both regular (sales) (12°P) and higher-gravity (16°P) worts. This strain proved to be capable of successfully fermenting both wort gravities but, because of its nonflocculent property, centrifugation was required in order to harvest the culture for yeast removal, beer clarification, and yeast collection for reuse. This strain has been employed for ale production by Labatt with high-gravity worts for the past 30 years and was one of the reasons for the introduction of centrifuges into Labatt plants (more details later).

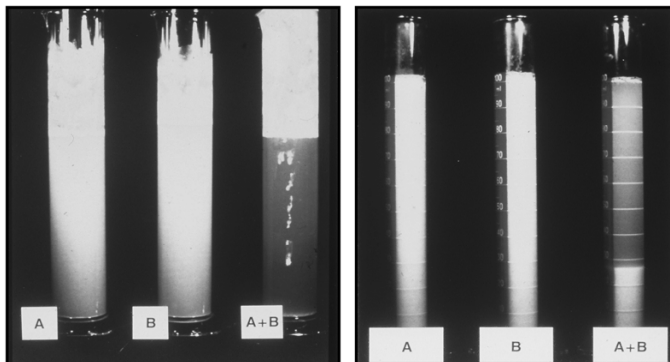


Fig. 2. Coflocculation between two ale yeast strains (A and B). **A**, 2-L static wort fermentations. **B**, Helm's sedimentation test. (Reproduced, by permission, from Stewart, 2009.) (71)

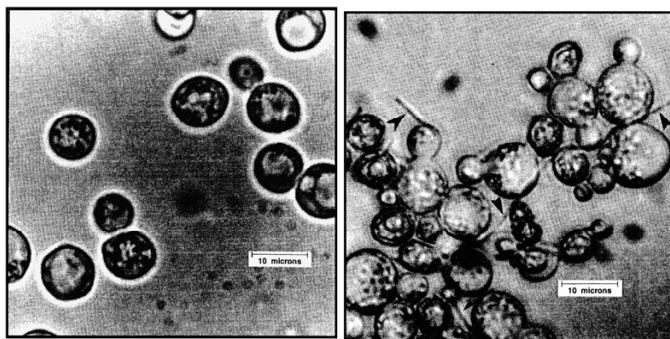


Fig. 3. Bacterial-induced yeast flocculation. *Lactobacillus fermentum*, strain 125. Arrows indicate bacterial bridges. (Reproduced from Stewart, 2014.) (76)

Pure-Strain Flocculation

A degree of confusion has arisen by the use of the term *flocculation* in the scientific literature to describe different phenomena in yeast behavior. Flocculation, as it applies to brewer's yeast, is "the phenomenon wherein yeast cells adhere in clumps and either sediment from the medium in which they are suspended or rise to the medium's surface" (89). It was logical to extend our studies to pure-strain flocculation. The term *nonflocculation* applies to the lack of cell aggregation and, consequently, a much slower separation of (dispersed) yeast cells from a static liquid medium. Flocculation usually occurs in the absence of cell division (but not always) during late logarithmic and early stationary growth phases and only under rather circumscribed environmental conditions involving specific yeast cell surface components (proteins and carbohydrates) and the interaction of calcium ions (details later). Although yeast separation often results in sedimentation (bottom-cropping), as already described in the discussion of coflocculation, it may also result in flotation because of cell aggregates attaching to CO₂ bubbles. These are top-cropping ale brewing strains (89).

Calcium Adsorption and Flocculation

The importance of calcium ions during yeast flocculation cannot be over emphasized (94,95). With many flocculent strains, the calcium can be removed from the yeast cell wall by washing with deionized water, and the culture will become reversibly nonflocculent (95). If calcium is added to this deflocculated culture, the cells become flocculent again (Fig. 4). Some flocculent strains are not deflocculated by washing with water; the cells need to be treated with a solution of a chelating agent such as ethylenediaminetetraacetic acid (EDTA) followed by washing with water to remove the EDTA. This treatment deflocculates these cultures, and the flocculation phenotype is restored upon addition of calcium ions. It has been suggested that cell walls isolated from flocculent cultures bind more Ca⁺⁺ ions than walls isolated from nonflocculent cultures. Employing radiolabelled Ca⁴⁵, we conducted studies to compare the calcium-binding ability of several ale and lager flocculent and nonflocculent brewer's yeast cultures (95). When the final calcium uptake of each culture was analyzed, it was clear that no direct correlation existed between the total cal-

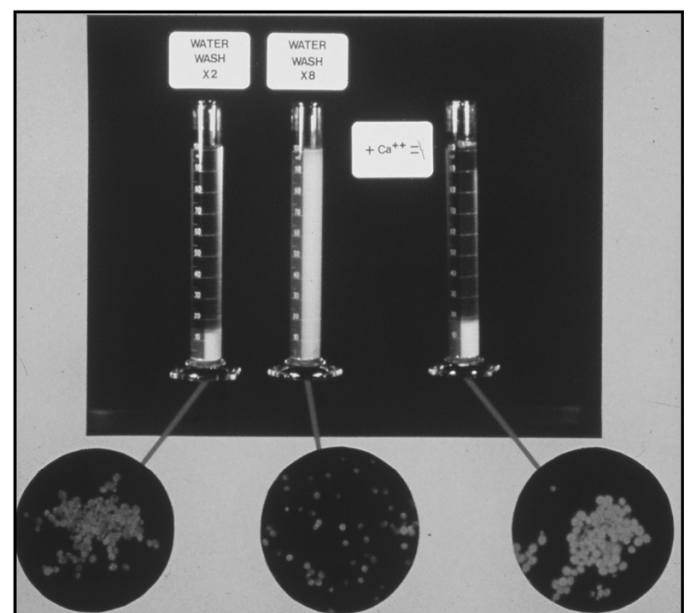


Fig. 4. Water wash deflocculation. (Reproduced, by permission, from Stewart, 2009.) (71)

cium adsorbed and flocculation. There is yeast strain-to-strain variation in calcium binding, and furthermore this variation does not correlate with flocculation and nonflocculation when one strain is compared to another. However, with the knowledge that many flocculent yeast cultures can be deflocculated by washing with deionized water (Fig. 4), we thought that if one could correlate the amount of calcium washed off a yeast culture with the visible loss of flocculation, an improved perspective of calcium-binding behavior in yeast and its relationship to flocculation might be obtained.

To test this hypothesis, aliquots of flocculent and nonflocculent yeast suspensions were taken after 120 min of incubation with the Ca^{45} solution and centrifuged. The yeast pellet was washed four times with 2 mL of deionized water, pH 4.0, for 15 s on a Vortex mixer and the activity of each centrifuged supernatant determined with a scintillation counter. Using standard curves relating calcium concentration to radioactivity, the amount of calcium removed with each washing was determined. The first wash did not deflocculate the flocculent yeast cultures but did remove loosely adhering calcium around and in the interstitial spaces between the yeast cells. This source of calcium should be relatively the same

TABLE I
Calcium Removed from Flocculent and Nonflocculent Cultures During Deflocculation Washings

Yeast culture	Flocculation characteristic	Total calcium washed off yeast (mg/100 mg of dry weight of yeast)
Ale	Nonflocculent	18
Ale	Nonflocculent	19
Ale	Flocculent	30
Ale	Flocculent	42
Lager	Nonflocculent	12
Lager	Nonflocculent	14
Lager	Flocculent	20
Lager	Flocculent	22

percentage of total calcium bound for each yeast culture and is in all probability not related to flocculation, since the visible observation of flocculation did not disappear during this first wash.

The subsequent washings gradually removed any observable flocculation. The sum of the calcium removed in washings 2 to 4 were expressed as a percentage of the total calcium removed during washing. When the results were expressed in these terms (Table I) for flocculent and nonflocculent cultures, the flocculent cultures were found to have bound 28 to 40% more calcium after four water washings than did nonflocculent cultures. As would be expected, there is strain-to-strain variation in calcium adsorption. This variation is in all likelihood a reflection of diversities in cell wall structure from strain to strain. In addition, this strain-to-strain variation in calcium adsorption *per se* does not correlate with the flocculation phenotype when one strain is compared to another. The only meaningful measure of calcium behavior that correlated with flocculation was the ease with which calcium was washed off the cell, and this coincided with the visible loss of flocculation.

Yeast Flocculation and Cell Surface Fimbriae

The yeast cell wall is a complex structure consisting of mannan, glucan, protein, chitin, lipid, and a number of other compounds (34). Flocculation requires the presence of cell surface proteins and mannan receptors (21). Studies in collaboration with Alan Day, of the University of Western Ontario, employed electron microscopy with shadowing by tungsten oxide to show that flocculent cultures possess a "hairy" outer surface due to the presence of fimbriae, whereas nonflocculent cultures do not (21) (Fig. 5). This observation has been reconfirmed by subsequent studies (62,103) in other laboratories.

Genetic Control of Yeast Flocculation

Genetic studies on yeast flocculation began more than 60 years ago. Such investigations involving brewer's yeast strains are

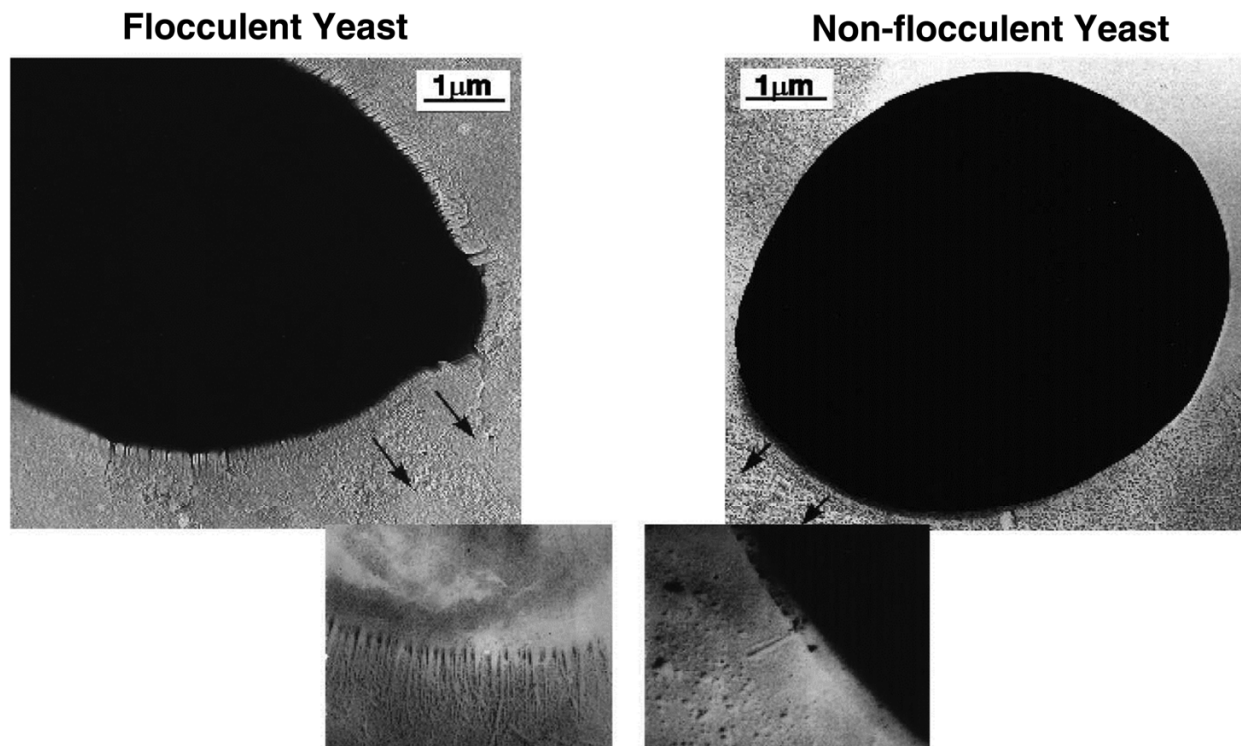


Fig. 5. Electron photomicrographs of *Saccharomyces cerevisiae* flocculent and nonflocculent shadows, cast with tungsten oxide and showing surface fimbriae. (Reproduced, by permission, from Stewart, 2009.) (71)

fraught with difficulty because of their frequent triploid, polyploid, or aneuploid nature (42). Consequently, we focused on haploid and diploid flocculent and nonflocculent strains. The flocculent haploid strain (coded 169) was of the mating type opposite to that of the nonflocculent haploid strain (coded 168). These two strains were mated using micromanipulation techniques, and the resulting diploid hybrid (169/168) was found to be flocculent, confirming previous findings that the flocculent character was dominant and stable (88).

Tetrad analysis of spores isolated from asci of the 169/168 hybrid revealed that the dominant flocculence of strain 169 was controlled by a single gene locus (i.e., 2:2 segregation). This gene has been coded *FLO1*. The next question was the location of the gene on one of the 16 chromosomes of *Saccharomyces*. A detailed discussion of the chromosome mapping procedures employed in this study is beyond the scope of this article. Suffice to say, we showed that *FLO1* is located on the right-hand side of chromosome I, 33 cM from the centromere (54).

The mapping of the *FLO1* gene employed traditional gene mapping techniques (mating, sporulation, micromanipulation, tetrad analysis, etc.). Today novel genetic techniques have been developed, the principle of which is the sequencing of the *Saccharomyces* genome (38). This has expanded our knowledge of the genetic control of yeast flocculation. Flocculation genes identified to date include *FLO1*, *FLO2*, *flo3*, *FLO4*, *FLO5*, *flo6*, *FLO7*, *FLO8*, *FLO9*, *FLO10*, *FLO11*, and *MUC1* (104). Of all the flocculation genes identified, *FLO1* is the most extensively studied and perhaps the most important and capable of conferring flocculation when transformed into nonflocculent *S. cerevisiae* strains.

Several years later, Robert Stewart (no relation), a National Sciences and Engineering Research Council (NSERC) post-doc, isolated and characterized *S. cerevisiae* cell wall proteins from *FLO1* yeast cells grown into the stationary growth phase. Preliminary results suggested that a putative *FLO1* protein (with a predicted molecular mass of 93 to 150 kDa) might have a regulatory role in flocculation, rather than mediating directly in lectin-carbohydrate interactions (100). Unfortunately, due to significant changes in the Labatt R&D philosophy (details later), this research project did not continue past the publication.

RESEARCH MANAGEMENT

One afternoon in late November 1973, I received a phone call requesting that I come to the office of Labatt Breweries Vice President of Production Fernand Loranger. When I arrived, Bert Shelton was also there. It was explained that Bert Shelton was going to become John Labatt's Director of Food Research (he had previous experience in the food industry working for Cadbury in the U.K.). As a consequence, there was a vacancy for the research manager's position in the Brewing Research Department. Would I be interested in assuming this position? The answer was an immediate yes, but, I said that I would like to continue my yeast research and, as there would now be a vacancy in the Brewing Research Department, could this be filled with a microbiology research technician reporting to me? They agreed to this, and on January 1, 1974, I became Labatt Breweries of Canada's research manager. Soon I employed Tom Goring, another medical laboratory technologist, to fill the vacancy.

There were three other contenders for the research manager's position, and I knew they would be disappointed. I tried to placate them, and, with all but one exception, they said they would support me. The exception said that he was very disappointed not to be appointed research manager and could not understand the decision. He informed me that he would seek a new position outside Labatt. Before the end of 1974, he left Labatt for another position in Winnipeg, MB.

I wanted to enlarge the department with more Ph.D. scientists and technical support staff, but I knew that, at that time, persuading the senior Labatt Breweries management to agree to this proposal would be difficult. Labatt Breweries had established its current Research Department in the late 1960s, and five years later, with a few minor exceptions (for example, the characterizing of the ale culture, the start of basic studies on high-gravity brewing, and some progress on the elucidation of the mechanisms of beer physical and flavor stability—details later), there was little to show for the significant expenditure employed so far! However, there was another way to obtain increased funds and personnel. The Canadian Federal Government (particularly the National Research Council [NRC] and NSERC) funded industrial research in Canada, particularly if it was in partnership with a university or a government research institute. It has been cynically said that “the only Canadian scientist in the 1960s and 1970s was one who had not been offered a job in the United States!” The government's financial assistance was to minimize this situation.

One of these programs was the NSERC Industrial Postdoctoral Fellowship (PDF), in which Canadian scientists with Ph.D.s would work in approved companies in Canada on a 2- to 3-year contract. NSERC paid 75% of their salary and the company contributed the rest of the salary and benefits, etc. We were able to make extensive use of this program, and over the next 20 years employed the following PDFs: Chandra Panchal, Michael Sills, Terry Dowhanick, Carl Bilinski, Tony D'Amore, Joseph Odumeru, John Williams, Yoseph Haj-Ahmed, Robert Stewart, Glen Austin, Luc Bordeleau, Sylvain Norton, and Xen Zheng. In addition, we received grants from NSERC to fund the employment of undergraduate students on cooperative assignments. We also received funding from the NRC's Industrial Research Assistance Program and the Agriculture Canada Research Council. This allowed us to significantly expand the research program (details later) to satisfy the expectations of the senior Labatt management with regard to their Research Department and keep the expenditures constant. Also, we published a number of peer-reviewed papers, books, review papers, and patents and presented papers at brewing conferences in North America, Europe, and the Far East.

Over the years, I have been fortunate to receive a number of awards (some of which are listed as a footnote at the beginning of this paper). Perhaps the most important award, particularly with respect to the position and reputation of the Labatt Breweries Research Department within the company and outside, was the Master Brewers Association of the Americas (MBAA) Schwartz Award, which I received in 1976. Notification of it came completely “out of the blue.” A letter from the president of the MBAA informed me of the award, and I was invited to present a lecture at the 1976 MBAA conference and to publish it in the MBAA *Technical Quarterly*. The 1976 conference would be held at Disney World in Orlando, FL. Apparently, the genesis of this award was a paper I had given in the 1975 MBAA Canada Western District summer meeting held in Winnipeg, MB.

Disney World in Orlando had only been operating for a couple of years and was regarded as a very iconic place to visit. A large number of Labatt plant managers and brewers, together with their families, attended the 1976 meeting. I had been research manager only for a couple of years and, although I knew most of them by name, I had met only a few of them. Quite frankly, several of them were very surprised that the most important presentation of the meeting was being given by someone from Labatt. The paper I gave had the rather unimaginative title of “Fermentation Yesterday, Today and Tomorrow” (67). All of a sudden, Labatt had a Research Department with a burgeoning international scientific reputation!

As the number of Labatt publications increased, scientists and brewers from all over the world asked if they could visit the de-

partment, and some even wanted to spend their sabbatical leave with us. I welcomed these approaches, but I was doubtful whether the Labatt Management Committee would agree to this. I was very surprised that they (with a minority of dissenters) welcomed this proposal as long as secrecy agreements were signed to ensure that results and information that visiting scientists became aware of were kept confidential. This did not cover their own research but details they acquired “on the side” (during coffee breaks, lunch, over a beer, etc.). During the ensuing years, we welcomed visiting scientists from Brazil, Argentina, Scotland, India, Ljubljana, Croatia, Cuba, Australia, England, the United States, Japan, and various parts of Canada.

The 1980s and 1990s were the decades of “the new biotechnology,” in which the potential of genetic manipulation techniques (particularly recombinant DNA) was being recognized. This was occurring in the pharmaceutical, agricultural, energy, environmental, waste disposal, food/beverage, and many other industries. The development of large-scale production techniques (substrates, media preparation, fermentation, downstream processing etc.) was (and still is) a particular challenge. The brewing industry had already solved many of these challenges. As a consequence, we were invited to discuss our experiences as a brewery, with an active R&D function, at biotechnology conferences in North America and overseas (43,69,80,87).

STORAGE AND PRESERVATION OF STOCK YEAST CULTURES

With the advent of the use of ale yeast single strains along with lager strains, some required for contract brewing, the implementation of a sophisticated yeast storage procedure was required. For long-term preservation of brewing yeast strains, not only is optimal survival important, but it is imperative that no change in the character of the yeast strain occurs. Many yeast strains are difficult to maintain in a stable state, and long-term preservation by lyophilization (freeze drying), which has proven useful for mycelial fungi and bacteria (33), has been found to give poor results with brewing yeast strains (32). Storage studies have been conducted with several ale and lager brewing strains (55,105). The initial studies on yeast storage in liquid nitrogen were conducted in collaboration with Angela Wellman of the University of Western Ontario (105).

After a two-year storage of yeast, we conducted wort fermentation tests, including fermentation rate and wort sugar uptake efficiency, flocculation characteristics, sporulation ability, formation of respiratory-deficient (RD) mutants, and ease of survival and compared the results with the characteristics of the stored control culture. Low-temperature storage appears to be the storage method of choice if cost and availability of the appropriate equipment is not a significant factor (105). Cultures stored at -70°C or in liquid nitrogen at -196°C (99) had the lowest death rates and were the easiest to revitalize. Also, the degree of flocculation, wort fermentation properties, sporulation ability, and proportion of RD mutants present were all unaffected by this storage procedure. Storage at 4°C on nutrient agar slopes, with subculturing every 6 months, was the next best method after low-temperature storage. Lyophilization and other storage methods revealed yeast instability, which varied from strain to strain. Currently, many breweries store their strains (or contract store them) in -70°C refrigerators. Routine subculturing on solid media every 6 months, although less desirable, is a very cost effective and acceptable storage method. Lyophilization of brewer's yeast cultures should be avoided (55)!

The Labatt Yeast Culture Collection continued to grow. In the early stages, the cultures were stored on slopes at 4°C and routinely subcultured. Subsequently, the cultures were stored in liq-

uid nitrogen until we acquired a -70°C refrigerator, which became the standard storage method. When I retired from Labatt in 1994 (details later), we had more than 2,000 yeast strains in the collection, under the efficient care of Inge Russell and Jadwiga Sobczak. It was Labatt policy to satisfy requests for strains from all over the world. Upon retirement, I mistakenly did not take any cultures with me to Edinburgh. All was well until Inge Russell prematurely retired from Labatt. After that I was informed that cultures were no longer available to me. I do not know what the state of the collection is today, but I fear the worst.

GENETIC MANIPULATION OF BREWER'S YEAST STRAINS

The behavior, performance, and quality of a yeast strain are influenced by two sets of determining factors, collectively called nature-nurture effects. The nurture effects are all the environmental factors (i.e., the phenotypes), to which the yeast is subjected from pitching onward. On the other hand, the nature influence is the genetic make-up (i.e., the genotype) of a particular yeast strain. Over the years, we have studied both nature and nurture effects of brewer's yeast strains during wort fermentation (76,96).

In 1988, I stated (70),

The use of manipulated yeast strains in brewing will become commonplace within the next decade with yeast strains specifically bred for such characteristics as extra-cellular amylases, β -glucanases, proteinases, β -glucosidase production, pentose and lactose utilization, carbon catabolite repression and production of a plethora of heterologous proteins. There is no doubt that before the introduction of such strains at the production level, the environmental and legal impact of such a move will have to be assessed.

Nearly 30 years later, genetically manipulated brewer's yeast strains are not employed commercially, due in large part to negative public opinion. Whether this will change, only time will tell. Nevertheless, genetic techniques have been extensively used to study the genetic composition and function of such strains (85).

Several methods can be employed in the genetic research and development of brewer's (68) and related yeast strains (28). Classic approaches to strain improvement include mutation and selection, screening, and cross-breeding (hybridization) (65,86). The use of hybridization to map *FL01* on chromosome I of the *Saccharomyces* genome has already been discussed (54). Mutation is any change that alters the structure of the DNA molecule, thus modifying the genetic material. The mutated strains often no longer have the desirable properties of the parent strain, exhibiting slower growth rates and producing undesirable taste and aroma compounds during fermentation (23). Mutagenesis is seldom employed with brewing yeast strains due to their polyploidy or aneuploid nature (42).

Spontaneous yeast mutations commonly occur throughout the growth and fermentation cycle, but they are usually recessive, due to functional loss of a single gene (38). Because of the aneuploid or polyploid nature of most strains, the dominant gene functions adequately in the strain as it is phenotypically normal. Only if the mutation takes place in all complementary genes is the recessive character expressed. However, if the mutation weakens the yeast (which is usually the case), the mutated strain is unable to compete and is soon outgrown by the nonmutated yeast population.

The RD mutation is the most frequent mutant found in brewing yeast strains (60). This mutant arises spontaneously when a segment of the DNA in the mitochondria becomes defective and forms a flawed mitochondrial genome. The mitochondria are then unable to synthesize certain proteins. This type of mutation is also called the “petite” mutation because colonies of such a mutant are

usually much smaller than respiratory-sufficient cultures (also called “grande”). The RD mutation usually occurs at frequencies of between 0.5 and 5% of the population, but in some strains, levels as high as 50% have been reported (71). RD mutants can also occur as a result of deficiencies in nuclear DNA, but these are much rarer.

Deficiencies in mitochondrial function result in diminished ability to function aerobically, and as a result, these yeasts are unable to metabolize nonfermentable carbon sources such as lactate, glycerol, or ethanol (Fig. 6) (75). Many phenotypic effects occur as a result of this mutation, including alteration in sugar uptake (particularly maltose and maltotriose), by-product formation and subsequent metabolism (for example, diacetyl), and intolerance to stress factors such as ethanol, osmotic pressure, and temperature. Also, further to the discussion of storage and preservation of stock yeast cultures, RD mutants are difficult to store, although liquid nitrogen and -70°C refrigeration have both been found to be the most effective storage matrices (55). Flocculation, cell wall and plasma membrane structure, and cellular morphology are affected by this RD mutation (23). In addition, beer produced with a yeast culture that is RD will have flavor defects and present fermentation problems during production. For example, beer produced using these mutants contained elevated levels of diacetyl and higher alcohols (59). Wort fermentation rates were slower; higher dead cell counts were observed; and biomass production and flocculation ability were reduced (20).

As mentioned, in the late 1970s and early 1980s, Labatt began installing centrifuges in their breweries. This was to harvest ale and lager yeasts at the end of fermentation and for environmental reasons. All of a sudden in 1988, one of these breweries with a centrifuge (300 hL/hr) reported that its ale fermentations were exhibiting slower and incomplete fermentations with a 16°P wort. Closer study by Terry Dowhanick from our Research Department, revealed reduced wort maltose and maltotriose uptake rates, with residual sugars when fermentation ceased. Consequently, the alcohol specification was not achieved. In addition, diacetyl levels were elevated at the end of fermentation because of difficulties with reabsorption of vicinal diketones (VDKs). Also, there was more yeast autolysis, resulting in reduced foam stability due to excreted protease (details later), and elevated unfilterable haze consisting mainly of mannoproteins from disrupted cell walls.

The centrifuged yeast exhibited decreasing cell viability (determined with methylene blue staining) during increasing repitching cycles. Also, the same cultures had a higher percentage of RD mutants, determined with the triphenyl tetrazolium chloride overlay method (40) (Table II). This increasing RD level and decreasing viability were due to centrifugation when the exit temperature was 30°C . When the bowl of the centrifuge was cooled and the

exit temperature reduced to 20°C , the cell viability increased, the RD level dramatically decreased, and the wort fermentation characteristics returned to normal (Table II), making the beer drinkable again (73)! This is an example of dual stresses upon yeast, where centrifugation and an elevated exit temperature cause mutation of mitochondrial DNA, increasing the level of RD mutants. The mutants are unable to ferment wort efficiently, and the resulting beer has poor quality and drinkability.

The advent of the “new biotechnology” stimulated the development of novel methods of genetic manipulation (70)—spheroplast (protoplast) fusion and recombinant DNA. Spheroplast fusion is a technique that has been employed by us in the genetic manipulation of brewer’s yeast and related strains (53,85). It does not depend on ploidy and mating type and consequently has great applicability to brewing strains because of their polyploid nature and absence of mating type characteristics.

Details of the procedure can be found in reference 53. We also employed rare mating to produce novel strains, particularly to manipulate the killer characteristics of brewing strains (42). We conducted extensive studies using spheroplast fusion, but all of the novel yeast strains produced were unsuitable for brewing because the beers produced were undrinkable. However, some of the spheroplast-manipulated strains exhibited faster fermentation, temperature tolerance, and dextrin use (53,107). Indeed, one of these strains has been patented and sold to a company that supplies yeast strains to the fermentation fuel alcohol industry (98).

Recombinant DNA techniques can also be used to make thousands of copies of the same DNA molecule to amplify DNA, thus generating sufficient DNA for various kinds of experiments or analysis. Although my colleagues and I have not employed these techniques to improve brewer’s yeast strains, other groups (28,41) have successfully conducted such approaches.

It is surprising that recombinant yeast strains are not commercially in use today in either brewing or distilling, and indeed there is evidence that adverse public opinion is changing. Since the early days of yeast genetic manipulation, considerable activity has occurred, which has been succinctly described by Boulton and Quain (5). Permission was granted more than a decade ago from the appropriate authorities in the United Kingdom for the use of a baker’s yeast strain that is genetically manipulated for more rapid maltose use, leading to enhanced baking properties (28), and for a brewing strain, cloned with DNA from *S. diastaticus*, that secretes glucoamylase to utilize wort dextrins and produce low-calorie beer (28,71). The future prospects for the use of recombinant DNA with brewer’s and distiller’s yeast in the industry are unclear.

The sequencing of the *S. cerevisiae* genome began in 1989 and was completed with the publication of the sequence in 1996 (25). Although a major achievement, the DNA sequence of *S. cerevisiae* is relatively small, with a genome size of only 13×10^6 Mb with nearly 6,000 genes compared to the human genome, with a size of 3.3×10^9 Mb and over 21,000 genes (25).

The sequencing of the yeast genome in conjunction with gene expression analysis has enabled the identification of genes that

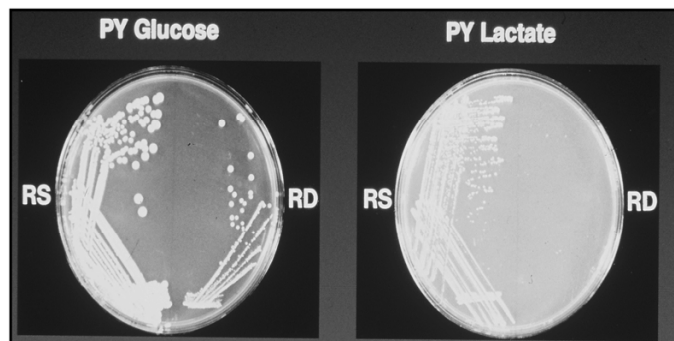


Fig. 6. Growth of respiratory-sufficient (RS) and respiratory-deficient (RD) cultures on fermentable (glucose) and nonfermentable (lactate) carbon sources. PY = peptone yeast extract. (Reproduced, by permission, from Stewart, 2009.) (71)

TABLE II
Effects of Centrifugation and Temperature on Respiratory-Deficient (RD) Mutants and Viability Levels of a Brewing Ale Strain

Treatment	RD ^a Mutants, %	Viability, ^b %
Freshly propagated culture	>1	98
Centrifugation with 30°C exit temperature (10 cycles)	28	72
Centrifugation with 20°C exit temperature (10 cycles)	8	84

^a With triphenyl tetrazolium chloride overlay.

^b By methylene blue.

have altered gene expression patterns in response to stressful environmental conditions (29). Tara Graves, an Alltech employee who completed an external Heriot-Watt University Ph.D. (26), studied the inhibitory effects of the stress factors most commonly encountered during alcoholic fermentation of corn mashes, in collaboration with International Centre for Brewing and Distilling (ICBD). The key stress-related yeast genes with different resistance levels to environmental stress were assessed. A “stress model” has been developed to assess yeast stress resistance and evaluate the suitability of a specific strain for use in industrial ethanol fermentations (27). This “stress model” could potentially be used for screening candidate yeast strains for relative stress resistance in the fuel ethanol industry and other fermentation industries where yeast encounters similar pressures.

Uptake and Metabolism of Wort Sugars, Amino Acids, and Peptides

Compared to other media employed for the production of fermentation alcohol (both industrial and potable), wort is by far the most complex. As a consequence of this, when yeast is pitched into wort, it is introduced into an intricate environment that consists of simple sugars, dextrins, amino acids, peptides, proteins, vitamins, ions (such as zinc, magnesium, manganese, calcium, sodium and potassium), nucleic acids, and other constituents too numerous to mention. One of the major advances in brewing science during the past 40 years has been the elucidation of the mechanisms by which the yeast cell utilizes, in an orderly manner, the plethora of nutrients in wort.

Wort Sugar Uptake

Wort contains the sugars sucrose, glucose, fructose, maltose, and maltotriose together with dextrin material. In the normal situation, brewing yeasts are capable of utilizing sucrose, glucose, fructose, maltose, and maltotriose in this approximate sequence (although some degree of overlap does occur), leaving maltotetraose and the other dextrins unfermented (20) (Fig. 7). A major focus of the Labatt Research Department for many years was the elucidation of mechanisms of wort sugar uptake (71,72,73,85).

Maltose and maltotriose are the major sugars in brewer's wort and, as a consequence, a brewing yeast's ability to use these two sugars is vital. This ability depends upon the correct genetic complement (81). Brewer's yeast cultures possess independent uptake mechanisms (maltose and maltotriose permeases) to transport the two sugars across the cell membrane into the cell (99,112). Once inside the cell, both sugars are hydrolyzed to glucose units by the α -glucosidase system. The transport, hydrolysis, and fermentation

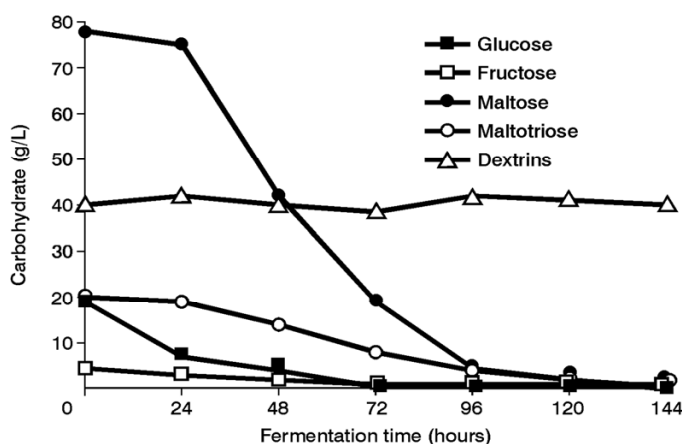


Fig. 7. Order of uptake of sugars by yeast from wort. (Reproduced, by permission, from Stewart and Russell, 2009.) (93)

of maltose are particularly important in brewing, Scotch whisky production, and baking, since maltose is the major component of brewing wort, spirit mash in Scotland, and wheat dough (101).

A major limiting factor in the fermentation of wort is the repressing influence of glucose (and possibly fructose) upon maltose and maltotriose uptake. The uptake of maltose commences only when approximately 50% (this is yeast strain and wort composition dependent) (81) of the wort glucose has been taken up by the yeast cells (Fig. 7). In other words, in most strains of *S. cerevisiae* and related species, maltose utilization is subject to control by carbon catabolite repression (57). In a similar manner, the presence of glucose represses the production of glucoamylase by *S. diastolicus*, thereby inhibiting the hydrolysis of wort dextrins and starch (24). Repression of this nature has a negative effect on overall fermentation rate.

We conducted studies in which glucose was added to fermenting wort when the yeast strain was metabolizing maltose and had already taken up all of the available wort glucose. The added glucose caused inhibition (repression) of the maltose uptake. Once this glucose had been taken up by the yeast culture the metabolism of maltose recommenced (20). To try to overcome this repression, the glucose analog, 2-deoxy-glucose (2-DOG) was successfully employed for the selective isolation of spontaneous mutants of yeasts (31) and other fungi. These mutants were derepressed for the production of carbohydrate-hydrolyzing enzymes employing this nonmetabolizable glucose analog, and derepressed

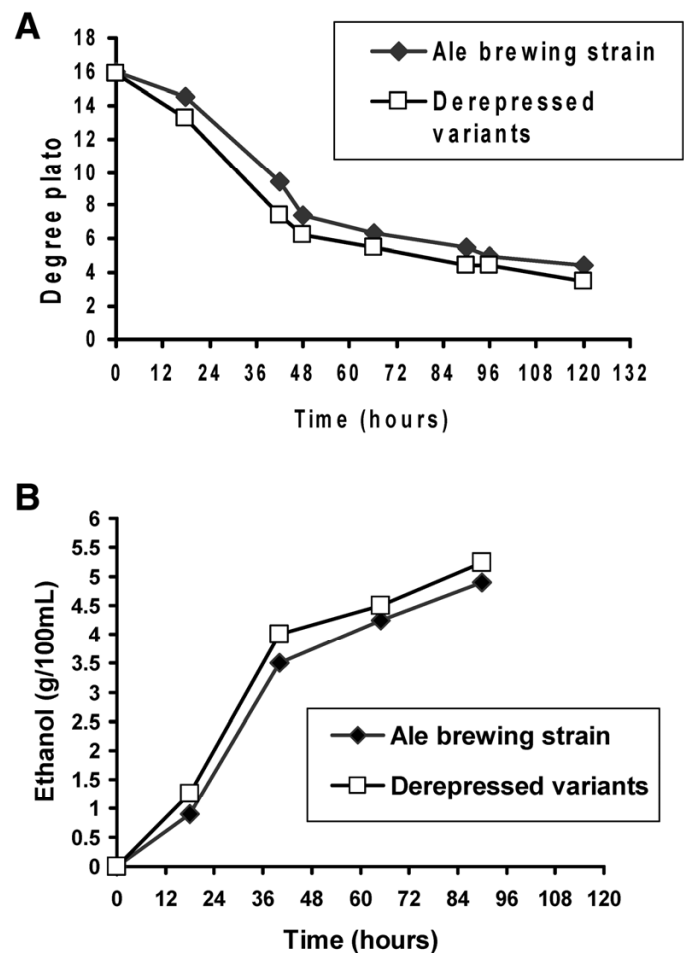


Fig. 8. Degree of Plato reduction (A) and increase in ethanol production (B) by an ale brewing strain and its 2-deoxy-glucose (2-DOG) derepressed variants. (Reproduced, by permission, from Stewart and Russell, 2009.) (93)

mutants of brewing and other industrial strains have been isolated that are able to metabolize wort maltose and maltotriose in the presence of glucose (Fig. 8). Fermentation and ethanol formation rates in 12°P wort were also increased in the 2-DOG mutants when compared with the parental strain. In addition, 2-DOG starch mutants of *S. diastaticus* have been isolated that exhibited increased fermentation ability in brewer's wort, cassava, and corn mash (31). Studies with 2-DOG spontaneous mutations were a major focus of Inge Russell's Ph.D. thesis (52). The research was conducted at Labatt, and the degree awarded by the University of Strathclyde in Scotland.

All of our studies with 2-DOG mutants were conducted with ale and distilling yeast strains. We were unable to isolate 2-DOG mutants from a range of lager strains screened. This is the major reason why large-scale trials were not conducted with 2-DOG mutants in a country (Canada) that, by the late 1980s, was predominantly a lager producer. Since our research, a major Spanish brewing company, with university collaborators, has reexamined the use of 2-DOG mutants to ferment 25°P wort, this time with a lager yeast strain (46). Stable 2-DOG mutants of their lager yeast strain were isolated. Their fermentation characteristics in 25°P wort using 2-L EBC tubes were assessed at 13°C. Improved fermentation capacity, where wort glucose did not repress maltose uptake, was achieved without changes in the beer flavor profile. However, the increased wort fermentation rate was not sufficient to introduce the 2-DOG mutants into commercial brewing.

Free Amino Nitrogen in Wort and Beer

Free amino nitrogen (FAN) is only a general measurement and is a "blunt" instrument for setting wort and, ultimately, malt specifications. At ICB, in collaboration with Scottish and Newcastle Brewery (now Heineken UK), we attempted to elucidate the role of different nitrogen wort components on yeast fermentation. We conducted static fermentations in 2-L cylinders using 15°P wort. Lager and an ale yeast strains were employed for fermentation at 13 and 20°C, respectively. Samples were taken throughout the fermentations, and yeast in suspension, specific gravity, total FAN ammonia, individual amino acids, di- and tripeptide levels, and proteinase activity were determined (36) (Fig. 9). A novel method for the determination of di- and tripeptides was developed (35). Following yeast removal and protein precipitation, the samples were filtered through an ultrafiltration membrane (molecular mass exclusion of 500 Da); hydrolysis

followed by HPLC was employed to determine the resulting amino acids.

The above results confirmed the Jones and Pierce studies, conducted in the 1960s (30), which showed that amino acid uptake can be divided into four groups (however, we propose that methionine uptake be moved from group B to group A, Table III) (84), with amino acid uptake completed, except for proline, within the first 48 h of fermentation. Peptide removal commenced during the first 19 h of fermentation and increased between 19 and 24 h of fermentation; between 24 and 67 h of fermentation, peptides decreased gradually (Fig. 9). The important finding is that yeast fermentation activity does not cease when wort FAN is depleted (83). During fermentation, oligopeptides are produced as a result of increased peptide hydrolysis due to protease excretion or secretion (Fig. 10). Both lager and ale yeast strains can simultaneously use amino acids and small peptides as sources of assimilable nitrogen. The implications of yeast proteinase secretion on beer foam stability, particularly during high-gravity brewing, is discussed later.

High Gravity Brewing

I have studied many aspects of high-gravity brewing research and brewing for most of the 45 years that I have been involved in the industry. When I arrived in Labatt in 1969, trials of high-gravity brewing of lager and ale were already ongoing. My Labatt colleague Egbert Pfisterer and I collaborated on studies of the influence of nitrogen concentration with high-gravity worts (45).

TABLE III
Revised^a Order of Wort Amino Acid Uptake During Fermentation

Group A: Fast absorption	Group B: Intermediate absorption	Group C: Slow absorption	Group D: Little or no absorption
Glutamic acid	Valine	Glycine	Proline
Aspartic acid	Leucine	Phenylalanine	
Asparagine	Isoleucine	Tyrosine	
Glutamine	Histidine	Tryptophan	
Methionine		Alanine	
Serine		Ammonia	
Threonine			
Lysine			
Arginine			

^a Revised from scheme of Jones and Pierce (30).

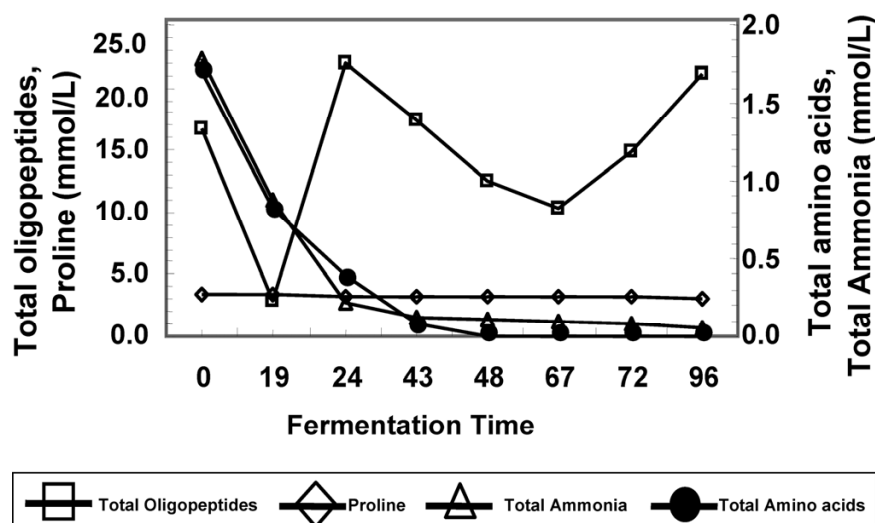


Fig. 9. Total nitrogen fermentation absorption profile for a typical lager yeast strain with 15°P wort. (Reproduced, by permission, from Stewart, 2009.) (71)

In addition, problems with the foam stability of high-gravity-brewed beers were identified together with stress effects on yeast (82). When I moved to Edinburgh, research on high-gravity brewing continued to be a major research focus (74), as we shall see.

CONTRACT AND LICENSED BREWING

When I became Labatt's technical director in 1986, one of the functions that became increasingly time-consuming was to oversee the technical aspects of the company's contract and licensing brewing commitments. Beer was brewed under license by Labatt on behalf of non-Canadian brewing companies for sale in Canada. During the 1970s, 1980s, and 1990s, Guinness, Skol, Carlsberg, Budweiser, and Suntory beer were brewed in a number of our breweries. The most successful and time-consuming beer brand was Budweiser, with a license from Anheuser Busch (A/B) of St. Louis, MO. The A/B relationship was very positive, enlightening, time-consuming, and sometimes frustrating.

First, as has already been discussed, centrifuges were installed by most Labatt breweries in the late 1970s and early 1980s for yeast cropping and clarification of fermented wort. Our A/B colleagues were apprehensive about this development and requested assurances that we would not centrifuge their beer at any stage of the production process. This assurance was readily given. Twelve months after this assurance, I received a telephone call from a senior A/B production executive enquiring about our use of centrifuges. I was very positive about their operation. I was then asked if we would be prepared to conduct centrifugation trials with their beer. I readily agreed to this request, and trials with Budweiser were successfully conducted in our brewery in Edmonton, AL. I believe that A/B has subsequently installed centrifuges in many of their U.S. breweries. This incident confirmed our impression that A/B sometimes considered some Labatt breweries to be pilot plants for their use and edification!

Second, when we performed brewing trials on Budweiser in our brewery in St. John's, Newfoundland, we encountered initial difficulties converting the rice in the cereal cooker. The temperature program was not right, and eventually we decided to suspend the trial. While we were discussing what to do next, the cooker's contents were inadvertently discharged into the sewer. The rice slurry flowed out of the brewery down a hill (St. John's is a very hilly city), and at its bottom the slurry forced open a manhole. The rice collected all over the road directly outside a Chinese restaurant. The owner of the restaurant was not amused!

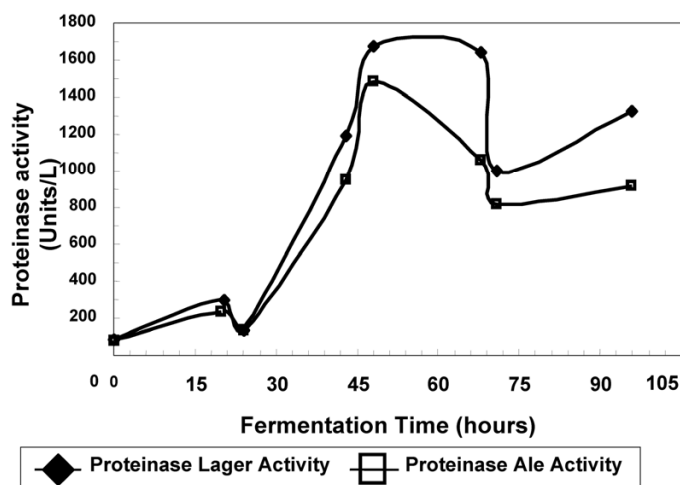


Fig. 10. Protease activity for ale and lager yeast strains during fermentation of a 15°P wort. (Reproduced, by permission, from Stewart, 2009.) (71)

We operated several contract agreements to brew Labatt's lager beer in the U.K. In this situation, Labatt leased time and space in some breweries, and we had responsibility for all other aspects of the brewing process. This included using the same lager yeast strain that was employed in Canada. Most of the contracted breweries employed vertical fermenters (some as unitanks). However, at that time, our breweries in Canada employed only horizontal tanks (as both fermentation and maturation tanks). This difference in tank geometry influenced the yeast culture's sedimentation characteristics. In vertical fermenters, this yeast culture was too nonflocculent (powdery), with too much yeast remaining in suspension at the end of fermentation (centrifuges were not available).

The studies that we had conducted in the 1970s on this strain's flocculation characteristics had indicated that cells, within the culture population, exhibited a spectrum of flocculation intensities. Consequently, Inge Russell and Jadwiga Sobczak isolated variants from this strain with more intensive flocculation characteristics, and one of these isolates was employed in the vertical fermenters. The result was less yeast in suspension at the end of fermentation. However, care had to be taken to ensure that the variant used was not too flocculent because underfermented wort and residual VDK levels could have been the result.

LEAVING LABATT AND CANADA

In 1988 I received a telephone call from an old friend, Charlie Brown, who at that time was head of the Department of Biological Sciences at Heriot-Watt University in Edinburgh. He informed me that the brewing group at Heriot-Watt, which had been in existence since 1903, was being reorganized and that distilling was formally being incorporated into the teaching syllabus and the research program. Also, new funding had been obtained from both brewing and distilling industries that had permitted the establishment of a center of excellence to be called the International Centre for Brewing and Distilling, which would have its own director and a Board of Management consisting of industry and university representatives. He inquired whether I would be interested in being considered for the director's position. I said I would give this proposal great thought and reply to him in a few days.

At that time, I was the Labatt Brewing Company's technical director with responsibility for research and development, quality assurance, technical training, government technical relationships and regulations, technical due diligence of potential acquisitions, and the sale of technology. As already discussed, my position description also included liaison with A/B because we brewed Budweiser under license and with other brewing companies. I was very busy and had an extensive travel itinerary. I was traveling over 100,000 miles a year. Also, we were about to have a new 16-hL pilot brewery installed into the R&D Centre in London, ON. The research program was intensive, with the generation of important new products (Genuine Draft, Ice Beer, .5 [a low alcohol beer], John Labatt Classic, and Suntory Beer), and results were directly relevant to the company. Also, our publication record was the envy of many in the industry. I discussed Charlie Brown's telephone call with my direct manager John Dunwell and with Sydney Oland, the president of Labatt Breweries. Both said that they wanted me to stay at Labatt and asked what could they do to convince me? I said that all I wanted was their continuing support. This was agreed upon, and I phoned Charlie Brown and said "thanks but no thanks." I thought this was the end of the matter, but a few months later "out of the blue" I was awarded some generous Labatt share options!

We live in an unstable and unsure world. In 1991 (only three years later) the John Labatt Ltd. situation was becoming con-

fused. This was not of direct consequence to the brewing division at the time. Indeed, matters could not have been much better: the launch of Ice Beer (already discussed) was a great success, the Labatt overseas ventures were, on the whole, thriving, and the Labatt Breweries owned a major league baseball team (the Toronto Blue Jays) that was en route to the World Series—which it won in both 1992 and 1993! The problems were coming from the Labatt food companies, whose sales were down and overhead costs were out of control. Also, most importantly, Brascan, which owed nearly 40% of Labatt stock, was encountering severe financial difficulties.

In order to understand this turn of events, regarding Brascan, a brief review of Labatt's history is appropriate. In the late 1940s, the Labatt Brewing Company became a public company with 49% of its shares becoming available on the Toronto Stock Exchange. In 1964, the Labatt family sold its shares (a controlling interest) to the Joseph Schlitz Brewing Company of Milwaukee (which at that time was the second largest brewing company in the United States). However, at that time Labatt possessed a controlling interest in a U.S. brewing company (the General Brewing Company) that operated in California and other states in the west of the United States. The U.S. Attorney General (Robert Kennedy) concluded that this was a conflict of interest (Schlitz also possessed considerable holdings in California) and stated that Schlitz would not be permitted to exercise any voting rights in Labatt nor would they be allowed to have voting directors on the Labatt board. Schlitz decided, after a period of uncertainty, to sell its Labatt shares. Jake Moore, the Labatt president and CEO (who had opposed the Labatt family sale to Schlitz) persuaded the Brazilian Traction, Light and Power Co. Ltd. (BTLP) to purchase Schlitz's shares.

BTLP was a Canadian company (founded in 1899) that provided electricity and tram services in São Paulo and Rio de Janeiro and that over the years had developed a broad spectrum of business interests in both Brazil and Canada (including a brewing company, Skol-Caracu). In 1969, BTLP changed its name to Brascan Limited and in 2005 to Brookfield Asset Management. The corporation is still based in Toronto.

In February 1992, Brascan, because of its financial difficulties, disposed of all its Labatt shares on the Toronto Stock Exchange to stabilize its cash flow (3). As a consequence, the ownership of John Labatt Ltd. was unclear, and eventually all of its shares had to be sold. To ensure an optimal share price, overheads were drastically reduced. This included the Labatt Breweries Research and Development Department. Consequently, in August 1993 I was asked to leave Labatt following 25 years of service, on the premise of constructive dismissal, and was offered a generous separation package that I could not refuse. Details of this package are beyond the scope of this review—suffice to say I was asked to stay until April 1994 to complete outstanding projects, including

due diligence assessments in Hungary, Cuba, China, and Italy as well as the sale of technology associated with Ice Beer.

As it happened, in late 1993, Charlie Brown (who by now was the University's dean of science) phoned again. He said that he had heard I was leaving Labatt and that the position of Director of ICBD was again vacant. Would I be interested in being considered this time? I immediately said yes and, following an interview in Edinburgh, became professor of brewing and distilling and director of ICBD on May 1, 1994. Labatt Breweries (from which all of the food companies had already been sold to other companies or were part of management buyouts) was acquired in 1995 by InterBrew, the Belgian brewing company, and is now part of Anheuser Busch Inbev.

THE INTERNATIONAL CENTRE FOR BREWING AND DISTILLING

For the next 13 years, my wife and I lived in Edinburgh (a beautiful city) and I worked from the Riccarton Campus of Heriot-Watt University. The wide-ranging job description included administration, promotion of and fundraising for the Centre, research, consulting, and teaching on campus and around the world. It is beyond the present scope to discuss all these activities in detail. However, I will focus on research in the university context; this overlaps into teaching, particularly at the postgraduate level, and funding.

Research, in the academic context, has two major objectives. First, to produce publishable results of peer-reviewed papers that will enhance the Centre's reputation and that will be employed in the Research Assistance Exercise (RAE) submission. Second, if the research is conducted by a postgraduate student, to supervise the project, oversee the writing of either the M.Sc. or Ph.D. thesis, and arrange for the appropriate examination.

The ICBD was financially well endowed when I arrived in Edinburgh, and I was able to add to it with funds from Labatt, which were a part of my agreed separation package. I decided to continue focusing on high-gravity brewing and related aspects such as beer stability (foam, flavor, and physical) and yeast stress effects. Also, I became interested in high-gravity procedures applied to Scotch whisky grain distilling. Allied to this would be a continuation of studies on the uptake of wort sugars and FAN by yeast.

The research was essentially conducted by a number of Ph.D. students: Dan Cooper, Steve Cunningham, Omar Younis, Kenny Leiper, David Finn, David Bright, Thomas Stoupis, Patricia Pratt, Stephen Brey, Stephen Martin, Chris Lekkas, Michaela Miedl, and Paul Chlup. Also postdoctoral researchers Liz Rees and Amanda Lyness and academic colleagues James Bryce, Annie Hill, Brian Eaton, and Fergus Priest were involved. We had a close and focused research team.

Yeast Morphological Changes Induced by High-Gravity Worts

Morphological changes of yeast vacuoles from the lager cultures were examined at specific times during fermentation in both 12 and 20°P all-malt worts, employing fluorescence microscopy and a fluorescent dye specific for vacuoles. These changes are depicted in Figure 11. The 20°P wort produced cells containing enlarged vacuoles compared to those grown on 12°P wort. The diameter of yeast vacuoles of these lager strains have been measured at specified times during static fermentation in 12 and 20°P all-malt worts. Figure 12 shows the effect of wort gravity on vacuolar morphology of one of the three lager yeast strains studied. At 0.5 h postpitching, water flowed passively from the yeast cells to the outside media, causing reductions in vacuolar volumes of yeast cells fermenting 12°P wort. Vacuolar morphology remained

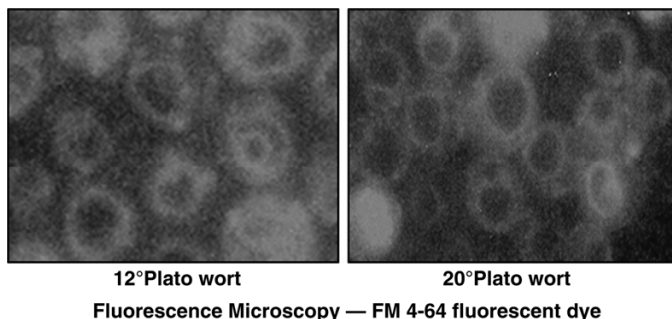


Fig. 11. Effect of wort gravity on vacuole size with an ale yeast strain. (Reproduced, by permission, from Pratt et al., 2007.) (48)

relatively constant during the lag growth phase, as the yeast cells adapted to the new environment. As budding commenced, at 6 h, small fragmented vesicles were distributed between mother and daughter cells, fusing to form large vacuoles. Consequently, vacuolar volumes increased until 18 h. No further changes in vacuolar dynamics occurred after this time (47).

A similar trend in vacuolar dynamics was observed in yeast cells fermenting 20°P wort. Budding was not initiated until 10 h, indicating that the abilities of these yeast cells to adapt to high solute concentrations and to ensure continued metabolic activity and cell growth were hindered by wort gravity. In the presence of elevated ethanol levels, continuous increases in vacuolar volumes occurred between 48 and 96 h in all six yeast (ale and lager) strains studied. (Figure 12 shows data for one lager strain only.)

The findings confirmed that the yeast vacuole plays an important role in the ability of yeast cells to successfully ferment high-gravity worts. The reduction in vacuolar volume at 24 h during normal- (12°P) and high- (20°P) gravity lager fermentations, was consistent with accepted theories of stress effects on vacuolar volumes, confirming that a relationship exists between vacuole integrity and yeast viability. The findings also confirmed that wort gravity has a significant negative effect on the yeast cell vacuolar volume of both lager and ale strains during fermentation (49).

In addition to studies on vacuolar volume, the effect of wort gravity on cell surface morphology of ale and lager yeast strains has been studied. To this end, the surface morphology of yeast strains was examined using scanning electron microscopy during static fermentation in 12 and 20°P worts. During the late stationary growth phase, cell surface features became apparent (Fig. 13). A more extreme effect of wort gravity on the yeast cell surface was observed in high-gravity fermentations, resulting in a wrin-

kly, prunelike, crenellated surface with numerous invaginations compared to the smooth surface of yeast cells fermenting normal-gravity worts.

Effect of Proteinase A Secretion and Wort Gravity on Beer Foam Stability

As already discussed, beers brewed at high gravities followed by dilution have poorer foam stability when compared to similar beers brewed at lower gravities (82) (Figs. 14 and 15). Specific hydrophobic polypeptides are known to play an important role in

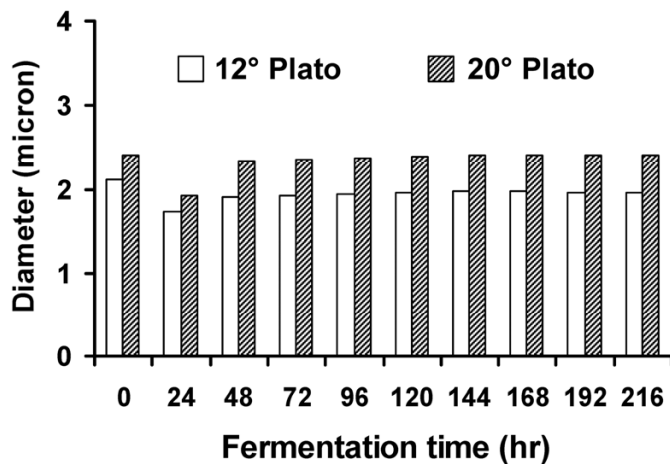


Fig. 12. Effect of wort gravity on vacuole size of lager industrial strain B. (Reproduced, by permission, from Pratt et al., 2007.) (48)

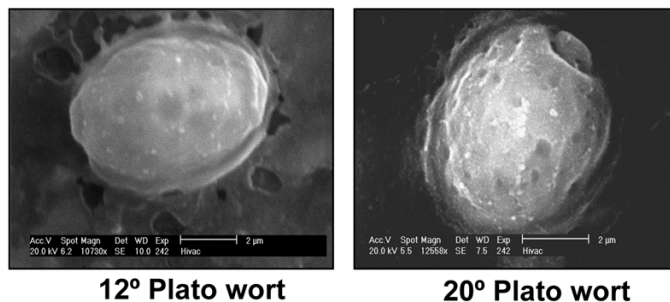


Fig. 13. Effect of wort gravity on the cell surface morphology of an ale yeast strain. (Reproduced, by permission, from Pratt et al., 2007.) (48)

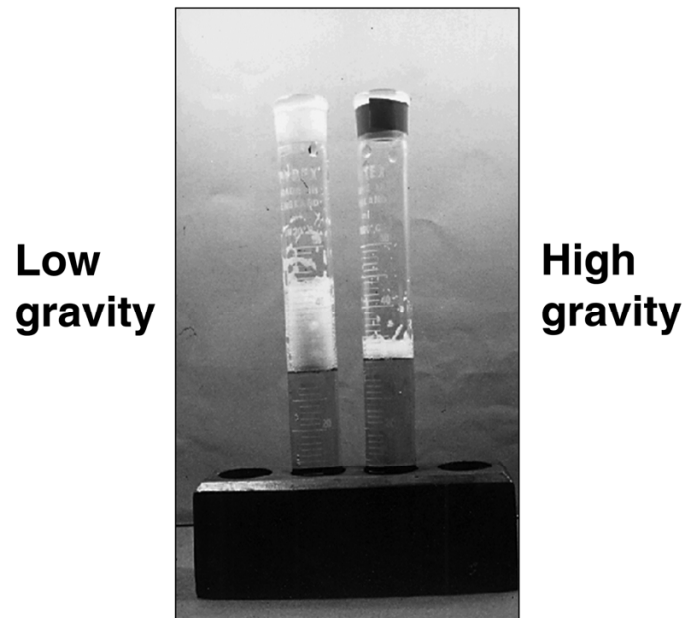


Fig. 14. Beer foam collapse characteristics 2 min after pouring. Beer on left produced with low-gravity (10°P) wort. Beer on right produced with high-gravity (20°P) wort. (Reproduced, by permission, from Bryce et al., 1997.) (10)

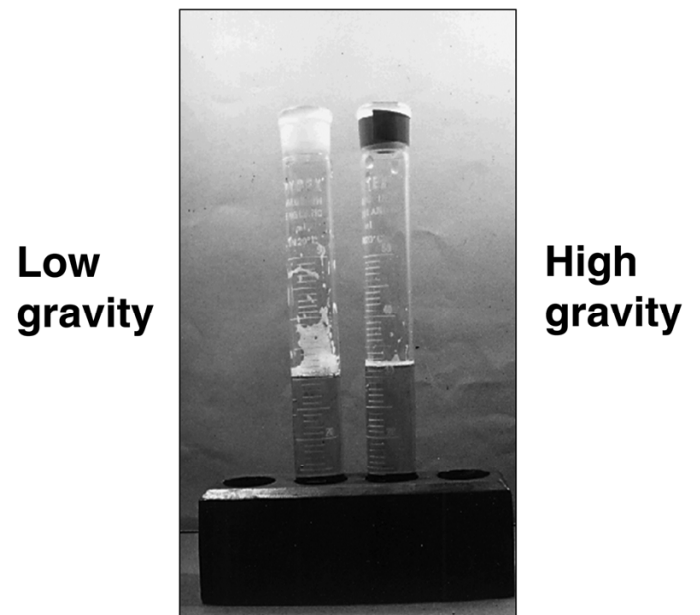


Fig. 15. Beer foam collapse characteristics 4 min after pouring. Beer on left produced with low-gravity (10°P) wort. Beer on right produced with high-gravity (20°P) wort. (Reproduced, by permission, from Bryce et al., 1997.) (10)

beer foam formation and stability (4). The level of hydrophobic polypeptides has been determined throughout the production and fermentation of high-gravity (20°P) and low-gravity (10°P) worts (17). During brewing, a proportionately greater loss of hydrophobic polypeptides occurs with the 20°P wort than the 10°P counterpart (Fig. 16) (17). When the high-gravity fermented beer was diluted to 4.5% alcohol by volume, equivalent to the low-gravity beer, it contained a level of hydrophobic polypeptides that was less than 50% of the level produced in a low-gravity produced beer (Fig. 14). The head retention of the diluted high-gravity brewed beer was much less than that of the low-gravity brewed beer (18).

Figure 17 illustrates that hydrophobic polypeptides are lost during brewing and fermentation. In brewing, foam-positive hydrophobic polypeptides are lost as a result of hot and cold break formation (6,7). Due to increasing polyphenol levels in high-gravity worts, a disproportionately greater amount of hydrophobic polypeptides are lost in high-gravity worts due to hot and cold break precipitation compared to peptides lost in lower-gravity wort (7,8). Fermentation is a key stage in which hydrophobic polypeptides are lost. At least two factors during fermentation account for this loss. First, losses occur due to fermenter foaming. During wort fermentation, a high gradient of hydrophobic polypeptides toward the surface has been shown to occur (6). This enhances adhesion of foam-active compounds to the side of the fermenter

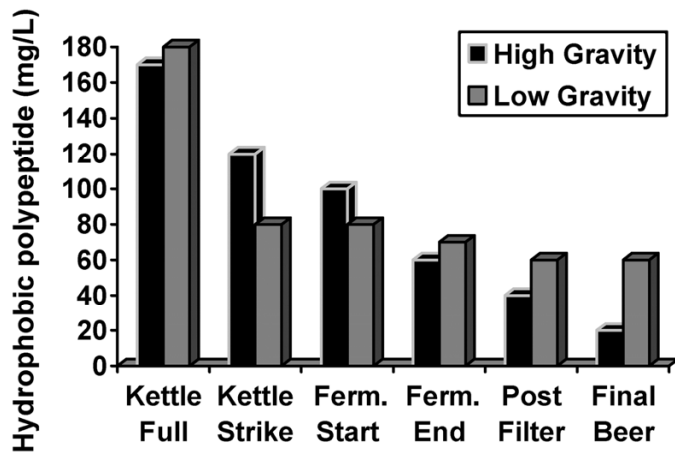


Fig. 16. Changes in hydrophobic polypeptide levels from kettle full to final beer. Final beers diluted to 4.5% alcohol by volume. (Reproduced, by permission, from Cooper et al., 1998.) (17)

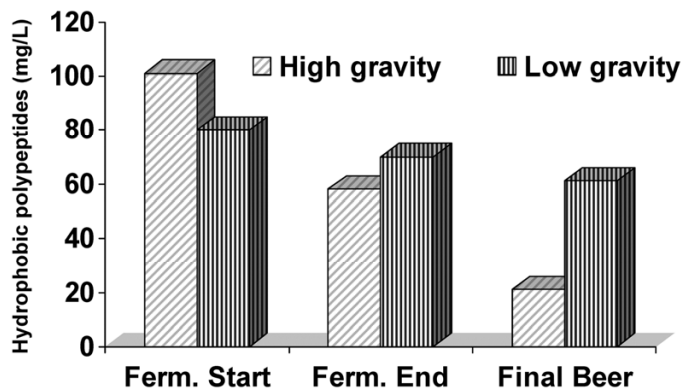


Fig. 17. Changes in hydrophobic polypeptide levels during the fermentation of low- and high-gravity worts. Final beers diluted to 4.5% alcohol by volume. (Reproduced, by permission, from Cooper et al., 2000.) (19)

vessel during transfer to the conditioning vessel. Second, yeast “secretes” proteolytic enzymes into the fermenting wort, and these appear to have a negative effect on the foam stability of finished beer due to polypeptide hydrolysis that occurs during fermentation in storage. Proteinase A (PrA) increased throughout fermentation (Fig. 18) (17). Higher amounts of PrA were released during a 20°P wort fermentation than during the 10°P wort fermentation, both of which were conducted on the 2-hL scale in the ICBD pilot brewery. During high-gravity wort fermentations, increased stress on the yeast, in the form of elevated osmotic pressure and ethanol concentration, appears to have stimulated the secretion of PrA into the wort (19). Several other factors such as thermal and mechanical stress can also promote PrA release in brewer’s yeast cultures (102).

Influence of Wort Sugar Spectrum and Gravity on Ester Formation

As discussed earlier, another disadvantage of high-gravity brewing is that fermentation of high-gravity worts induces the production of disproportionately high levels of esters (Table IV) (2). Varying the wort sugar source has been reported (109) to modify the level of many metabolites, including esters, although reasons for these differences are unclear. Entry of the hexose sugars, glucose and fructose, into the yeast cell is facilitated by the same transport system. However, utilization of glucose occurs more rapidly than fructose when the two sugars are fermented separately, possibly due to the differing affinities of the sugars for the transporter (20). It has already been discussed that the disaccharide maltose (the sugar with the largest concentration in most worts) is taken up by the cell only when 40 to 50% of the glucose has been removed from the wort (71) and occurs via an active transport system (a requirement for energy), whereas the uptake of glucose and fructose is by passive transport (no energy requirement). The production of ethyl acetate and isoamyl acetate in maltose-grown cells has been shown to be lower than in glucose-grown cells (109,110).

It is generally agreed that a reduction in ester levels, particularly ethyl acetate and isoamyl acetate from high-gravity brewed beers, would be welcome. Two adjunct-containing 20°P worts

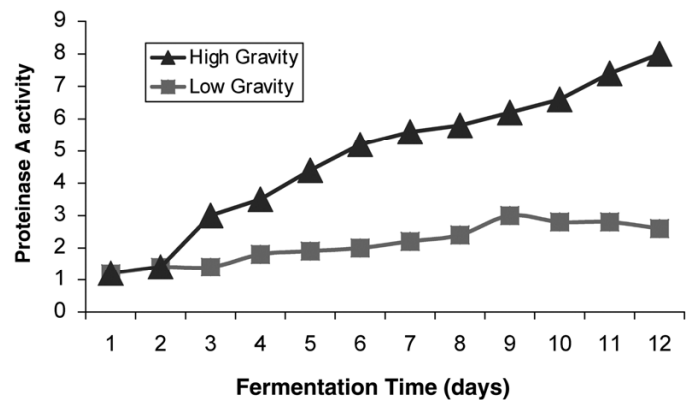


Fig. 18. Effect of wort gravity on proteinase A release during fermentation of low- (12°P) and high- (20°P) gravity worts. (Reproduced, by permission, from Cooper et al., 2000.) (19)

TABLE IV
Influence of Wort Gravity on Beer Ester Levels

Component	12°P	20°P
Ethanol (v/v)	5.1	5.0
Ethyl acetate (mg/L)	14.2	21.2
Isoamyl acetate (mg/L)	0.5	0.7

were prepared, one containing 30% maltose syrup (MS) and the other containing 30% very-high-maltose syrup (VHMS) (in studies in collaboration with Corn Products of Brazil). The sugar compositions of the two brewing syrups are shown in Table V. In addition, a 12°P wort containing 30% (w/v) MS was prepared and used as a control. The sugar spectra of the three worts are shown in Figure 19. The maltose plus maltotriose concentration in the 20°P VHMS wort increased compared to that in the 20°P MS wort, with a corresponding decrease in the concentration of glucose plus fructose (109).

The three worts were fermented in the ICB 2-hL pilot brewery with a lager yeast strain at 13°C, and the concentrations of ethyl acetate and isoamyl acetate were determined throughout the fermentation (Figs. 20 and 21). The profiles were similar for both esters. The concentration of both esters in the 20°P (MS) fermented wort was twice that observed in the 12°P (MS) fermented wort. However, the ester concentration in the 20°P (VHMS) was approximately 25% reduced compared to that in the 20°P (MS) wort. This confirms that maltose fermentations produce less ethyl acetate and isoamyl acetate than glucose fermentations (110).

Influence of High-Gravity Wort (Wash) on the Production of Grain Whisky

In 2005, a grain distillery in Scotland employing continuous fermentation was successfully fermenting 21°P grain wort yielding 11% (v/v) alcohol in the fully fermented wort (73,74). This situation prevailed early in 2006, but in late 2006 problems began to be encountered: alcohol yield decreased to 9.6 (v/v) due to incomplete utilization of maltose and particularly maltotriose (Fig. 22). This equated to a reduced alcohol yield from 385 L of alcohol to 370 L of alcohol per metric ton of grain. In addition, because of the residual wort maltose and maltotriose, the resulting distillers dried grain (DDS) had a sticky consistency and was not

TABLE V
Sugar Composition (%) of Brewing Syrup^a

Sugar	Maltose syrup (%)	Very high maltose syrup (%)
Glucose	15	5
Maltose	55	70
Maltotriose	10	10
Dextrins	20	15

^a From a study on sugar uptake (109).

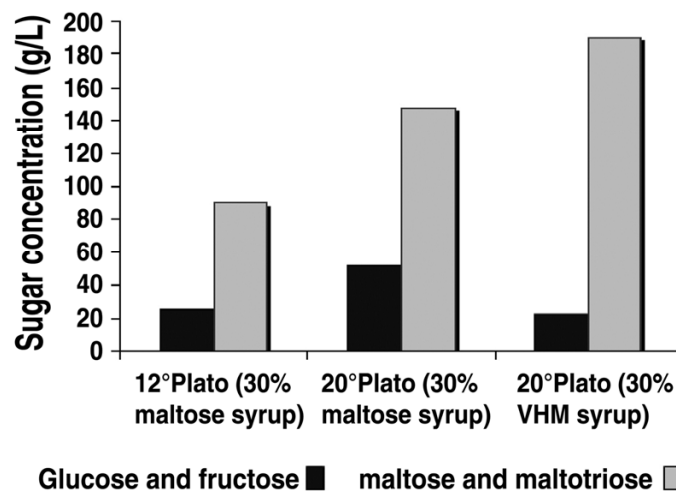


Fig. 19. Wort sugar profiles of 12 and 20°P worts containing either 30% (w/v) maltose syrup or 30% (w/v) very high maltose (VHM) syrup. (Reproduced, by permission, from Younis and Stewart, 1998.) (109)

acceptable for use as an animal feed. In an attempt to overcome this problem, the original gravity of the wort was reduced to 19°P (Table VI). This resulted in complete fermentation of the wort with no residual maltose and maltotriose and improved the consistency of the DDS. However, the distillery's overall alcohol yield was reduced below budgeted productivity levels. The reasons for the deterioration in yeast efficiency regarding maltose and maltotriose uptake in 2006 are still unclear.

It would appear that the 21°P wort exerts stress effects on the pitching yeast, with inhibitory effects on the uptake of maltose and particularly maltotriose. Stress effects on sugar uptake, especially maltose and maltotriose, have been described previously (112). The exact reasons for this inhibition are not completely clear, but the fact that maltose and maltotriose require energy (active transport) to be taken into the yeast cell cannot be ignored.

High-Gravity Brewing, Yeast Centrifugation, and Flow Cytometry

As already discussed, the use of centrifuges has become an established way to enhance brewery throughput as they increase beer clarification times. Centrifuges can have several different roles within a brewery (16):

- Cropping of nonflocculent yeast at the end of primary fermentation.
- Reducing the yeast quantity from green beer before the start of secondary fermentation.

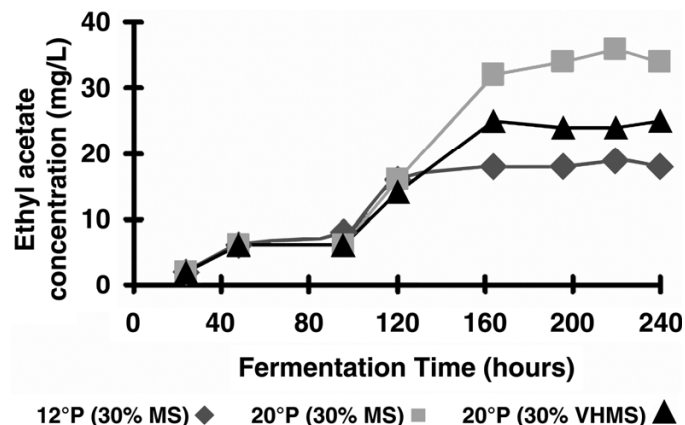


Fig. 20. Ethyl acetate concentration (mg/L) in fermenting worts of differing gravities and sugar compositions. MS = maltose syrup, VHMS = very-high-maltose syrup. (Reproduced, by permission, from Younis and Stewart, 1998.) (109)

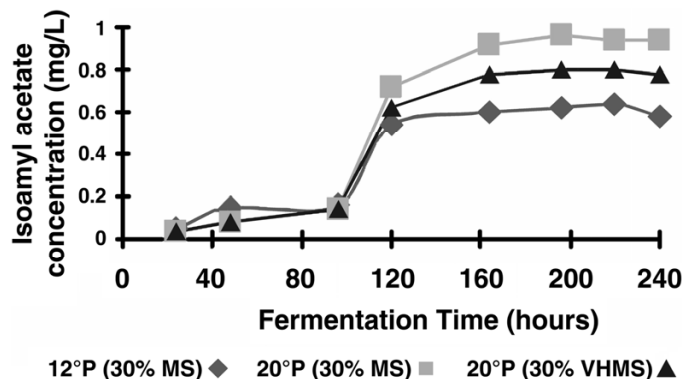


Fig. 21. Isoamyl acetate concentration (mg/L) in fermenting worts of differing gravities and sugar compositions. MS = maltose syrup, VHMS = very-high-maltose syrup. (Reproduced, by permission, from Younis and Stewart, 1998.) (109)

- Beer recovery from cropped yeast.
- Separation of hot break (trub) after wort boiling.
- Removal of cold break (trub) at the end of maturation.

Yeast that passes through a centrifuge experiences mechanical and hydrodynamic shear stresses (15). We have shown that these stresses can cause a decrease in cell viability and flocculation, cell wall damage, increased extracellular PrA levels, hazier beers, and reduced beer foam stability (11). Despite evidence of cell damage, little was reported until recently (12) about the effect on yeast and beer quality of repitching yeast that has been cropped using a centrifuge, especially in a high-gravity wort environment.

During the course of these studies, flow cytometry was employed to assess several yeast cellular parameters before and after centrifugation cycles at high *g*-force using a 5- to 6-hL/h disk stack centrifuge (Westfalia Separator). Funding for this equipment was obtained from the Institute of Brewing and Distilling (IBD) Grants Committee and was used in tandem with the ICB 2-hL pilot brewery. Increasing *g*-force exposes yeast cells to the detrimental effects of hydrodynamic forces. Analysis by scanning electron microscopy (Fig. 23) provided visual evidence of yeast

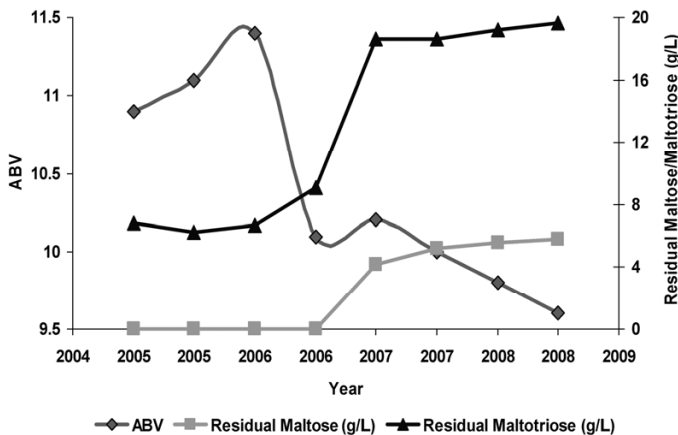


Fig. 22. Fermentation trends for a 21°P grain wort (July 2005–September 2008). ABV = alcohol by volume. (Reproduced from Stewart, 2010.) (73)

TABLE VI
Fermentation Characteristics of 19 and 21°P Grain Worts During 2008^a

Wort	Alcohol (v/v)	Residual maltose (g/L)	Residual maltotriose (g/L)
21°P	9.6	5.8	19.6
19°P	10.2	4.3	6.5

^a In a distillery in Scotland. 21°P wort caused problems with distiller's dried grain consistency in 2007 and 2008.

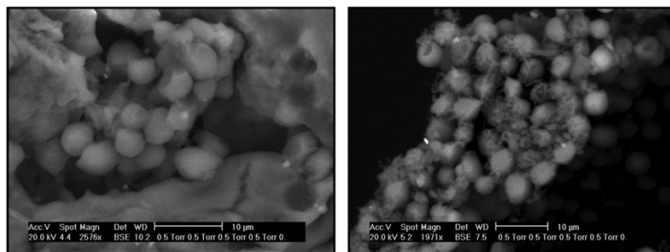


Fig. 23. Environment scanning electron microscope analysis of a centrifuged yeast culture. Left, cells before centrifugation; right, cells following centrifugation at high *g*-force. (Reproduced, by permission, from Chlup et al., 2008.) (15)

damage and the release of cellular wall components as a result of disc stack centrifugation at high *g* force (11).

Flow cytometry utilizes technology that simultaneously measures and analyzes multiple physical characteristics of single particles or cells (for example, yeast cells) as the flow in a fluid stream through a beam of light (14). Flow cytometry and confocal imaging (funding for both instruments came from the U.K. Biotechnology and Biological Science Research Council and the IBD Grants Committee) were used in this study to measure the following in yeast cells: cell viability, damaged cells, intracellular pH, mannan, PrA, and intercellular trehalose and glycogen (13,58).

Two series of fermentations were conducted in the ICB 2-hL pilot brewery. One set was a 12°P all-malt wort and the other a 20°P all-malt wort. A Meura 2001 42-kg capacity pilot mash filter (together with a hammer mill) was used for mash separation. The wort was collected in the kettle and boiled for 1 h, achieving 8% evaporation. A lager yeast strain donated by a local production brewery was used at a fermentation temperature of 13°C.

The yeast cellular characteristics before and after 12 and 20°P fermentations (determined by flow cytometry) are shown in Table VII. As would be expected (11) the stress effects of the 20°P wort exhibited a more deleterious effect on the yeast in terms of viability, damaged cells, intracellular pH, bud index, and intracellular glycogen compared to the stress effects in the lower-gravity wort. Also, an elevation in trehalose levels confirmed the stress imposed upon the yeast by the 20°P wort.

Centrifugation of the culture after nine times recycling in 20°P wort demonstrated that the yeast had been damaged in a number of ways (Table VIII). These included a reduction in viability and an increase in damaged cells together with a reduction in glycogen and trehalose levels. Also the hydrodynamic shear imposed by the centrifuge resulted in mannose (a yeast cell surface component) being released, together with a PrA increase resulting in a reduction in wort hydrophobic polypeptides and poor beer foam stability. The reasons for the trehalose reduction levels are unclear because the stress effects imposed upon the culture are illustrated by the reduced cell viability.

TABLE VII
Fermentation Characteristics of Cells and Beer Before and After Centrifugation (Nine Cycles) at High *g*-Force with 20°P Wort

Characteristic	Before	After
Viability (%)	85	42
Extracellular pH	4.2	4.4
Intracellular pH	5.8	5.3
Damaged cells (%)	4	15
Glycogen (ppm)	18	8
Trehalose (ppm)	22	6
Mannan released (counts)	400	1,000
Proteinase A (units/mL)	3.1	6.2
Hydrophobic polypeptides (mg/L)	48	25
Beer foam stability (NIBEM)	110	82

TABLE VIII
Fermentation Characteristics of Cells During 12 and 20°P Wort Fermentations Determined by Flow Cytometry

Characteristic	12°P		20°P	
	Start	End	Start	End
Viability (%)	80	96	80	94
Damaged cells (%)	7	2	7	5.5
Extracellular pH	5.2	4.0	5.2	4.2
Intracellular pH	6.9	6.0	6.9	5.6
Budding cells (%)	38	15	38	10
Glycogen (ppm)	22	22	22	16
Trehalose (ppm)	28	26	28	36

Although centrifugation can exhibit negative effects, the positive effects of centrifugation on brewery production and on effluents cannot be overstated. However, yeast is subjected to numerous factors that individually or collectively impose stress on yeast cells. Typically, the effects of environmental conditions and beer production equipment may have been underestimated or even ignored. An understanding of yeast biological response to interactions with cell physiology and brewing equipment is an important criterion for maintaining beer quality.

SYMPOSIA, COURSES, WORKSHOPS, AND GOVERNMENT/INDUSTRY/SCIENTIFIC SOCIETY COMMITTEES

During my employment with Labatt and then ICBD, I had the privilege of helping organize symposia, courses, and workshops all over the world considering yeast technology and the brewing and distilling process. In addition, several two-day seminars reviewing proteinases (92), microbial stress, microbial temperature tolerance (61), food beverage stability, and amylases (91) were held in the London, ON, brewery in collaboration with the Departments of Biochemistry, Microbiology and Plant Sciences, the University of Western Ontario (UWO). In 1985, we organized a symposium called the Biochemistry and Molecular Biology of Industrial Yeasts in collaboration with The Upjohn Company at Upjohn's conference facilities in Kalamazoo, MI. Sixty presentations (oral and posters) were given and all the papers have been published in a three-volume monograph (97).

In 1980, Labatt employees played a major role organizing the VI International Fermentation Symposium and the V International Yeast Symposium (90), which were jointly held on the UWO campus. This event was financially supported by Labatt and attracted more than 1,650 delegates from all over the world.

Yeast technology workshops were conducted in 10 countries with the assistance of Inge Russell. They were funded by the Canadian International Development Agency (now part of the Department of Foreign Affairs, Trade and Development), the Royal Society of Canada, UNESCO, the Commonwealth Secretariat, and the International Union of Microbiological Societies (IUMS). The Brewing Process courses were sponsored by the Central Food Technology Research Institute in Mysore, India, IBD (Asia Pacific Section), SAB Miller, and the ICBD. They were conducted by John Andrews, Charlie Bamforth, Trevor Roberts, and myself. I have often called them my "dream team" because their collective knowledge of brewing practice, raw materials, technology, and engineering were (and still are) unprecedented. A number of anecdotes of these events have been documented in my biographical article considering Charlie Bamforth's election as the current president of the IBD (77).

During my 25 years in Canada, I served on a number of federal government and international committees. Although I made a large number of friends and contacts during this time, I would question the value of these committees at that time. In most (not all) instances, they served as a "rubber stamp" for bureaucrats and politicians! Consequently, when I moved to Britain, I decided that I would not accept invitations to join committees of this nature. However, I did join the U.K. Technology Foresight Panel on Food and Drink, which to this day I regret. I do not know what it achieved—it was another waste of time and taxpayers' money.

My time spent on industry and scientific society committees was, in my opinion, different. With few exceptions, they invariably achieved a majority of their objectives. I look back on my time as president of the IBD (1), chairman of the International Commission for Yeasts, chairman of the Product Integrity Task Force of the Brewer's Association of Canada, and president of the Brewing and Malting Barley Research Institute of Canada with

pleasure and satisfaction. The exception is my 8 years as treasurer of IUMS. For many (not all) members of its Executive Committee, it was a "gravy train." Trying to control the expenses of many committee members was a nightmare.

FINAL THOUGHTS

I retired from Heriot-Watt University at the end of 2007. I was 65 years old in March of that year, but I had stayed an additional 9 months in order that some of my publications could be included in the 2007 Research Assessment Exercise. Olga and I moved back to Cardiff from Edinburgh for a variety of reasons. I had been away from the city of my birth for 44 years. I had lived in Bristol, Bath, Fareham (near Portsmouth), London, Ontario, and finally Edinburgh.

I have been asked a number of times, "During your time associated with the brewing and distilling industries, what areas of development made you most proud?" There are three areas worthy of mention (not in any particular order):

- Although I encountered opposition from some academic colleagues (but full support from many other colleagues), I was able to introduce, in the late 1990s, a distance-learning postgraduate degree in brewing and distilling into the ICBD's curriculum. This initiative resulted in a course that currently has more than 100 students registered and more than 75 graduates.
- I have successfully supervised 25 Ph.D. students and have been able to publish a large number of peer-reviewed papers, many of which are cited in this document.
- When I became research manager of Labatt in 1974, the research function and the other technical functions were separate physically and in their philosophy. When I retired from Labatt in 1994, there was only one technical function! I like to think that we operated as a single team.

If I had my time over again, what would I do differently? There are obviously many issues, but first and foremost, I would make much greater efforts to incorporate the distilling industry in Scotland into the activities of the ICBD. I know my successor as director of the Centre (Alex Speers) is making every effort to rectify the situation. I hope the recent publication of the second edition of *Whisky: Technology, Production and Marketing* assists in this objective (56).

Since my move to Cardiff, my time has been spent writing, editing, consulting, examining, traveling and presenting papers, etc. I look back on my 45-year involvement in brewing (and distilling) with great satisfaction. The industry has been very good to me, and I hope I have been good for it. I have said many times that it has presented me with three great opportunities:

1. To conduct reasonable research and publish it. It may not be worthy of a Nobel Prize, but nevertheless I am proud of it (at least most of it). It has given me a degree of scientific respectability and reputation.
2. To travel the world—most of the time at someone else's expense. My greatest satisfaction in this regard has been to visit at least 65% of the countries in the Commonwealth (I fervently believe in this organization), as well as the United States, China, Russia, Brazil, Mexico, Argentina, EU countries, and many more!
3. Last, and most importantly, to meet and work with a large number of fascinating people. Indeed, teamwork has invariably been the order of the day. I have friends and acquaintances all over the world, but unfortunately, with the march of time, many are no longer with us—may they rest in peace.

However, my primary interest never was brewing *per se*. It was yeast! I was seduced by this unicellular fungus all those years ago, and my fascination is undiminished.

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During my 45 years associated with the brewing industry, I have been supported by a large number of excellent scientists. Administrators and secretaries have also been an invaluable part of this endeavor. Worthy of particular mention and thanks are Karen Smith (now Karen Thomas) (who was my secretary at Labatt for 13 years) and Anne Anstruther (who was my secretary at Heriot-Watt University for 3 years immediately before my retiring and still functions as my personal assistant). Also, I have been supported by Inge Russell since 1970. Her encouragement, enthusiasm, friendship, and critical approach cannot be overemphasized. Finally, thank you to Olga, whose help, support, patience, and encouragement have been invaluable for nearly half a century.

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