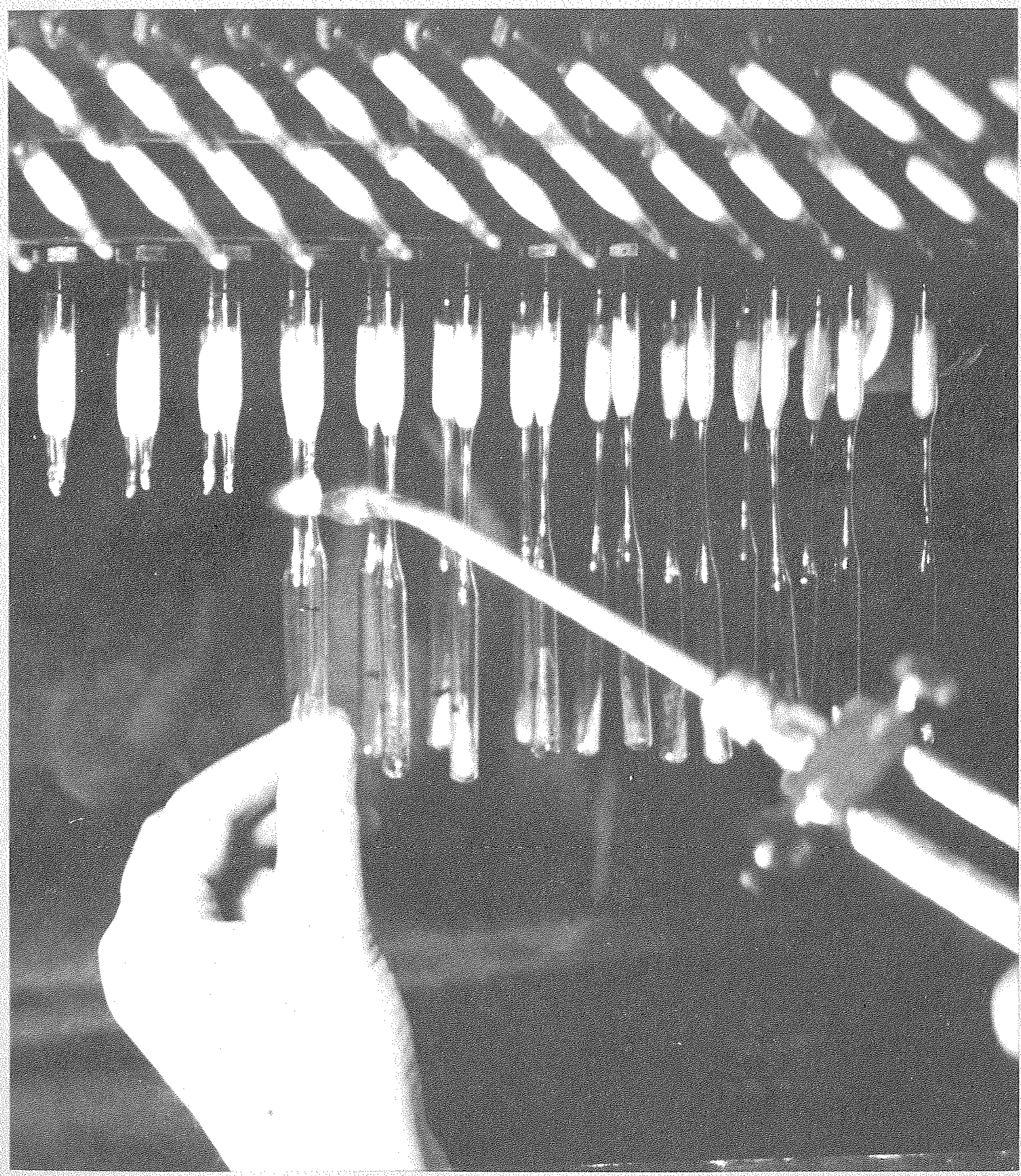
Vol. 45 No. 2 June 1985

CSIRO Food Research Quarterly



Economic feasibility of adsorptive de-acidification and debittering of Australian citrus juices

By R. L. Johnson and B. V. Chandler

CSIRO Division of Food Research, North Ryde, New South Wales, 2113

Data from 'resin-in-cage' pilot contactors for de-acidifying and debittering citrus juices are used to estimate the likely capital and running costs of an adsorption unit for reducing the concentration of acid and bitter principles. Lowering the acid content of a juice by 5 g/l or reducing the limonin content of a bitter juice from 15 to 5 mg/l should cost less than one cent per litre. Debittering is a process for improving quality, and therefore debittered navel orange and grapefruit juices should have added value. Acid reduction, when used to raise the sugar : acid ratio of citrus juices, is cost-competitive with sucrose addition. Operation of both processes for three months of the year each should repay capital in less than one year.

Introduction

Consumer resistance to the excessive acidity and bitterness of Australian grapefruit juice (Chandler and Johnson 1981) is reflected in the low minimum price of $82 per tonne set by the Fruit Industry Sugar Concession Committee (FISCC) for grapefruit delivered to factories in rural areas during 1983/84 (Australian Citrus Growers Federation 1984). This is to be compared to the FISCC minimum price of $120 per tonne for Valencia oranges. Early season Valencia oranges may also produce juices that are excessively acid, but as the season progresses this defect becomes less of a problem. High acidity in juices from mid-season oranges also inhibits the utilization of these cultivars to expand the orange juice processing season (Chandler and Nicol 1982).

Bitterness in freshly extracted grapefruit juice is caused by the flavonoid bitter principle naringin, but bitterness due to the dilactone bitter principle limonin may also develop to an unacceptable extent after processing (Chandler and Nicol 1975). Part of the appeal of fresh grapefruit juice lies in the slight but pleasant bitterness induced by the presence of moderate amounts of naringin, but excessive amounts of naringin and the presence of limonin cause the bitterness to become unpleasantly intense.

Furthermore, bitterness due to limonin diminishes the acceptability of navel orange juice to consumers and inhibits the full utilization of navel oranges, one-third of the Australian orange crop, in the production of orange juice. The bitterness defect of navel orange juice is reflected in the 1983/84 FISCC prices for navel and Valencia oranges of $100 and $120 per tonne respectively (Australian Citrus Growers Federation 1984).

To balance the excessive acidity of citrus juices, sugar is added to raise the sugar : acid ratio of the juice to a level more appealing to consumers, and food regulations permit Australian processors to add up to 4% sucrose by weight, without identification of the product as sweetened (National Health and Medical Research Council 1973). However, the acidity of Australian grapefruit juice and early season Valencia orange juice is often so high that even juices sweetened by 4% added sucrose meet consumer resistance. More than 4% sucrose is therefore often added to these juices, and currently no limit is imposed on the amount of sucrose that can be added to juice labelled 'sweetened'.

Sucrose addition imposes extra costs on the processor, the practice is nutritionally undesirable and it complicates the authentication of fruit juices by methods based on the natural ratios of the juice sugars (Bielig *et al.* 1983). Finally, European and North American countries do not allow 'undeclared' addition of sweetener to fruit juices at the 4% level and this concession is unlikely to last indefinitely; proposed new standards set 1.5% as the maximum amount of sucrose that

can be added to juice without declaration as sweetened, in conformity with international standards (Joint FAO/WHO Food Standards Programme 1982a and 1982b).

Partial de-acidification of citrus juices by sorption of acid on to weak base resins offers an alternative to sucrose addition as a means of raising the sugar : acid ratio. Since there is no technical limit to the amount of acid that may be adsorbed, provided the juice retains its characteristic composition, the sugar : acid balance of even the most acid juices can be successfully adjusted to an optimum for consumer acceptance by this method. Bitter principles can also be adjusted to more acceptable levels by specifically adsorbing the bitter substances on to adsorbent resins (Johnson and Chandler 1982).

Because adsorptive processes offer the prospect of eliminating acidity and bitterness problems in citrus juices, the Division of Food Research undertook research aimed at developing a low cost method for adsorptive treatment of juices (Chandler and Johnson 1981). The 'resin-in-cage' process is one product of this research. In this process, whole uncentrifuged juice is passed through contactors consisting of tanks in which adsorptive resin is enclosed in stainless steel mesh cages which are oscillated up and down to ensure full contact between resin and juice (Chandler and Johnson 1981). With the support of the Australian Citrus Industry and CSIRO Development Fund, the 'resin-in-cage' concept has been brought to the pilot plant stage. From data obtained from pilot contactors a simple conceptual model for an industrial unit has been developed. Since all required components of this unit are commercially available at known prices and the operating costs of the process can be estimated, this model allows a preliminary calculation of the likely costs of reducing the acid and bitter principle contents of citrus juices. The details of this costing are now presented.

Basis for costing

The adsorptive apparatus is to be used for debittering navel orange juice (May to July) and for de-acidifying grapefruit juice and early season Valencia orange juice (August to October); in this way, the capital cost of the apparatus is amortized over 130 working days each year (Table 1).

The gross daily cost of juice treatment is the sum of the costs listed in Table 2. The costs 1 to 3 are the same for both de-acidification and debittering and these general costs are calculated first. The specific costs of de-acidification are then calculated and combined with costs 1 to 3 (Table 2) and the daily juice throughput – which is 38 400 l per 10-hour day – to provide the cost of juice de-acidification in cents per litre. For debittering, similar calculations are then applied and combined with the throughput of 26 880 l per 10.5-hour day to obtain the cost of juice debittering in cents per litre.

TABLE 1

Annual schedule for use of 'resin-in-cage' contactors

Use	Months	Time of year
Debittering		
Navel orange juice	3	May, June, July
De-acidification		
Grapefruit juice	2	August, September
Valencia orange juice	1	October
Total	6	
Number of operating days = 130[A]		

[A]The calculation of the number of working days is based on a five-day week, with two public holidays in the 26-week period May to October.

Capital outlay

The proposed adsorptive apparatus, similar to that used in the 'resin-in-pulp' method for mineral recovery (Hollis and McArthur 1956), is sketched schematically in Figs 1, 2 and 3. The unit has four contactors; each consists of a 500 l stainless steel tank with a 160 l stainless steel mesh cage enclosing an 80 l bed of resin; the cages are raised and lowered in their tanks by 200-watt geared motors, one for each pair of cages.

TABLE 2

Cost factors in adsorptive treatment of citrus juices

	Daily costs for	
	De-acidification ($)	Debittering ($)
1 Loan repayments	67.42	67.42
2 Maintenance	13.48	13.48
3 Labour	21.90	21.90
4 Regeneration	177.60	18.94
5 Resin replacement	17.58	10.98
6 Evaporation of diluent water	30.15	12.06
Total	328.13	144.78

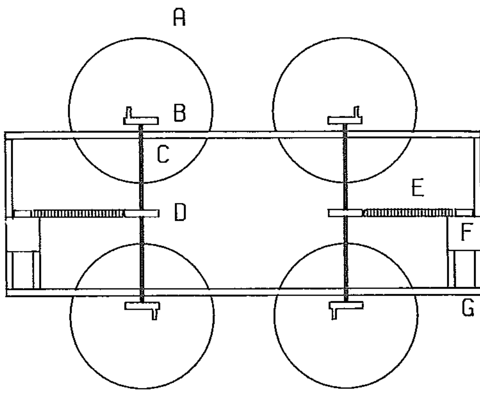


Fig 1. Plan view of apparatus
[A]500-litre tank.
[B]Pulley wheel; used for raising and lowering cage (not shown) by means of a connecting rod (not shown).
[C]Driving shaft.
[D]Sprockets.
[E]Chain connecting motor sprocket to drive shaft sprocket.
[F]200 watt geared motor.
[G]'Millstrut' scaffolding.

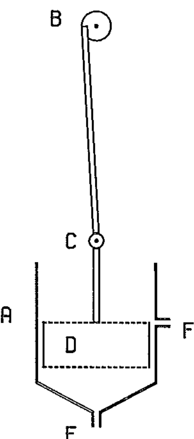


Fig 2. Side elevation of a contactor.
[A]500-litre tank.
[B]Pulley wheel.
[C]Joint in connecting rod.
[D]Cage.
[E]Tank inlet.
[F]Tank outlet.

TABLE 3

Capital Outlay

Item	Number	Unit cost ($)	Cost ($)
Hardware			
7500 litre tank[A]	1	6216	6216
500 litre tank[B]	4	823	3292
Pumps	2	1195	2390
200 watt geared motor[C]	2	501	1002
Cages[D]	4	2000	8000
Programmable controller	1	1600	1600
Solenoid valves	24	300	7200
Level switches	4	60	240
'Millstrut' scaffolding[E]	—	—	270
Piping[F]	—	—	942
Assembly[G]			4104
Resin[H]			3712
Floor space[I]			4860
Total capital outlay			43828

[A]Stainless steel tank, conical bottom with 50 mm outlet, equipped with lid and mild steel stand, used for holding caustic soda solution.

[B]Stainless steel tanks, conical bottoms with outlets and equipped with mild steel stands, used for contactors.

[C]Includes sprockets, chain, 4 pulley wheels, 4 bearings and 2 metres of shafting.

[D]The cost of the specially made cages is estimated by considering a cage to consist of a stainless steel cylinder – 760 mm diameter and 350 mm height – fashioned from stainless steel sheet with stainless steel screens fitted at each end. The amount shown is the estimated combined cost of the sheeting, its fabrication and fitting with the screens, with $500 allowed for each screen.

[E]30 metres of channels, 42 angles and 100 fasteners.

[F]23 metres of 20 mm rolled welded stainless steel pipe and 40 'Tee' joints.

[G]Two men with skills equivalent to plumber and electrician should be able to assemble the equipment within ten days (38 hour working week), taking three days to place the tanks in position and erect the scaffolding, two days to install piping and pumps and two to test the equipment; three days are allowed for contingencies. With contracted skilled labour at about $27/h (Anon. 1983), assembly cost would be $4104.

[H]With a total resin bed volume of 320 l, initial costs would be $2285 for the de-acidifying resin ($7.14/l) and $1427 for the debittering resin ($4.46/ l).

[I]Calculated from the estimated space occupied by the equipment (4.5 × 4.5 metres) and the cost of factory premises ($240/m²) (Anon. 1983).

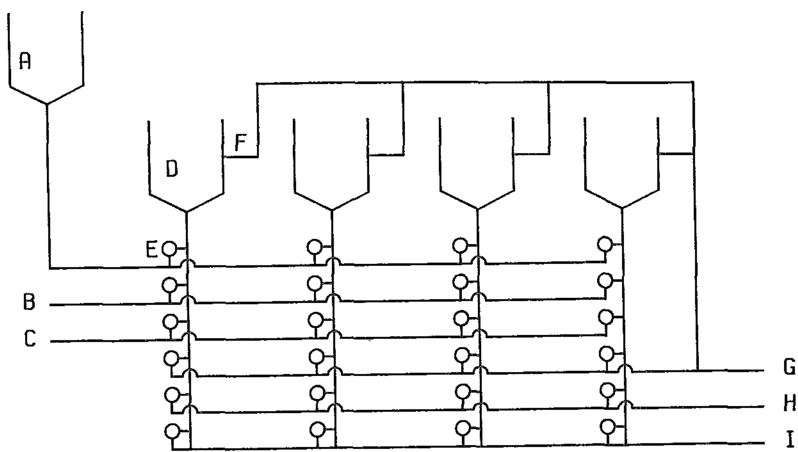


Fig 3. Flow diagram for proposed apparatus.
[A]7500-litre tank for alkali storage.
[B]Juice inlet.
[C]Water inlet.
[D]500-litre contactor tank.
[E]Solenoid valve.
[F]Outlet of contactor.
[G]Juice outlet.
[H]Alkali outlet.
[I]Waste outlet.

The capital required (total $43 828) is calculated (Table 3) as the sum of the costs of hardware, assembly, initial charge of resin and the floor space allocated to the apparatus.

Loan repayment and depreciation

The capital to construct the unit is borrowed at a reducible interest rate of 15% per annum. Since the Taxation Commissioner estimates ten years as the effective lifetime of equipment in fruit canneries, breweries and butter factories (CCH Australia 1982), a loan repaid over ten years allows for the unit to depreciate to zero. The annual repayment is equal to 20% of the capital which is equivalent to $67.42 per operating day (130 days per annum).

Maintenance

Buchanan (1966) recommends that maintenance be estimated at between 2.5 and 8% of the capital per annum; the lower figure is applicable to plant in which wear and corrosion are small, whilst the upper value is applicable to plant operating under extremely harsh and corrosive conditions. The higher figure is used here and for operating the unit for six months of the year, the annual maintenance cost, exclusive of resin replacement, is taken to be 4% of the capital, i.e. $1753 per year or $13.48 per operating day.

Labour cost

Provision has been made in the capital outlay for programmable control; the unit therefore requires only part-time attendance of a factory worker (General Hand Class 2) for two hours a day at a daily labour cost of $21.90 (Table 4).

De-acidification of citrus juice

Experimental results indicate that, over one hour, approximately 0.12 kg acid is adsorbed per litre of resin from a juice stream with an initial acid content of 17.5 g/l fed at the rate of 24 l juice/l resin/hour. The corresponding result for a unit of four contactors (80 l resin each) is a reduction in acidity of 5 g/l at a daily throughput of 38 400 l, a total removal of 192 kg citric acid/day. This would bring the acidity of the most acid Australian grapefruit juice (17.5 g/l) to just above the average acidity (11.3 g/l) of Florida grapefruit juice (Chandler and Johnson 1981).

Regeneration of de-acidifying resin

Theoretically, for every kilogram of citric acid adsorbed on to the resin, 0.625 kg sodium hydroxide is required for regeneration. Because effective regeneration of a weak base resin normally requires a two-fold excess of alkali (BDH Chemicals Ltd 1981), the costing assumes resin regeneration to use 1.25 kg sodium hydroxide per kg acid removed from

TABLE 4

Calculation of hourly labour cost

Item	Cost ($)
Annual wage[A]	11515.90
Annual oncosts[B]	2303.18
	13819.08
Hourly labour cost	
Annual labour cost/Productive hours in year[C]	8.76
Add in cost of supervisor[D]	2.19
Total hourly labour cost	10.95
Daily labour cost at two hours per day	21.90

[A]The Federal Fruit Growing Industry Consolidated Award wage for a General Hand Class 2 is $220.40 for a 40-hour week or $11 515.90/year.

[B]Oncosts are taken to be 20% of the annual wage (Australia-Dept of Industry and Commerce 1980).

[C]Based on eight-hour day, three weeks leave, five days sick leave, 52 weekends and nine public holidays per annum, i.e. 365 less 133 equals 232 days or 1856 hours per annum. An allowance of 15% is made for nonproductive time which gives 1578 productive hours per annum.

[D]Based on a labour cost of the supervisor, which is twice that of the worker, and one hour of supervision per day.

the juice. The bulk price of sodium hydroxide is 74 cents per kg; the cost of alkali for regeneration is then $177.60 per operating day.

Replacement of de-acidifying resin

As yet, data on the operational life of the resins are incomplete. However, under conditions equivalent to those for operation of the pilot contactors, a small bed of the weak base resin showed no loss in adsorptive power for titratable acid after 200 de-acidifying cycles with grapefruit juice. It is assumed that the de-acidifying resin will last for 600 cycles, the resin having to be replaced once every two years at a cost of $2285, i.e. $17.58 per operating day.

Evaporation of diluent water

After each regeneration, ca. 0.5 l of water is held in each litre of resin. By necessity, this water is entrained in the juice stream.
At the end of each juice run, ca. 0.5 l of juice is held in each litre of resin; this juice may be recovered by washing the resin twice with 2 l of water per litre of resin. The resulting diluted juice - 0.48 l juice plus 3.52 l water - is added to the treated juice.
Thus, a total of ca. 4.02 l of water per litre of resin is added to the treated juice 3 8400 l.
Since 24 l of juice are treated per litre of resin in each cycle, over one day 6432 l of water dilutes the treated juice in this way.
Taking the cost of steam to be 1.5 cents per kg, and assuming 3.2 kg (i.e. ca. 3.2 l) of water to be evaporated by 1 kg of steam, the daily cost of evaporation of diluent water is calculated to be $30.15

Cost of citric acid removal

The daily cost ($328.13) of citric acid removal is the sum of loan repayments ($67.42), maintenance ($13.48), labour ($21.90), regeneration ($177.60), resin replacement ($17.58) and evaporation ($30.15). This corresponds to 0.85 cents/l acid-reduced juice and is equivalent to $1.71/kg acid removed from the juice. The percentage distribution of this cost is: loan repayment 21, maintenance 4, labour 7, regeneration 54, resin replacement 5 and evaporation 9 respectively.

Debittering of citrus juice

From the pilot plant data, passage of an objectionably bitter navel orange juice (initial limonin content 15 mg/l) through a contactor at 14 l juice/l resin/hour would remove about 10 mg limonin/l of the treated juice over three hours. This operation yields a non-bitter juice with a limonin content of 5 mg/l; the flow rate corresponds to a daily throughput of 26 880 l.

Regeneration of debittering resin

The adsorption of limonin on to the polymeric matrix of the resin during debittering must be reversed for the resin to be re-activated; this is achieved by dissolving the limonin in alkali. Limonin is soluble in 0.25 molar alkali (10 g NaOH/l), 4 l/l resin being adequate to regenerate the resin. The daily cost of alkali for regeneration is then $18.94.

Replacement of debittering resin

The operational life of the debittering resin is unknown; however, under conditions similar to those in the pilot plant a small bed of debittering resin has undergone 160 debittering cycles with a negligible loss in debittering power. It is assumed that the debittering resin will have a useful life of 240 cycles, the resin having to be replaced once every two calendar years. The cost per operating day of resin replacement is $10.98.

Evaporation of diluent water

As in de-acidifying, 4.02 l water/l resin are added to the juice stream in each debittering

Fig 4. Contacting pattern for de-acidifying.
Full lines indicate contactor in use, dotted lines indicate contactor being regenerated. Each contactor has one hour in contact with the juice followed by one hour of regeneration. Contactor 1 is started first; after 30 minutes contactor 2 is placed in parallel with 1; at the end of one hour, contactor 1 is subjected to regeneration and contactor 3 is connected in parallel with contactor 2. Ninety minutes into the shift, contactor 2 joins contactor 1 in regeneration and contactor 4 is started. At two hours, contactor 3 joins contactor 2 in regeneration and contactor 1 comes back into service. This cycling of the contactors continues throughout the shift.

cycle; since 42 l of juice are treated per litre of resin in each cycle, a total of 2573 l of water has to be evaporated each day at a cost of $12.06.

Cost of debittered navel orange juice

The daily cost of debittering ($144.78) is the sum of the loan repayment ($67.42), maintenance ($13.48), labour ($21.90), regeneration ($18.94), resin replacement ($10.98), and evaporation of diluent water ($12.06). The cost per litre of debittering juice is then 0.54 cents. The percentage distribution of this cost is loan repayment 47, maintenance 9, labour 15, regeneration 13, resin replacement 8 and evaporation 8.

Return by saving in sucrose addition

One advantage of the de-acidification process is the saving it would allow by removing the need for sucrose addition to improve the sugar : acid balance of citrus juices. As an example of such savings, consider an average Australian grapefruit juice with total soluble solids content 9.0 °Brix, acid content 14.1 g/l and Brix : acid ratio 6.38 (Chandler and Johnson 1981). This juice very likely would be marketed after addition of sucrose at less than 40 g/kg in the final juice which then would not require the label description 'sweetened'. If sucrose addition is limited to 38 g/kg, the product would have a final ratio of 9.06 (allowing for density changes), just slightly above the permitted minimum (9.0) for U.S. Grade A sweetened grapefruit juice (United States Department of Agriculture 1968). To achieve this ratio without sucrose addition, the acid content of the original juice would have to be reduced by 4.17 g/l to 9.93 g/l. Such a reduction of the acid could be achieved by taking 100 volumes of the juice, reducing 83.4 volumes by 5 g/l and blending the de-acidified juice with the other 16.6 volumes. The cost per litre of doing this may be calculated as the cost of reducing the acidity of 0.834 l of juice by 5 g/l, i.e., 0.71 cents/l.

To obtain 1 l of sweetened juice (12.5°B, sp. gr. 1.0505), 40 g sucrose would be added to 0.975 l of juice (9°B, sp. gr. 1.0359), the juice costing 23.52 cents as calculated from juice yield and fruit price (Australian Citrus Growers Federation 1984). With sugar at 50.2 cents per kg, the cost of added sugar to produce 1 l of sweetened juice would be 50.2 × 0.040, i.e. 2.01 cents; total raw material would therefore be 25.3. For comparison, acid reduction of 1 l of juice (raw material 24.12 cents) would cost 0.71 cents, i.e. total cost 24.83 cents per l, 0.70 cents less than sugar addition. A plant processing 38 400 l of average Australian grapefruit juice daily for 65 days would therefore save about 38 400 (l per day) × 65 (days) × 0.70 (cents per l) cents, i.e. $17 472 annually. On this basis alone, the capital ($43 828) invested in the contactors could be recovered in about 2.5 years

Return by increased value of debittered juice

The FISCC price for navel oranges for the 1983/84 season was $100/tonne (Australian Citrus Growers Federation 1984); given the

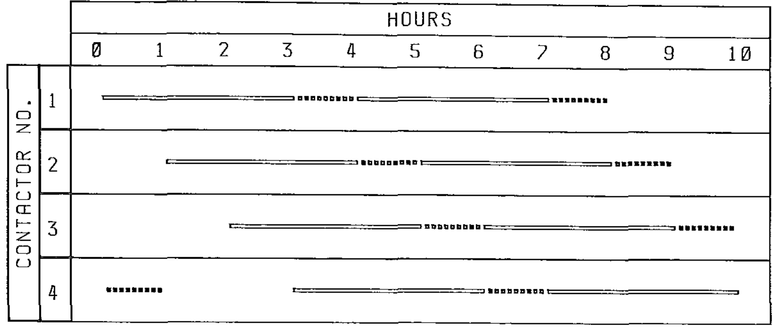


Fig 5. Contacting pattern for debittering.
Full lines indicate contactor in use, dotted ones indicate contactor being regenerated. Contactor 1 is started first: after one hour, contactor 2 is placed in parallel with 1, at two hours contactor 3 is connected in parallel with 1 and 2, and at three hours contactor 1 is subjected to reactivation and contactor 4 is placed in parallel with 1 and 3. Once the cycle is established, three contactors are in use and one contactor is being regenerated at any one time.

juice yield to be 455 l/tonne fruit (Australian Citrus Growers Federation 1984), the juice cost to the factory is then 21.98 cents/l. For Valencia orange juice ($120/tonne, juice yield 455 l/tonne) the cost is 26.37 cents/l, 4.39 cents/l more than for navel orange juice.

With the bitterness defect eliminated, navel orange juice may be expected to command a price equal to that of Valencia orange juice, especially considering its generally higher total soluble solids content and more acceptable sugar : acid balance. At parity pricing with Valencia orange juice, the increased value of navel orange juice per litre would be 4.39 cents less 0.54 cents (the cost of debittering), i.e. 3.85 cents. For a daily output of 26 880 l, the increased value would be 26 880 × 3.85 cents, i.e. $1035 and on this basis alone the capital outlay ($43 828, Table 3) could be repaid within 43 operating days.

The cost advantages of the application of the adsorptive processes to grapefruit juice are less easy to calculate. A possible basis for this calculation, the comparable prices for local and imported juice, show very marked seasonal fluctuations; from consumer comments the imported juice is always preferred even at greater cost. The calculations are also complicated by the fact that de-acidifying and debittering would both be required. Nevertheless, the above calculations for de-acidifying and for debittering will allow industry to assess the value added by the application of adsorptive processes.

Discussion

The removal of titratable acid and bitter principles from citrus juices is now technically feasible; the application will depend not on further technical development but on economic and regulatory considerations, and we have tried to show that adsorptive de-acidification and debittering have the potential to be profitable undertakings.

A return on capital can come from either of two sources. Acid reduction may be looked upon as an alternative to sucrose addition for improving the sugar : acid balance in the juice; in the example given, annual sucrose savings would be about $30 000. The second source of return could be from value added to debittered juice; for example, parity pricing of navel orange juice with Valencia orange juice should return the capital within one year.

In preparing this costing, we have had to make some arbitrary assumptions and have not considered some potentially advantageous operating variables, e.g. simultaneous de-acidification and debittering of grapefruit juice by passage through a single unit, and addition of the calculated amount of concentrate to compensate for diluent water. The costing has also been based on the assumption of only one shift per day, although some factories would operate 24 h/day during the peak of the season. On this latter point, operation for 106 h/week would reduce the cost of de-acidification of grapefruit juice to 0.63 cents/l and the payback period – based on elimination of sugar addition – to 69 days of operation. For navel orange juice, this mode of operation would reduce the cost of debittering to 0.49 cents and the payback period to 18 days of operation. Since caustic soda accounts for 54% of the cost of acid removal from citrus juice there is scope for savings by reducing this

portion of the cost, e.g. by optimizing the regeneration procedure, by recycling regenerant solution with make-up alkali and by using a cheaper alkali such as soda ash or ammonia.

Account has not been taken of the possibility of recovering citric acid from the spent regenerant, which, for a ten-hour working day, would contain 192 kg of citric acid with a potential market value of $2.28 per kg; citric acid worth $438 could therefore be recovered from the process each day, at a value of $26 000 annually allowing for 60 operating days. There would, of course, be recovery costs involved and further discussion of this aspect of the process is beyond the scope of this paper.

References

Anon. (1983). Cordell's building cost book; new construction; New South Wales. Cordell's Building Publications, Northbridge, NSW, vol. 13 no. 3.

Australia. Dept of Industry and Commerce (1980). Costing and pricing for small manufacturers. Managing the small business series: manufacturing no. 3, p. 12. (AGPS: Canberra).

Australian Citrus Growers Federation (1984). Annual report. p. 24, App. C, App. D. (Aust. Cit. Grow. Fedn: Adelaide, S.A.).

BDH Chemicals Ltd (1981). Ion Exchange Resins. 6th Ed. (BDH Chemicals: Poole, England).

Bielig, H. J., Faethe, W., Kock, J., Wallrauch, S., and Wucherpfennig, K. (1983). Standard values and ranges of specific reference numbers for grapefruit juice. *Flussiges Obst.* **50**, 671-82.

Buchanan, R. H. (1966). Operating cost estimation. In "Costs and economics of the Australian process industries". (Eds R. H. Buchanan, C. G. Sinclair). (West Publishing Corp.: Sydney).

CCH Australia (1982). Taxation aspects of plant equipment and buildings. 4th Ed. pp 339-73. (CCH Australia Ltd: North Ryde, N.S.W.).

Chandler, B. V., and Johnson, R. L. (1981). New adsorptive processes for improving the acceptability of processed grapefruit juice. *Proc. Int. Soc. Citriculture,* **2**, 885-8.

Chandler, B. V., and Nicol, K. J. (1975). Debittering citrus products with enzymes. *CSIRO Food Res. Q.*, **35**, 79-88.

Chandler, B. V., and Nicol, K. J. (1982). Alternative cultivars for orange juice production. *CSIRO Food Res. Q.*, **42**, 29-36.

Hollis, R. F., and McArthur, C. K. (1956). The resin in pulp process for recovery of uranium. U.N. Int. Conf. Peaceful Uses of Atomic Energy, Vol. VIII, pp 54-63. (United Nations: New York).

Johnson, R. L., and Chandler, B. V. (1982). Reduction of bitterness and acidity in grapefruit juice by adsorptive processes. *J. Sci. Food Agric.*, **33**, 287-93.

Joint FAO/WHO Food Standards Programme (1982a). Codex Alimentarius. Codex stand. 45-1981. Codex standards for orange juice preserved exclusively by physical means. (FAO: Rome).

Joint FAO/WHO Food Standards Programme (1982b). Codex Alimentarius. Codex stand. 46-1981. Codex standards for grapefruit juice preserved exclusively by physical means. (FAO: Rome).

National Health and Medical Research Council (1973). Report 76th Session. (AGPS: Canberra).

United States Department of Agriculture (1968). United States standards for grades of grapefruit juice. (U.S.D.A. Food Quality Safety Service: Washington, D.C.).

How do food emulsion stabilizers work?

By L. R. Fisher and N. S. Parker

CSIRO Division of Food Research, North Ryde, New South Wales, 2113

Introduction

An emulsion without a stabilizer is like a fool and his money – rapid separation is inevitable. The rapidity is due to the fact that every collision between emulsion droplets leads to coalescence. Since collisions are frequent the 'natural' lifetime of, say, oil in water emulsions ranges from a few seconds to an hour or so, depending on the concentration of the oil (Carroll 1976).

Emulsions are intrinsically unstable because any mixture of oil and water can reduce its total energy by decreasing the interfacial area between the oil and the water. The lifetime of an emulsion can be greatly increased, though, by the addition of emulsion stabilizers. Emulsion stabilizers are compounds that coat the droplet surfaces to provide an energy barrier which reduces the chance of coalescence of colliding droplets. Emulsion lifetimes may also be increased by adding agents (e.g. gums) that increase the viscosity of the continuous phase, thus reducing the frequency of collisions between droplets. In practice, both approaches are used simultaneously, and a given stabilizer may act by both mechanisms.

The choice of an emulsion stabilizer for a given application is largely a matter of experience at present, and likely to remain so for some little time. Recent advances in surface science, however, offer hope of a more rational basis for the choice between food emulsion stabilizers and for the production of a wider range of materials for this purpose. This is not to say that *ad hoc* approaches are not valuable, they are, and have been so since ancient times. A favourite Roman sauce, for example, consisted of olive oil dispersed in *liquamen* – the yellow liquid which is all that is left of salted fish after it has been fermented in the open for several months. The resulting emulsion was reasonably stable, since *liquamen* is no more than a salty solution of amino acids and polypeptides, which are fairly efficient emulsion stabilizers. (It is interesting that *liquamen*, under other names, is still manufactured by a similar process in many South-East Asian countries, and is sold in Australia as fish sauce.)

While *ad hoc* approaches have their value, and are often the quickest way to solve a short-term problem, long-term progress depends on the establishment of fundamental principles. The basic forces governing emulsion stabilization were stated many years ago by Verwey and Overbeek (1948), and understanding of the forces governing emulsion stability has been refined and extended to an astonishing degree in the last 15 years. These advances, and their bearing on food science, are discussed in this article.

Required properties of food emulsion stabilizers

The first essential property of an emulsion stabilizer is that it should be adsorbed (accumulated) strongly at the oil/water interface (Fig. 1). Molecules which do this are generally amphiphiles, which have both a polar and a non-polar region. Often the molecule has a polar head, and one or more hydrocarbon chains forming a non-polar tail, as with fatty acids and their esters, lecithin, cholesterol and the mono- and di-glycerides. Other arrangements are possible, though. For example, proteins have a polar backbone with a range of side-chains, some of which are polar and some non-polar. Emulsions can also be stabilized by small particles, such as mustard grains, the type of emulsion formed (oil in water or water in oil) depending on whether the particle is more readily wet by water or oil respectively.

Once at the interface, emulsion stabilizers act by slowing down one or more of the basic processes responsible for emulsion breakdown (Fig. 2). They cannot generally prevent these processes from occurring; emulsions, with the exception of the recently fashionable microemulsions (Friberg and Venable 1983), are thermodynamically unstable. The first step in emulsion breakdown is drainage of bulk liquid from between the droplets. As mentioned above, gums can slow down this process by increasing the viscosity of the liquid. Most of the common food emulsion stabilizers also slow down the drainage process, either by creating a relatively thick liquid crystalline film or, as for

CSIRO Food Res. Q. 1985, **45**, 33-39

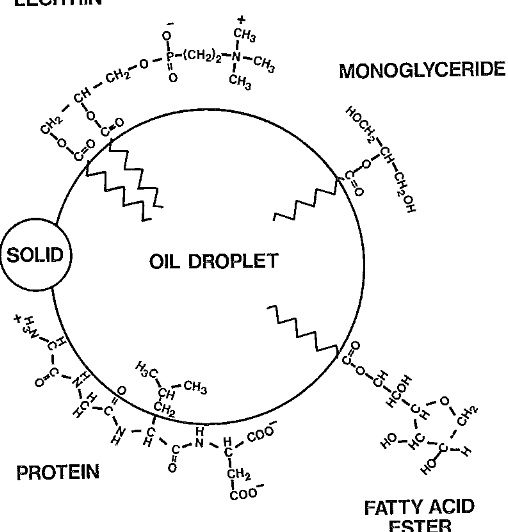


Fig. 1. Adsorption of the first monolayer for some typical food stabilizers at the interface between an oil droplet and water. The polar 'heads' of the molecules protrude into the aqueous phase, while the hydrocarbon 'tails' (represented by zig-zag lines; most tails contain between 12 and 20 carbon atoms) remain in the oil phase. Solid particles act by being more wettable by one phase than the other. The examples illustrated apply to oil-in-water emulsions, where oil droplets are dispersed in a continuous water phase. Water droplets can also be dispersed in a continuous oil phase to give a water-in-oil emulsion.

proteins, by having parts of the molecule protruding into the continuous phase. The drainage process takes a perceptible time, but few measurements of drainage rates have been made, and none of the influence of food emulsion stabilizers on drainage rates.

We are attempting to remedy this situation at the Food Research Laboratory, using apparatus newly developed for the purpose and capable of accurately measuring the very small distances involved (from 80 nm down to contact). As drainage proceeds, the droplets are increasingly pulled together by Van der Waals forces of attraction, and these forces must be balanced by some force of repulsion if the droplets are not to contact and coalesce. If the polar part of the adsorbed material is charged, an electrical repulsion between approaching droplets exists (Fig. 3(a)). This is a major factor in the stabilization of emulsions by charged surfactants such as sodium dodecyl sulphate and also appears to be a factor in the stabilization of emulsions by proteins (Halling 1981). The balance between Van der Waals and electrostatic forces was the first to be discovered and is well described by DLVO theory (Verwey and Overbeek 1948), the letters being the initials of its originators.

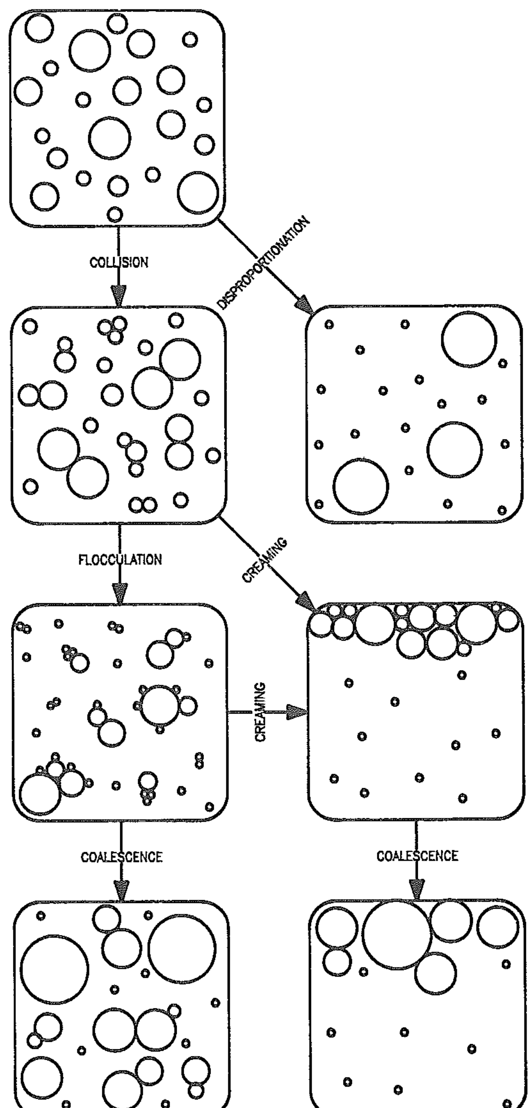


Fig. 2. Steps in the breakdown of an emulsion. The dispersed phase is mobile and on collision the droplets may form aggregates, which can be loosely bound and relatively stable flocs, or the individual droplets may coalesce. When the densities of the two phases are different they will tend to separate by a process called creaming from the analogy with the separation of cream in milk. The rate of separation depends on the size of the individual droplets or flocs. Disproportionation is the growth of the larger droplets and the shrinkage of smaller droplets which can occur when the disperse phase is slightly soluble in the continuous phase.

Most food emulsion stabilizers are not charged, and some other balancing force is needed. Recent work has shown that a major factor is steric repulsion (Phillips 1975), a repulsion arising when the headgroups try to overlap (Fig. 3(b)). Steric repulsion has two contributions: an entropic contribution (interleaving of the headgroups reduces the number of possible configurations of the headgroups thus reducing the entropy) and an osmotic contribution (the local osmotic pressure increases as the local concentration of headgroups increases). An additional short-range repulsion has been discovered recently (LeNeveu *et al.* 1977; Pashley 1981). This is hydration repulsion, and is due to overlap of the hydration shells of the polar headgroups. It is particularly important in maintaining the integrity of biological systems.

There are two further factors that play major parts. One is the fact, briefly noted above, that many of the stabilizers used can form liquid crystalline phases (Fig. 4) (Larsson 1976) in aqueous solution, with the result that, rather than a single monolayer of adsorbed material being present at the droplet surface, many layers may arise – in other words, there is a relatively thick layer of the liquid crystalline phase at the interface. The interaction between the outer layers of the liquid crystalline coatings on different droplets is still governed by the forces described above, but bridges may be formed between adjacent droplets and a three-dimensional gel-like structure developed.

A second factor is that packing of the molecules at the interface may profoundly affect the properties of the adsorbed film. It is known that surfactants in solution aggregate to form a variety of structures (Fig. 4), that are essentially either globular (micelles) or lamellar (vesicles and liposomes). The type of structure formed by a particular surfactant depends on its molecular geometry – in particular, whether or not the headgroup has a larger cross-sectional area then the hydrocarbon tail region (Mitchell and Ninham 1981). Similar considerations may well apply to the packing of adsorbed molecules at the oil/water interface, and hence to the design of more efficient emulsion stabilizers, but this exciting field of enquiry is still in its infancy.

Interpreting the behaviour of food emulsion stabilizers will be much easier as a consequence of these recent advances in colloid science, and the concepts have already been used in our laboratory and other laboratories to design and perform experiments on materials closely related to currently used food stabilizers – lecithin, proteins and the monoglycerides in particular. Such work also bears on important

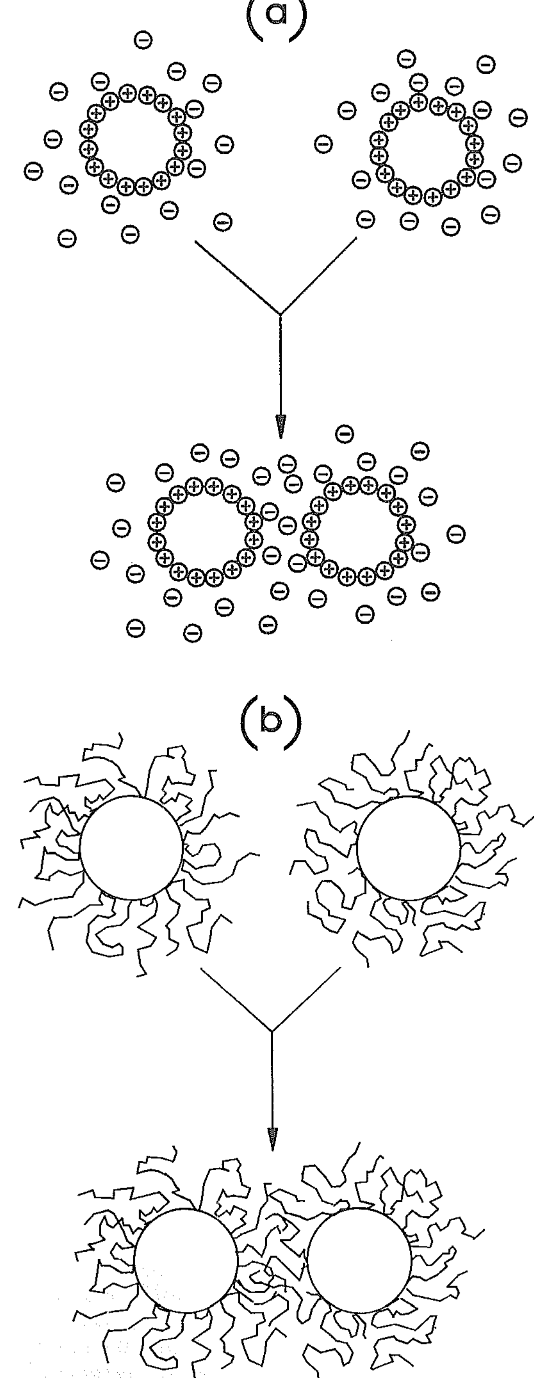


Fig. 3. (a). Repulsive electrostatic forces arise when the clouds of ions surrounding similarly charged droplets begin to overlap.
(b). Repulsive steric forces arise when hydrophilic groups of molecules adsorbed at the surfaces of the droplets begin to overlap.

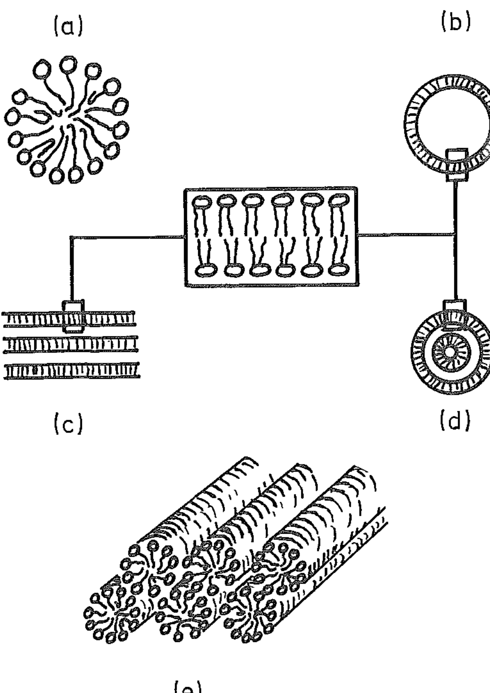


Fig 4. Amphiphilic molecules may aggregate in a number of ways, depending on molecular geometry (a) Micelles (b) Single-walled vesicles (c) Multilamellar stacks of bilayers (d) Concentric multilamellar shells of bilayers (liposomes) (e) Stacks of rod-shaped micelles. (c) and (e) are respectively lammelar and hexagonal liquid crystalline phases which some amphiphiles can adopt at the oil/water interface.

biological questions, since phospholipids and proteins in particular play a major part in biological cell membrane recognition, adhesion and fusion, the fundamental processes being very similar to those that occur in emulsion droplet interactions.

Mode of action of food emulsion stabilizers

The common types of emulsifiers recommended by the National Health and Medical Research Council (1983) as food additives are listed in Table 1. The discussion will be confined to the four main classes of emulsion stabilizer which act by adsorption at the oil/water interface – fatty acids and their esters, lecithin, the mono- and di-glycerides and the proteins. The gums (polysaccharides) usually act simply by increasing the viscosity of the continuous phase (Glicksman 1982), although there is some recent evidence that these materials also adsorb at the oil/water interface (Fig. 5 and Robins, M. (personal communication)). A recent article by Oakenfull (1984) describes some of the properties of polysaccharides.

It is first necessary to be clear what is meant by 'oil'. In most surface chemistry, 'oil' is equated with 'liquid hydrocarbon', and adsorption of all sorts of materials, including food emulsion stabilizers, has been studied extensively at the hydrocarbon/water interface. In food science, though, 'oil' means 'triglyceride', and adsorption studies at the triglyceride/water interface are scarce and often unreliable. Even the most fundamental information on this interface is lacking. Interfacial tension values, for example, are a guide to the ease with which emulsions can be formed, since the lower the interfacial tension the easier it is to deform and break up a droplet. The extent of adsorption of added stabilizers can also be calculated from the change in interfacial tension when the stabilizer is added. Reliable values for triglyceride/water interfacial tensions, though, have been sparse, and worse, inconsistent (Fisher *et al.* 1985). It has thus frequently been necessary to use measurements of adsorption made at the hydrocarbon/water interface in the hope that these might have some relevance to the triglyceride/water interface. Parallels do indeed exist (Dickinson 1984), but there are also important differences. For a start, the triglyceride/water interfacial tension is about 25 mNm^{-1}, which is around one-half of the value for the hydrocarbon/water interface. Adsorption can also be very selective, and while both the fatty acids and the monoglycerides adsorb strongly at the hydrocarbon/water interface, only the latter do so at the triglyceride/water interface.

These differences arise because the triglycerides are 'polar' oils, having a distinct head and tail region (Fig. 6). Fatty acids, having a similar head and tail structure, tend not to

TABLE 1

Emulsifiers recommended for use in foods.*

Ammonium salts of phosphatidic acids
Diacetyl tartaric acid ester of mono- and di-glycerides
Glyceryl lactostearate
Mono- and di-glycerides of fat forming fatty acids
Phospholipids derived from natural sources (including lecithin)
Polyoxyethylene (20) sorbitan monostearate (polysorbate 60)
Polyoxyethylene (20) sorbitan tristearate (polysorbate 65)
Polyoxyethylene (20) sorbitan monooleate (polysorbate 80)
Sorbitan monostearate
Sucrose esters of fatty acids

*from 'Model food legislation' (National Health and Medical Research Council 1983).

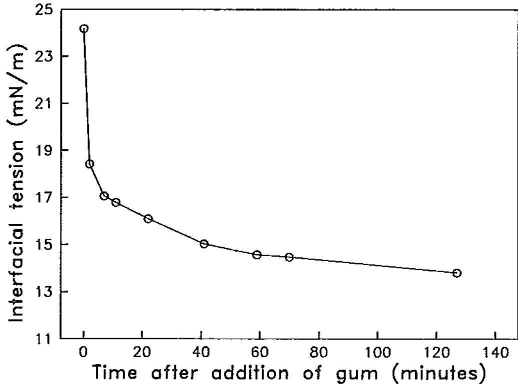


Fig. 5. The effect of 0.1% guar gum in the aqueous phase on the interfacial tension between water and olive oil from measurements made at this laboratory.

adsorb at the triglyceride/water interface. A current project in the Food Research Laboratory is to measure systematically the interfacial tensions with water of a range of triglycerides, and also to study the adsorption of common food emulsion stabilizers at the oil/water interface. The experimental approach adopted is the pendant drop method which has already produced some useful results (Fisher *et al.* 1985), including the fact that all the major pure natural triglycerides have very similar interfacial tensions with water, the value being around 25 mNm^{-1}.

Fatty acids and their esters

Fatty acids and their esters adsorb at the oil/water interface to form a monolayer, and may stabilize either oil-in-water (i.e. oil droplets in a continuous water phase) or water-in-oil emulsions depending on circumstances. The concept of 'hydrophile-lipophile balance' (HLB) gives an indication of the type of emulsion that may be formed. Briefly, a longer hydrocarbon chain makes the compound more 'lipophile' and more likely to promote oil-in-water rather than water-in-oil emulsions. The situation is complex, though, and the detailed structure of the emulsion stabilizer can be crucial; it can, for example, make a difference whether the alkyl chain is saturated or unsaturated (Petrowski 1976). The constraints imposed by molecular packing are obviously important here, but have so far been largely ignored by workers in the field.

For oil-in-water emulsions stabilized by fatty acids and their esters, interaction between the headgroups of the adsorbed molecules on different droplets is of prime importance. If the headgroups are charged, the main interaction is electrostatic, which can be simply described by DLVO theory (Dickinson and Stainsby 1982). If the headgroups are not charged, then steric interactions predominate and these can again be predicted with some accuracy by theory. For water-in-oil emulsions, it is the interactions between the protruding hydrocarbon tails of the stabilizer that are important. Again, these interactions are steric in nature.

Monoglycerides

Monoglycerides adsorb strongly at the triglyceride/water interface (Kuhrt *et al.* 1950), often forming relatively thick liquid crystalline layers, which may be viewed as consisting of an anchoring monolayer at the oil surface, upon which is stacked a set of bilayers, so that the outer monolayer has its polar headgroups pointing outwards (Fig. 4(c)). Depending on the conditions of formation of the emulsion, the stack may extend as a bridge to an adjacent droplet, or individual droplets may have their own stacks, in which case the polar groups will face each other. We have recently found in this laboratory that when the polar groups of monoglyceride bilayers approach each other closely there is a significant repulsion, presumably due to hydration forces, which keeps the bilayers apart. This finding implies that liquid crystalline bridges are unlikely to develop as an emulsion ages, and are more likely to have appeared during the initial stages of emulsion formation.

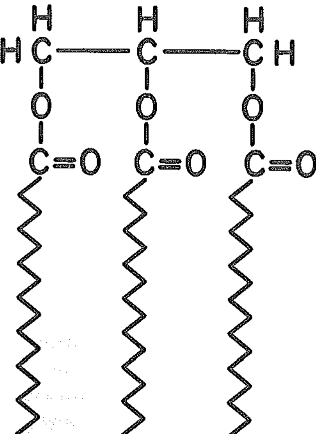


Fig. 6. Molecular structure of a triglyceride. The ester linkages connecting the three hydrocarbon chains (represented by zig-zag lines) to the glycerol residue have some affinity for water. The hydrocarbon chains may be of different length and different degrees of saturation.

Phospholipids

Phospholipids, in particular lecithin, are ubiquitous constituents of cell membranes and also occur in high concentrations elsewhere e.g. about 10% in egg yolk. Their mode of action as emulsion stabilizers is similar to that of the monoglycerides, since they can also form lamellar liquid crystalline phases. In fact, it is only necessary to shake lecithin gently in water to generate multilamellar 'liposomes' (Fig. 4(d)), whose obvious similarities to the lipid bilayer structure of the cell membrane have led to extensive study of their properties. It is particularly interesting that the interlamellar distance can be changed by exposing the liposomes to different osmotic conditions, thus shrinking or swelling them, and the degree of shrinking or swelling at different osmotic pressures can yield the distance between the phospholipid sheets as a function of applied pressure (Parsegian *et al.* 1979). Direct measurements have also been made of the forces between phospholipid layers (Marra and Israelachvili 1985), and there is thus a considerable body of information about these important stabilizers, although this information has not so far been applied to their function as food emulsion stabilizers.

Two particular difficulties arise in studies of systems containing phospholipids. One is the rate at which effects may occur. Both the growth of the liquid crystals and their swelling behaviour can be very slow, leading to complex time-dependent effects. Further, the phospholipids, such as those from soybeans, used in commercial practice are grossly impure, a major impurity being lysolecithin, which is well known to disrupt the lamellar phase (because it disrupts the packing behaviour), and is in fact used in biochemical practice to lyse cells.

Proteins

Protein stabilization of emulsions is the subject of an outstanding recent review (Halling 1981), as is the behaviour of proteins at interfaces in general (MacRitchie 1978). Proteins are large and complicated molecules, and in nature each protein performs a precise and different function. Thus, it is not surprising, although certainly disconcerting, to find that there is no consensus on how proteins stabilize emulsions. A common idea has been that proteins form a protective skin around the emulsion droplets, but this is difficult to reconcile with several properties of protein-stabilized emulsions (Halling 1981), and there is at present no clear correlation between any interfacial properties of proteins and their effectiveness as emulsifiers.

Halling (1981) has pointed out that part of the difficulty in determining the mechanism of action of proteins as emulsion stabilizers is that it is very difficult to compare results from different laboratories. The conditions of preparation of emulsions are often different, and crucial parameters, particularly droplet size and size distribution, are frequently left unmeasured. This difficulty is not unique to protein studies, the trouble being that the most useful parameters, in particular drop size distribution, are very difficult to measure compared with such parameters as emulsifying capacity and creaming rate which, however, yield less useful information.

Even so, it is disappointing that so little is known about the emulsion stabilizing functions of proteins, since the incorporation of proteins into emulsions as stabilizers would, for example, allow a wider range of possibilities for introducing protein from novel sources into the diet. Since studies on large, complex proteins have produced so little useful information, the authors have embarked on a study of the adsorption behaviour of small polypeptides (parts of proteins) and low molecular weight proteins of known structure, the aim being to find out how the detailed structure of a protein affects its adsorption behaviour. This study is being carried out in parallel with a study of the effect of adsorbed proteins on the drainage of aqueous solution from between approaching oil droplets.

Conclusions

Simple emulsion stabilizers, such as the fatty acids and their esters, act by balancing the Van der Waals forces of attraction between approaching emulsion droplets with electrostatic or steric forces of repulsion. These forces are well understood, although application of the knowledge to particular food problems is made difficult by the complexity of most food emulsions. The main hurdle, though, is that the necessary basic experimental data, particularly the adsorption isotherms at the triglyceride/water interface, are simply not available except possibly in industrial files.

The mono- and di-glycerides and lecithin show somewhat more complicated behaviour, in that they can form liquid crystalline multilayers at the interface between oil and water. The basic forces stabilizing the system are augmented by the recently characterized hydration force, a very strong short-range force of repulsion, which is important not only in emulsion stabilization but also in maintaining the integrity of biological systems, and which is consequently receiving a lot of attention at the present time.

While the basic forces due to the presence of emulsion stabilizers are now quite well understood, calculation and measurement of these forces in actual food emulsions is at an early stage. There is very little substantial evidence as to how proteins stabilize emulsions, and much of this is conflicting. The same forces must be involved, but the interfacial behaviour of proteins may well depend on the details of the protein structure, so that a program of adsorption studies on very simple protein analogues is necessary to elucidate the actual mechanisms.

Work at the Food Research Laboratory is focused on three areas: measurement of adsorption isotherms at the triglyceride/water interface (particularly those of simple proteins and simple protein analogues); measurement of the effect of food emulsion stabilizers on the drainage rate of liquid from between approaching droplets; and measurement of the effect of adsorbed stabilizer on the forces between droplet surfaces. Some of this work has been published, but we are really only at the beginning of these long-term but essential studies whose aim is to improve the use of present stabilizers and guide the production of new and more efficient ones.

References

Carroll, B. J. (1976). The stability of emulsions and mechanisms of emulsion breakdown. *Surf. Colloid Sci.* 9, 1-67.

Dickinson, E. (1984). Effect of polar and non-polar interactions on the thermodynamics of triglyceride mixtures. *Chem. Phys Lipids* **34**, 171-83.

Dickinson, E., and Stainsby, G. (1982). 'Colloids in Food.' (Applied Science: London.)

Fisher, L. R., Mitchell, E. E., and Parker, N. S. (1985). The interfacial tensions of commercial vegetable oils with water. *J. Food Sci.* (in press).

Friberg, S. E., and Venable, R. L. (1983). In 'Encyclopedia of Emulsion Technology'. (Ed. P. Becher.) (Dekker: New York.)

Glicksman, M. (1982). 'Food Hydrocolloids. 'Vol. I. (CRC Press: Boca Raton, Florida.)

Halling, P. J. (1981). Protein-stabilized emulsions and foams. *Crit. Rev. Food Sci. and Nutr.* **15**, 155-203.

Kuhrt, N. H., Welch, E. A., and Kovarik, F. J. (1950). Molecularly distilled monoglycerides I. Preparation and properties. *J. Am. Oil Chem. Soc.* 27, 310-3.

Larsson, K. (1976). In 'Food emulsions.' (Ed. S. Friberg.) (Dekker: New York.)

LeNeveu, D. M., Rand, R. P., Parsegian, V. A., and Gingell, D. (1977). Measurement and modification of forces between lecithin bilayers. *Biophys. J.* **18**, 209-30.

MacRitchie, F. (1978). Proteins at interfaces. *Adv. Protein Chem.* **32**, 283-326.

Marra, J., and Israelachvili, J. N. (1985). Direct measurement of attractive, adhesive and repulsive forces between phosphatidylcholine and phosphatidylethanolamine bilayers in aqueous electrolyte solution. *J. Colloid Interface Sci.*, submitted for publication.

Mitchell, D. J., and Ninham, B. W. (1981). Micelles, vesicles and microemulsions. *J. Chem. Soc. Faraday Trans. II* 77, 601-29.

National Health and Medical Research Council (1983). 'Model food legislation.' (Australian Government Publishing Service: Canberra.)

Oakenfull, D. G. (1984). Food gels. *CSIRO Food Res. Q.* **44**, 49-55.

Parsegian, V. A., Fuller, N., and Rand, R. P. (1979). Measured work of deformation and repulsion of lecithin bilayers. *Proc. Natl. Acad. Sci. USA* **76**, 2750-4.

Pashley, R. M. (1981). DLVO and hydration forces between mica surfaces in Li^+, Na^+, K^+, and Cs^+ electrolyte solutions: a correlation of double-layer and hydration forces with surface cation exchange properties. *J. Colloid Interface Sci.* **83**, 531-46.

Petrowskj, G. E. (1976). Emulsion stability and its relation to foods. *Adv. Food Res.* **22**, 309-59.

Phillips, M. C. (1975). In 'Water: A Comprehensive Treatise 5' (Ed. F. Franks.) (Plenum: New York.)

Verwey, E. J. W., and Overbeek, J. Th. G. (1948). 'Theory of the Stability of Lyophobic Colloids'. (Elsevier: Amsterdam.)

Recent developments in the genetics of lactic acid bacteria*

By R. R. Hull

CSIRO Division of Food Research, Dairy Research Laboratory, Highett, Victoria, 3190

Introduction

Lactic acid bacteria (*Pediococcus, Lactobacillus, Streptococcus and Leuconostoc*) are used in the manufacture of a wide range of traditional fermented foods (Table 1). Their production of organic acids not only contributes to the desired taste and flavour of the final product, but it also makes the substrate unfavourable for proliferation of, and spoilage by, other undesirable microorganisms. At the same time, the acids make the substrate more suitable for growth of desirable microorganisms which improve the properties of the food. The genetics of lactic streptococci used in cheese manufacture will be discussed in this paper.

TABLE 1

Lactic ferment products

Substrate	Products
Milk	Cheese
	Yoghurt
	Cultured butter
Meat	Salami
Fish	Fish sauce
Vegetables	Pickles
	Olives
	Sauerkraut
Grains	Sour dough bread

Cheese manufacture

Cheese quality is critically dependent upon reliable performance of starter culture streptococci in producing lactic acid during cheesemaking and in contributing to the development of desirable flavours as the cheese matures.

One of the main factors contributing to low quality Cheddar cheese is a reduced level of acid in the cheese as a result of impaired starter activity. Such cheese would be expected to contain higher than normal amounts of lactose, providing an energy source for the growth of spoilage and other undesirable microorganisms. A number of factors adversely affect starter activity, and are listed in Table 2. They can be considered in two categories, as intrinsic or extrinsic factors.

Intrinsic factors

Intrinsic variation within starter streptococci is a major factor affecting their rate of lactic acid production. Variation is due to both physiological factors and genetic instability.

Physiological factors

Lactic streptococci produce lactic acid at a rate of more than 10% of their weight per minute. This rapid production of acid is used to

TABLE 2

Factors influencing starter activity in cheese manufacture

Intrinsic properties of starter

1. Physiological state
2. Phenotypic and genotypic changes during culturing

Extrinsic factors

1. Manufacturing conditions
2. Milk composition
   - mastitis
   - colostrum
   - mineral deficiencies
3. Milk inhibitors
   - antibiotics
   - antibodies
   - dissolved oxygen
   - disturbing phage
   - free fatty acids
   - inhibitory bacteria
   - lactoperoxidase system
   - sanitizers

*A paper presented to the Australian Society of Microbiology - New Zealand Microbiology Society Annual Scientific Meeting held in Sydney, 1984.

advantage in cheesemaking but is a marked disadvantage when maintaining cultures, and when producing bulk starter cultures that can be stored. Loss of activity in overripe bulk cultures is well recognized but the problem of acid injury producing undesirable changes during culture maintenance is less well defined.

Heterogeneity within lactic streptococcal cultures is known to exist for acid production (Pearce *et al.* 1974; Efstathiou and McKay 1976) and phage sensitivity (Hunter 1947; Limsowtin and Terzaghi 1977; Limsowtin, Heap and Lawrence 1978). Many of the observed changes in cultures may well be a result of the selection of a variant subclass from heterogeneous cultures. Elucidation of the genotypic and phenotypic basis of culture heterogeneity may provide a solution to the problem of maintaining starter cultures without undesirable changes.

Genetic instability

The production of lactic acid is dependent upon at least two host functions:

- the lactose transport and hydrolysing enzyme complex and
- the protease/peptidase enzyme complex which hydrolyses milk proteins releasing amino nitrogen required for bacterial growth.

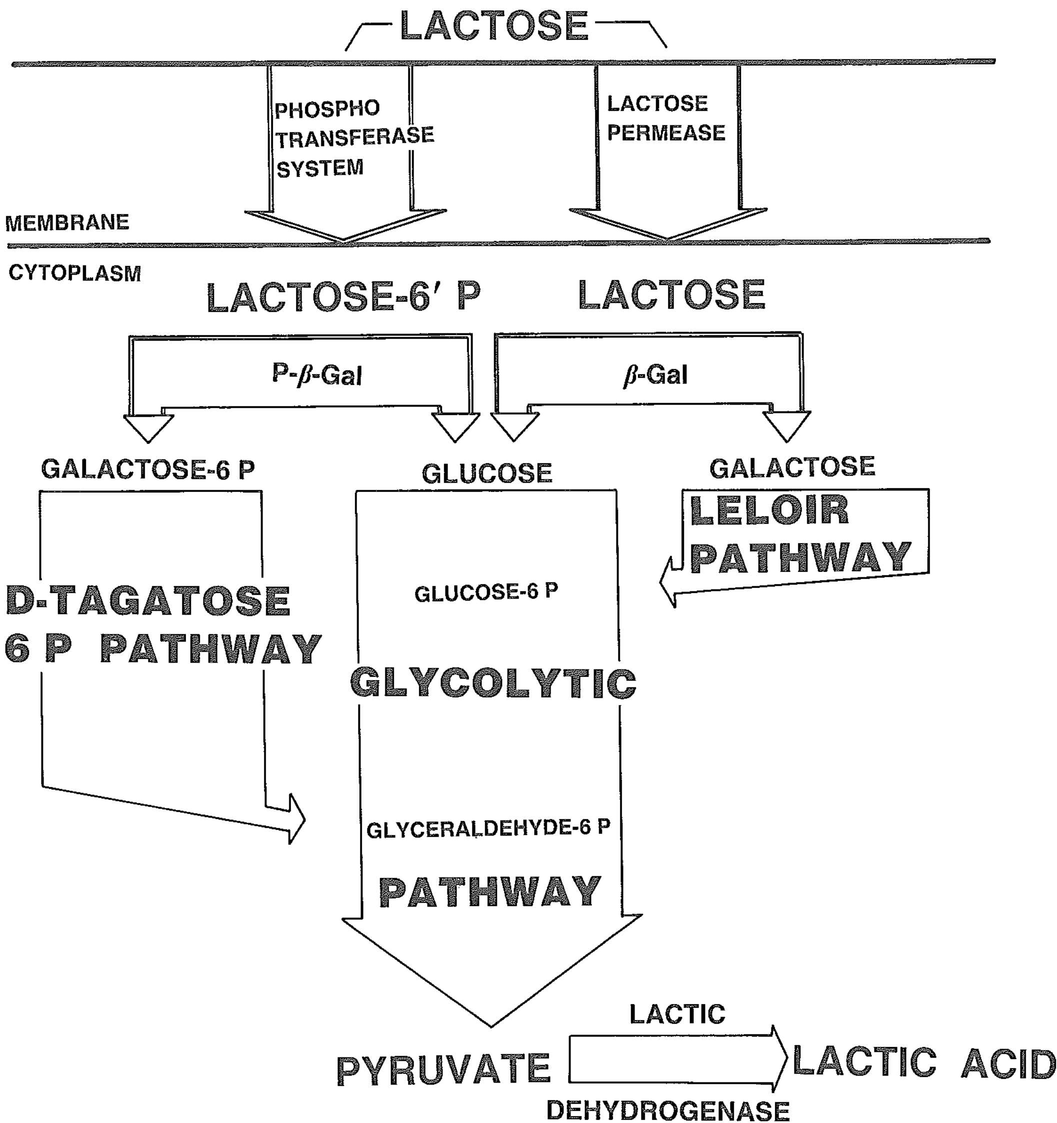


Fig. 1. The pathways involved with lactose utilization.

Lactose utilization

The group N streptococci, to which most of the industrial starters belong, take up lactose as lactose-phosphate (lactose-P) via a phosphoenolpyruvate-dependent phosphotransferase system (PEP:PTS). A β-D-phosphogalactosidase (β-P-gal) then hydrolyses the lactose-P to glucose and galactose-6-P from where glucose is metabolized by the normal glycolytic pathway, and galactose-6-P is metabolized by the tagatose diphosphate pathway. Ultimately, both the hexose moieties are converted completely to lactic acid, and this provides the organisms with their energy requirement (Fig. 1).

Group N streptococci spontaneously produce mutants which are unable to utilize lactose. These *lac*⁻ mutants cannot synthesize two protein components of the PEP:PTS and the β-P-gal enzyme (Pearce *et al.* 1974; Efstathiou and McKay 1976; Crow and Thomas 1984). The instability of lactose utilization has led to the suggestion that the genes coding for these PEP:PTS proteins and the β-P-gal are plasmid encoded and such a suggestion is supported in *S. lactis* by genetic and biochemical studies (McKay and Baldwin 1974; 1975; Klaenhammer *et al.* 1978).

Utilization of milk proteins

The lactic streptococci require free amino-acids for growth, but the concentration of free amino-acids in milk is too low to support the starter growth required for milk fermentation. Group N streptococci use cell-bound extracellular proteinases to digest casein to peptides. The peptides are transported into the cell and then hydrolysed intracellularly to their constituent free amino-acids. Some strains have the additional capability of hydrolysing the peptides extracellularly.

Cultures of group N streptococci contain a small number of variant cells (*Prt* variants) which grow very slowly in milk, but at a normal rate in media supplemented with free amino-acids or peptides. These variants presumably lack one of the proteinase functions making up the *Prt*⁺ phenotype. There is evidence suggesting that the genes specifying these functions may be located on different plasmids or on the chromosome (McKay 1983).

Plasmids

Many strains of lactic acid bacteria contain an unusually large complement of plasmid DNA (see Davies and Gasson 1981, for review). However, the number of plasmid bands observed on gels may well be an overestimate of the number of plasmid species present. Plasmids may exist in monomeric or multimeric forms and also as open circular, closed circular, or linear DNA depending upon the preparation techniques used (Davies and Gasson 1981; Yu *et al.* 1982). Most of the plasmids are cryptic, in that only a few have been shown to encode known functions. Plasmid locations have been shown for genes specifying some or all of the functions of lactose fermentation (McKay and Baldwin 1974), proteinase production (McKay and Baldwin 1975), citrate metabolism (Kempler and McKay 1979), and resistance to inorganic salts (Efstathiou and McKay 1976). Some of these plasmids are thermolabile so that incubation of lactic streptococci just below the lethal temperature leads to a rapid loss of plasmids in culture. This method has successfully been used to assign functions to particular plasmids, including lactose metabolism, proteolysis, citrate utilization, bacteriocin production and restriction/modification activity (Teuber 1982).

The plasmid pattern of individual strains varies widely with some strains containing no plasmids and others containing up to 12 plasmids. Molecular weights and some functions tentatively assigned to some plasmids are shown in Table 3.

TABLE 3

Plasmids and associated functions in lactic streptococci

Function	Plasmid m.wt. ($\times 10^6$)
Lactose transport and hydrolysis	30-45
Milk proteolysis	30-45
	2.2, 8.5, 12.5
Citrate permease	5.5
Bacteriocin WM4	88
Restriction and modification (KH)	15
Phage resistance (TS)	40
Sucrose, glucose, mannose, xylose, galactose metabolism:	Uncertain
Resistance to inorganic ions	

Extrinsic factors

Extrinsic factors affecting starter activity can be considered in three categories:

- Variations in manufacturing conditions, e.g. temperature and salting rate
- Variations in milk composition, e.g. mastitis, mineral content and period of lactation, and
- Variation in levels of natural and added inhibitors, e.g. bacteriophage and sanitizers.

Of these factors, bacteriophage appears to be the main factor causing variability in the activity of starters, and therefore the quality of cheese.

Bacteriophage

Bacteriophage (or phage) are bacterial viruses, and their properties make them extremely troublesome in fermentation industries. Viruses are the smallest of living things varying in size from 0.03-0.1μm. They are parasites in the extreme, consisting only of nucleic acid and protein. They are metabolically inert, resistant to heat and desiccation and because of their very small size, readily become airborne. They depend upon the metabolic activity of their host for multiplication.

This multiplication cycle commences with adsorption to a specific receptor on the surface of a sensitive host bacterium. Irreversible adsorption of the phage to the cell triggers a number of steps which lead to the transport of the viral nucleic acid into the cell cytoplasm. Once inside the cell the viral nucleic acid (chromosome) becomes metabolically active, transforming the host cell from its normal cellular functions to one of synthesizing components for many new virus particles. Completion of virus assembly leads to lysis of the cell and release of approximately 100 new virus particles (Fig. 2 – Lytic cycle). All of this occurs in approximately one generation time of the host bacterium. Therefore, in one generation time of the cell, one virus particle multiplies to 100, in two generation times to 10^4 and in five generation times to 10^{10} particles; more than enough to lyse the complete bacterial culture.

Bacteriophage can enter into alternative relationships with bacteria – such as the passive lysogenic situation (Fig. 2 – Lysogenic cycle). In such a situation the phage chromosome integrates itself into the bacterial chromosome and is inherited along with all other bacterial genes by all progeny bacteria. Under certain circumstances, such latent phage can be induced to become metabolically active, lysing the bacterium and releasing new phages.

Starter cultures for cheesemaking are available from various commercial suppliers and Government agencies such as CSIRO, however, historically they were handed from one generation of cheesemaker to the next with the original source being unknown.

Starter preparation

Generally, starter cultures are prepared in the factory by growing streptococci in milk (Fig. 3), which is then used at a 1% inoculum level in cheese manufacture. Typically, a factory fermenting 0.5 megalitres of milk a day to

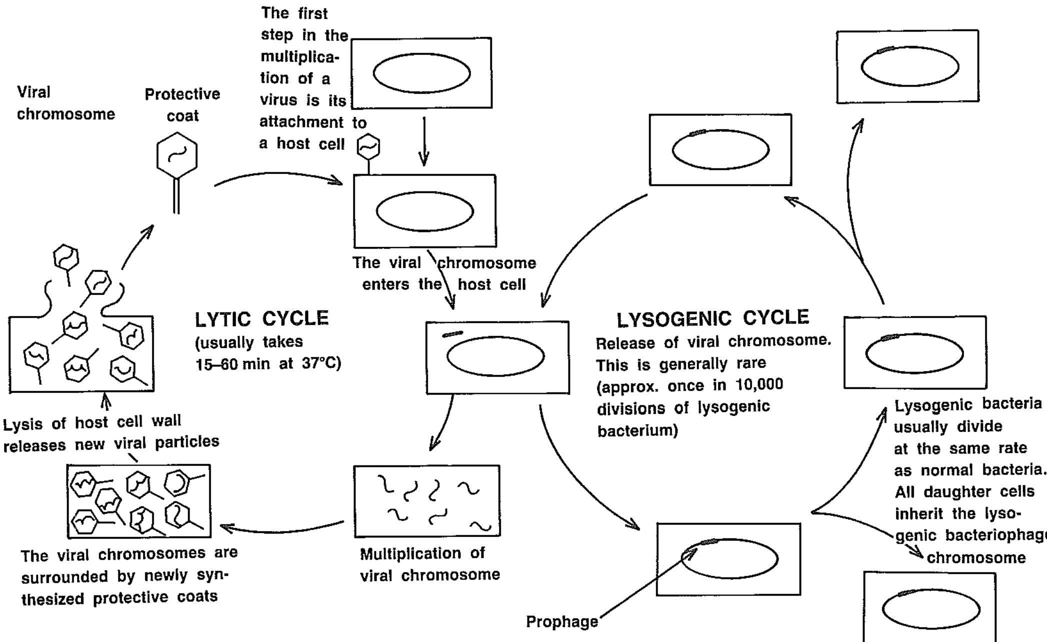


Fig. 2. The life cycle of a lysogenic bacterial virus.

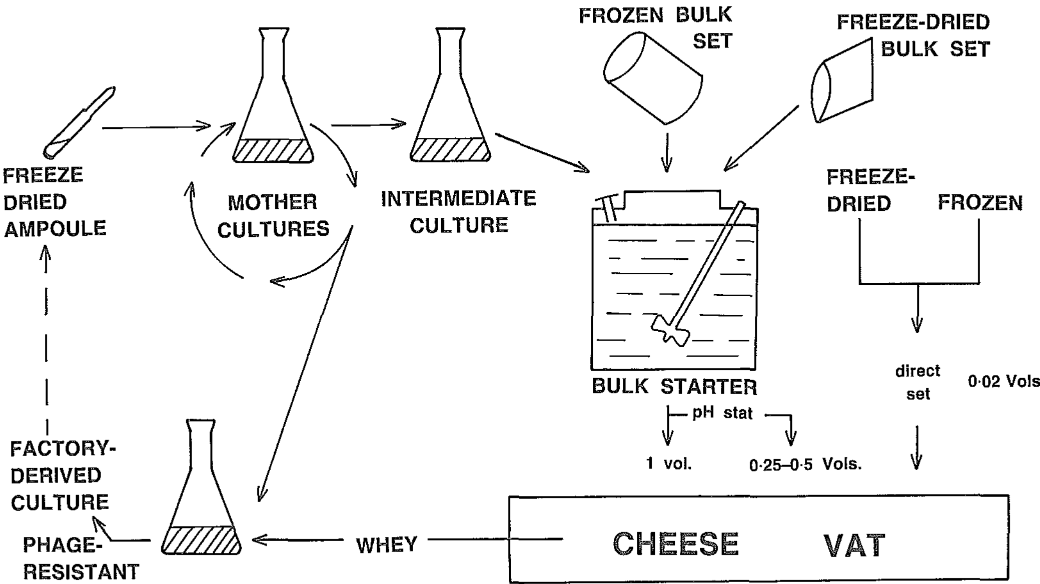


Fig. 3. The various forms of starter available.

cheese will require 5000 litres of starter culture (bulk culture).

In the absence of phage there is no difficulty in preparation of the bulk culture or in its use in cheese manufacture. However, in the presence of phage the whey produced from cheese manufacture may contain up to 10^{12} phage per ml or in excess of 10^{20} phage per day. These phages pose two problems for the factory technologist: to prepare the bulk starter culture without phage infection, and to manufacture cheese without phage infection.

Phage control

Elaborate procedures based on two approaches have evolved over the last 40 years to control phages. The first uses aseptic techniques to exclude phages during starter preparation and cheesemaking. The theory was to keep the phages away from the starter culture.

The second uses so-called "phage unrelated" strains (strains showing no cross-sensitivity to phage) on a rotational basis. Typically, in such schemes, a factory may use up to twelve phage unrelated cultures in a rotation to provide sufficient time before reusing any of the strains. The time period from the beginning to the end of a rotation should allow phage numbers to be reduced to safe levels by way of normal factory hygiene.

This system of phage control has never been fully effective because of the difficulty of applying aseptic techniques on a large scale, and because strains with completely different phage sensitivities have not been found. Indeed, the use of a large number of "phage unrelated" strains actually aggravates the phage problem because most strains are lysogenic and during manufacture release their phage(s) into the factory environment. This increases the genetic diversity of the phage population of the cheese factory providing a larger gene pool from which new types of virulent phages can arise by mutation or recombination.

Phage control by using phage-resistant mutants

Bacteriophage-resistant mutants of streptococci have been isolated by a number of workers, but this approach was always abandoned because the phage adapted to the resistant mutants, i.e. host range phage mutants or other new phage appeared. These failures caused this approach to be abandoned in the 1940s and again in the 1950s and early 1960s.

S. lactis strain 101 is sensitive to at least 18 different phages – different on the basis of host range and plaque morphology. All phages were isolated from cheese whey except 900 and 901, which are lysogenic phages for *S. lactis* strain C6. We have isolated bacterial mutants resistant to each of the 18 phages and checked their cross-resistance to all other phages. On the basis of the cross-resistance of these phage-resistant mutants, it is possible to group the phages in four sets.

These four sets of phage correspond to morphological subgroups (Table 4), suggesting that the members of each set are variants of a

TABLE 4

Morphology of bacteriophages attacking S.lactis

Bacteriophage resistance class			
I	II	III	IV
sk3 10III	10n 10p m18 900 901	drc1 10w wm4 c6B	c6A c6 drc1C 10I 10II sk2 c8T

100 nm

single type of phage.

Many of the bacterial mutants resistant to sets 3 and 4 phages at 30 °C, showed varying degrees of sensitivity when challenged at 37 °C, with some strains reverting to complete sensitivity. Sets 3 and 4 phages also show a wider host range at 37 °C which appears to be due, in part, to a breakdown in the efficiency of host restriction-modification systems at higher temperatures (Sanders and Klaenhammer 1984) and to some other host functions that are yet to be determined.

Commercial phage-resistant strains

The results of our study of the genetics of phage resistance, and other aspects of biology of the phage-host relationship, has led to the conclusion that phages of this type (sets 3 and 4) are a major cause of disrupted fermentations, using both *S. lactis* and *S. cremoris*. This has led to the development of a method for the isolation of commercial phage-resistant strains by natural selection. Purity, activity and phage resistance are all checked before these 'factory-derived starters' are used commercially (Hull 1983). Such strains are currently being used to manufacture more than half (60 000 tonnes p.a.) of Australia's Cheddar cheese.

Conclusion

The lactic acid bacteria have been of great importance in the fermentation of various foods, particularly milk, for many centuries. All of these fermentations rely on the organisms producing large amounts of lactic acid. Various reasons exist for the starter bacteria being slow acid producers, the major one being bacteriophage. Efforts in the past have not satisfactorily solved this problem.

The genetic approach to the phage problem has yielded a procedure for the selection and use of phage-resistant cultures in commercial manufacture. However, the problem of genetic instability of other important technological properties, such as rapid acid production, remains for resolution.

In the last decade, research into the genetics of lactic acid bacteria has also made significant progress. A realization that genetic studies may find application in the modification of cheese starters has led to an expansion of research effort in this area, particularly overseas.

References

Crow, V. L., and Thomas, T. D. (1984). Properties of a *Streptococcus lactis* strain that ferments lactose slowly. *J. Bact.* **157**, 28.

Davies, F. L., and Gasson, M. J. (1981). Genetics of lactic acid bacteria. *J. Dairy Res.* **48**, 363.

Efstathiou, J. D., and McKay, L. L. (1976). Plasmids in *Streptococcus lactis*: evidence that lactose metabolism and proteinase activity are plasmid linked. *Appl. Environ. Microbiol.* **32**, 38.

Hull, R. R. (1983). Factory-derived starter cultures for the control of bacteriophage in cheese manufacture. *Aust. J. Dairy Technol.* **38**, 149.

Hunter, G. J. E. (1947). Phage-resistant and phage-carrying strains of lactic streptococci. *J. Hyg. Camb.* **45**, 307.

Kempler, G. M., and McKay, L. L. (1979). Characterization of plasmid deoxyribonucleic acid in *Streptococcus lactis* subsp. *diacetylactis*: evidence for plasmid-linked citrate utilization. *Appl. Environ. Microbiol.* **37**, 316.

Klaenhammer, T. R., McKay, L. L., and Baldwin, K. A. (1978). Improved lysis of group N streptococci for isolation and rapid characterization of plasmid deoxyribonucleic acid. *Appl. Environ. Microbiol.* **35**, 592.

Limsowtin, G. K. Y., and Terzaghi, B. E. (1977). Characterization of bacterial isolates from a phage-carrying culture of *Streptococcus cremoris*. *N.Z. J. Dairy Sci. Technol.* **12**, 22.

Limsowtin, G. K. Y., Heap, H. A., and Lawrence, R. C. (1978). Heterogeneity among strains of lactic streptococci. *N.Z. J. Dairy Sci. Technol.* 13, 1.

McKay, L. L., and Baldwin, K. A. (1974). Simultaneous loss of proteinase- and lactose-utilizing enzyme activities in *Streptococcus lactis* and a reversal of loss by transduction. *Appl. Microbiol.* 28, 342.

McKay, L. L., and Baldwin, K. A. (1975). Plasmid distribution and evidence for a proteinase plasmid in *Streptococcus lactis* C2 *Appl. Microbiol.* 29, 546.

McKay, L. L. (1983). Functional properties of plasmids in lactic streptococci. *Antonie van Leeuwenhoek* 49, 259.

Pearce, L. E., Skipper, N. A., and Jarvis, B. D. W. (1974). Proteinase activity in slow lactic-acid producing variants of *Streptococcus lactis. Appl. Microbiol.* 27, 933.

Sanders, M. E., and Klaenhammer, T. R. (1984). Phage resistance in a phage-insensitive strain of *Streptococcus lactis*: temperature-dependent phage development and host-controlled phage replication. *Appl. Environ. Microbiol.* 47, 979.

Teuber, M. (1982). Jahresber. Bundesanst. Milchforsch. Kiel B91.

Yu, R. S. T., Hung, T. V., and Azad, A. A. (1982). Rapid screening of highly purified plasmids in lactic streptococci. *Aust. J. Dairy Technol.* 37, 99.

News from the Division

Visiting scientist

Although the Division derives little pleasure from the declining number of its research scientists over the past several years, happily the number of distinguished Visiting Scientists shows no sign of a similar decline. It is particularly pleasing to be able to welcome back Emeritus Professor Sir Rutherford Robertson, AC, FRS, FAA, currently a Guest Worker in FRL's Food Structure Group. 'Bob' Robertson was appointed to the Homebush laboratories of the Division of Food Preservation in 1945 to take charge of the fruit and vegetable work and in 1950 became the first Leader of the Plant Physiology Unit, then based at the University of Sydney. From 1959-1962 he served on the CSIRO Executive. He was Professor of Botany at the University of Adelaide, 1960-69, Master of University House at the Australian National University from then until 1973, when he was appointed Director of the Research School of Biological Sciences at ANU, the position he held until he officially 'retired' in 1978. Professor Robertson's interest in the inner mitochondrial membrane, in which electrons and protons are transported, eventually forming water by combination with oxygen, began some 30 years ago in CSIRO, at which time he was working on the respiration and ion balance of plant mitochondria. In the following paragraph Dr Bruce Cornell explains how this life-long interest has become part of a collaborative project at FRL.

"Many of the projects currently under investigation in the Division of Food Research ultimately depend upon an understanding of the molecular interactions which occur within biological membranes. In order to cope with the many aspects of this complex problem it is necessary to draw on a wide range of expert knowledge. For this reason we were extremely pleased when Professor Sir Rutherford Robertson agreed to collaborate with us on the study of the ubiquinone cycle in mitochondria. Work in this area began in our section in 1982 during the visit of Dr Peter Quinn of the University of London. Since then our interests have broadened to encompass the function of a related compound, photoquinone, which performs a similar role in plant chloroplasts. The work on plant chloroplasts has involved the additional collaboration of Dr Roger Hiller and Ms Adele Post, both of Macquarie University. With the visit of Dr Max Keniry, from the University of Illinois, in 1983-84 and Professor Philip Westerman, from Kent State University, in 1984-85 we now have available a number of specifically labelled ubiquinone and ibiquinone analogue molecules which permit the use of solid state nuclear magnetic resonance techniques to study in detail the location and dynamics of ubiquinone in mitochondrial extracts and ultimately intact mitochondria. The involvement of Professor Robertson provides a most timely complement to our approach and will allow us to immediately extend our work into biologically active systems."

Awards

Mr H. H. N. Panhuber (Chemical Bases for Food Acceptance Group, FRL) was awarded the M.Sc. degree by Macquarie University.

Mr G. R. Chaplin (Plant Physiology Group, FRL) was admitted to the Ph. D. degree by the University of New South Wales for the thesis 'Storage behaviour of avocados at low temperatures'.

A Post-graduate Studentship that is funded by the Division was awarded to Ms Vijoleta Braach-Maksvytis. Ms Braach-Maksvytis, who graduated with First Class Honours in Biochemistry from the University of New South Wales in 1984, is working with Dr Bruce Cornell in FRL's Food Structure Group. Her research is into receptor mechanisms, with the possible investigation of receptor mechanisms in olfaction, in collaboration with Drs David Laing and Graham Bell.

Mr Ian Eustace from MRL's Microbiology Group has been granted an Australian Meat Research Committee Overseas Study Award, which will enable him to spend 12 months in Britain and the USA.

He will work for six months with Dr Paul Gibbs' Applied Microbiology Section at the Leatherhead R.A. Laboratory in Surrey, then a further six months with Dr Tony Kotula of the Meat Science Research Laboratory of the US Department of Agriculture at its Animal Science Institute, Beltsville, Maryland. He will study new and rapid methods for estimating and identifying microbial contamination of meat and meat products.

Visits to FRL

Twenty-three members of FICA (Food Industry Council of Australia) held a meeting at FRL on 26 February.

In March FRL was host to: the Minister of Agriculture, Animal Husbandry and Sciences of the People's Republic of China, His Excellency Mr He Kang and party; to a delegation from the Chinese Academy of Agricultural Sciences (led by Professor Lu Liang Shu) and to two specialists from the Bile Fermentation Research Institute, Guandong.

Also in March, 10 Regional Directors from Thailand visited FRL as part of a World Bank National Agricultural Research Project whose ultimate aim is the organization of the Department of Agriculture in Thailand.

Visiting worker

Dr Setsuo Koike left FRL in February 1985 after working for one year with the Plant Physiology Group on the involvement of glutathione in chilling injury in plants. He has returned to the Hokkaido National Agricultural Experiment Station, Japan, where he works on chilling injury in rice.

Overseas visitors for the ACIAR projects

Dr Helen Nair, who took her Ph.D. with Dr Brady when the Plant Physiology Group was located at Sydney University, has returned to the PPG as a guest worker for 9 months (from November 1984 to August 1985) with Dr N. Wade on the ACIAR Banana Project. She comes from the Botany Department, University of Malaya, Kuala Lumpur, Malaysia where she is an Associate Professor. Her work in Sydney is concentrating on the regulation of the softening of banana fruit which is a serious problem in Malaysian varieties of banana.

Dr Tan Soon Chye, an Associate Professor in the Department of Biochemistry, National University of Malaysia, is also working with Dr N. Wade for 10 months (January to October 1985) as a guest worker on the ACIAR Banana Project. He is concentrating on storage disorders of the fruit.

Ms C. Warisaiho, a Scientific Officer with the Department of Primary Industry for Papua New Guinea has recently arrived at FRL to work for 3 months with Mr K. J. Scott on the ACIAR-PNG Project. She will be collaborating in several aspects of the project to determine the handling characteristics of mixed loads of fruits and vegetables and the storage and transport of dessert and cooking bananas.

Retirement – W. G. Murrell

Dr W. G. Murrell has retired from CSIRO as a Chief Research Scientist after nearly 37 years in the Division. Following wartime service in the RAAF as a pilot, Bill Murrell resumed his interrupted studies at the University of Sydney and graduated Bachelor of Science in Agriculture with First Class Honours in Microbiology. He was recruited to the Bacteriology section of the Division of Food Preservation as a Research Officer in 1948, to begin a long career devoted to research on bacterial spores and their relevance to food processing and food safety.

In the course of his research, Dr Murrell investigated aspects of the composition, physiology, biochemistry and physical chemistry of bacterial spores, seeking throughout to establish the basis of the remarkable heat resistance of the spore and hence to enhance the stability and safety of heat processed foods. He became a leading authority in the field, in strong demand as a contributor to meetings and as an author of critical reviews. He founded and produced a Spore Newsletter which has been for many years a means of rapid communication for spore researchers throughout the world. As a research manager he was a most effective group leader, with a

critical mind and a never-ending flow of ideas.

In recent years Dr Murrell added to his spore studies and his untiring efforts to help the food industry solve its bacteriological problems an interest in infant botulism and in the ecology of the causal organism.

Dr Murrell's research was recognized academically by awards of the degrees Doctor of Philosophy from Oxford University and Doctor of Science in Agriculture from the University of Sydney. In retirement he has returned to the latter campus to continue his research on botulism. The Division acknowledges with gratitude his very many major contributions to science and to CSIRO.

J.H.B.C.

Retirement – B. J. Stewart

Ms Betty Stewart is another very experienced microbiologist to leave the Division recently. She joined CSIRO as a Junior Assistant and stayed with us for almost 41 years, retiring as a Senior Experimental Scientist.

Over the years, Betty Stewart participated in virtually every major bacteriological research program of the Food Research Laboratory. She played major roles, with Dr W. J. Scott, in the pioneering studies on microbial water relations, involving research on both growth and survival, and in an extensive program on the preservation of bacteria in the dry state. For much of her career, Ms Stewart was deeply involved in research on bacterial spores, predominantly with Dr W. G. Murrell and particularly in respect of biophysical analysis. Most recently she has carried a very heavy load of microbiology and of toxin testing in the investigation of cases of suspected infant botulism.

However, in spite of all these activities, it can be argued that Ms Stewart's greatest contribution to the Division and to the Australian food industry was in the microbiological examination of foods. For very many years she carried the main responsibility for the investigation of a wide range of foods which were, for some reason or another, microbiologically suspect. This demanding activity, of the greatest importance to the consumer and to the food industry, was carried out with infallible care and precision, and will be difficult to maintain in her absence. She leaves with her colleagues' deep appreciation and best wishes.

J.H.B.C.

Retirement – D. L. Ingles

Dr David Ingles joined the Division of Food Research in 1955 as a member of the Organic Chemistry Section which then comprised Dr T. Reynolds and Dr E. Anet. The work of this group was concerned with non-enzymic browning and its inhibition by sulphur dioxide. It became known as 'the Australian group' as workers in laboratories throughout the world were attracted to this interesting problem. The sulphite aspects of the work were primarily developed by Ingles.

In 1961-2 Dr Ingles spent a year working as a Senior Fulbright Fellow with Professor R. L. Whistler's group at Purdue University, Lafayette, U.S.A. The first syntheses of sugars with sulphur as a ring hetero atom were devised during this period. Subsequently, in Australia, Dr Ingles developed the first syntheses of sugars containing basic nitrogen as the ring hetero atom.

Bisulphite adducts of carbonyls were also extensively studied and several new classes were defined. The acyclic nature of the sugar-sulphite adducts was proved chemically by Ingles by the preparation of acetate and acetonyl derivatives. This he considered to be his best work.

Work on browning reactions within the Division was terminated in 1967 and Dr Ingles turned to a study of oxidation by free radicals and reactions of Fenton's Reagent (ferrous ion and hydrogen peroxide).

In 1976 Dr Ingles joined forces again with Dr Anet in a study of amines in food and their relation to migraine. This association, unlike that in the Organic Chemistry Section which had continued for almost 15 years, was terminated by the sudden death of Dr Anet in December of that year. This work, published during the period 1976-85, appears likely to provide a new perspective of the problems of estimation of trace amounts of amine in foods and their physiological significance.

In addition to his research activities, Dr Ingles acted as Divisional Editor for some 3 years and was a member of the editorial committee of this journal for several years. He maintained an active interest in the affairs of the CSIRO Officers Association and acted as an advocate for research scientists in CSIRO in a major salary case before the Commonwealth Arbitration Commission in 1969.

Dr Ingles' retirement at age 60 in March 1985 was occasioned partly by a change in the climate of CSIRO which he likened to "the second ice age" and partly by "having worked at the coal-face long enough". Finally, after spending so long pursuing the growth of crystals from complex mixtures, the prospect of continuing in this field, unfettered, in a contiguous cottage industry concerned with crystalline pottery glazes had some attraction to him.

K.C.R.