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The two-factor triangle test

J. M. HARRIES* AND G. L. SMITH†§

Summary

The triangular test, commonly used in sensory tests of food quality, is generally interpreted on the assumption that the probability of a correct selection is the same for all assessors, and independent for separate trials leading to the use of the binomial distribution in setting up tests of significance. Only when there is no real discrimination is it true that using one taster *n* times is the same as using *n* tasters once, the basis for the column heading 'number of tasters or tastings' in published tables of significance. This paper attempts to remove this assumption by the use of the beta-binomial distribution, and suggests an interpretation in which the chance of success is different between and within assessors so that a two-factor analysis becomes possible. A set of data relating to the flavour of fish has been reinterpreted in these terms.

Introduction

The triangular test has come to be regarded as the archetype of the direct difference test in the sensory assessment of food quality. In a survey of the methods used by food companies in the U.S.A. Brandt and Arnold (1977) found that the triangle test was the most often used method and there is every reason to suppose that the situation is much the same in the U.K. (Muller, 1977). Yet, to judge by published results, some difficulty is often experienced in the interpretation of the findings. The method was extensively analysed by Frijters (1980) who made no reference to the problem discussed in this paper.

The same assumptions have been made in all interpretations of triangle test results from Helm and Trolle (1946) to Woodward and Schucany (1977) who state that they use the same model as Hopkins and Gridgeman (1955), i.e. that

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the probability of a correct answer is the same for all subjects and is independent from trial to trial (the binomial distribution). Under the null hypothesis, this probability is one-third, and the assumption is the basis for the now familiar column heading in tables of significance, 'number of tasters or tastings' (e.g. B.S.I. 1980). Only when there is no discrimination is it true that using one taster n times is the same as using n tasters once. As soon as we approach the 'threshold' however (and the definition of the threshold may vary with circumstance) difficulties arise. Inevitably, as soon as the limits of discrimination are reached the assumption of equal and independent probabilities becomes untenable. The panel will consist of a mixture of discriminators and non-discriminators, which will change as the magnitude of the difference between the stimuli increases, until presumably all members, eventually, will be discriminators.

This paper considers the situation in which the probability of success in a triangle test is not equal for all assessors. The theory developed is applicable to all direct difference tests, but attention is concentrated on the triangle test because of its practical importance.

Alternative models

There has been some discussion in the statistical literature of a deterministic approach to paired comparison tests, when used to study consumer preferences. Horsnell (1969) argues that results can be examined adequately with a model in which some responses are deterministic (i.e., probabilities of 0 and 1 alone are possible) and some are random with a common probability of choice. He considers that a stochastic distribution 'is an unnecessary assumption in the conditions of product testing' and is not such a good basis for inference as the composite one. Wierenga (1974) uses the stochastic model, that is, the probability that an individual in a paired comparison test will prefer a particular one of the alternatives is not necessarily 0 or 1, but may also lie somewhere between these extremes. We agree with Hutchinson (1979) who considers that Horsnell's model is in conflict with psychological ideas about the processes of perception and decision. Indeed, it is not practicable to think of such direct difference tests as the triangle test in other than stochastic terms.

The idea of a stochastic model for preference has been examined by Wierenga (1974) using the beta-binomial model (Skellam, 1948) in which the parameter p is not constant but has the beta distribution. Chatfield and Goodhardt (1970) also used it as a model for consumer purchasing. The beta-binomial model has not, however, been applied to direct difference tests, where it has the clear advantage over the binomial of permitting a relaxation of the constraint that the probability of a correct answer should be invariant.

This alternative model supposes that p, the probability of correct selection,

has the beta distribution

$$b(p) = \frac{p^{\alpha-1}(1-p)^{\beta-1}}{\mathrm{B}(\alpha, \beta)} \qquad (0 \leq p \leq 1)$$

where $\mathrm{B}(\alpha, \beta)$ is the beta function with parameters α and β,

$$\text{i.e., } \mathrm{B}(\alpha, \beta) = \int_0^1 u^{\alpha-1}(1-u)^{\beta-1}\, du$$

The distribution of r, the number of correct selections in a panel of n, is then given by

$$P(r|n, \alpha, \beta) = \int_0^1 \binom{n}{r} p^r(1-p)^{n-r} \frac{p^{\alpha-1}(1-p)^{\beta-1}}{\mathrm{B}(\alpha, \beta)}\, dp$$

$$= \binom{n}{r} \frac{\mathrm{B}(\alpha+r, \beta+n-r)}{\mathrm{B}(\alpha, \beta)} \qquad (0 \leq r \leq n)$$

and it follows that the mean and variance of r are respectively

$$\mathrm{E}(r) = \frac{n\alpha}{\alpha+\beta} \text{ and } \mathrm{Var}(r) = \frac{n\alpha\beta\,(n+\alpha+\beta)}{(\alpha+\beta)^2(\alpha+\beta+1)}$$

For the binomial model, for which p is fixed,

$\mathrm{E}(r) = np$ and $\mathrm{Var}(r) = np(1-p)$.

With suitable choice of α and β almost any distribution of a variate bounded by 0 and 1 can be satisfactorily approximated by a beta distribution. The effect of any variation at all in p will be to increase the variance of the distribution of r from that of the binomial distribution having the same mean, but just how dramatic this can be is shown (Fig. 1) by three examples of the beta distribution constructed in a similar manner to those in Wierenga (1974), but with α and β chosen to give a mean of ⅓ instead of 0.6. Each distribution has the same value of $\alpha + \beta$ as the corresponding one given by Wierenga, and Table 1 shows values of the mean and variance of the beta-binomial distribution and the binomial distribution with $p = 1/3$, and $n = 20$.

For distribution I, where p is fairly compactly clustered around ⅓, the variance of 6.2 is not much greater than the figure of 4.4 for the binomial, but for the more widely scattered II and particularly for distribution III (which in the case of preference testing approximates to the extreme but quite feasible situation of some subjects preferring one product with probability 1.0 and the others choosing the other with certainty) the variance is considerably increased.

Practical implications

There is no indication from the published literature of the order of magnitude of the parameters α and β. They may vary enormously, depending upon the sense

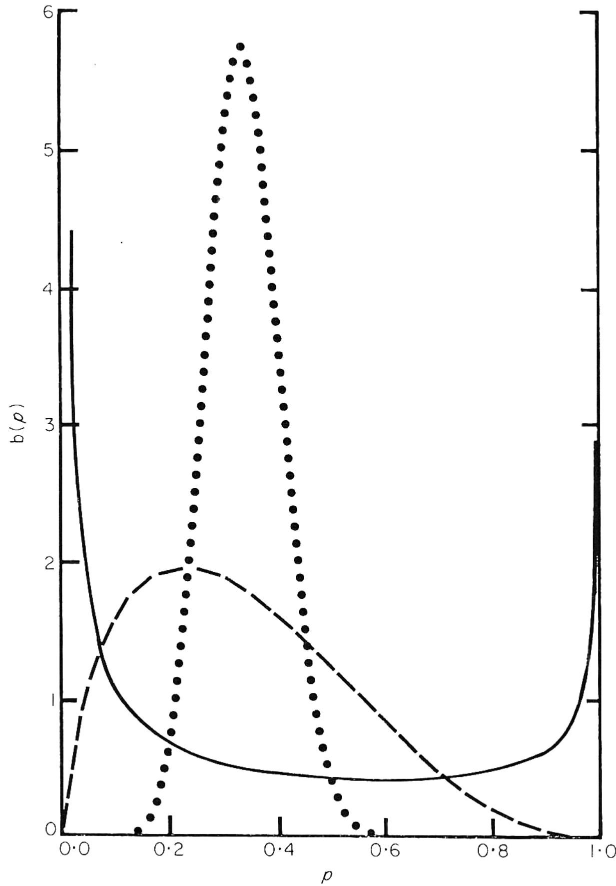

Figure 1. Some examples of the beta distribution with mean = ⅓; . . . ., case I, $\alpha = 15.67$, $\beta = 31.33$; — — —, case II, $\alpha = 1.67$, $\beta = 3.33$; ———, case III, $\alpha = 0.2381$, $\beta = 0.4762$.

Table 1. Means and variances of some examples of the beta-binomial distribution

Distribution	n	α	β	E(r)	V(r)
I	20	15.67	31.33	6.7	6.2
II	20	1.67	3.33	6.7	18.5
III	20	0.2381	0.4762	6.7	53.7
Binomial, $p = ⅓$	—	—	—	6.7	4.4

modality and the magnitude of the sensory difference. However, a sensory difference situation in which the conditions represented by type III above would obtain, i.e. some subjects always correct, the others always wrong, is most unlikely in practice. It has been well established that people vary in their

sensitivity to most stimuli, and in some contexts that there is a greater variation between subjects than within (i.e. between different occasions for the same subject) e.g. Amerine, Pangborn and Roessler (1965). Provided the sensory difference is great enough, the value of p will be unity, and the assumption made in published tables of the triangle test – that number of tastings and number of tasters can be interchanged – will again hold good (as it does when there is no discrimination). The variance will now be 0, but this is a situation of no interest to the practical investigator, and may not even exist in practice.

Reports of investigations in which a sensory difference is gradually increased until all the assessors are able to identify it are rare. An interesting example, however, is that of O'Connor, Stevenson and Holmes (1974), who used triangular tests to study thermal perception in the mouth. Ten subjects twice compared water differing by 5°C at each of fourteen temperature steps. They used 100 people altogether with groups 'selected informally'. A histogram of the results shows that at two temperature steps (10°C–15°C and 30°C–35°C) they achieved 100% correct responses.

The values of α and β are likely to vary with the sensory context. Values can be calculated for any set of data, but published sets which give separate frequencies for individual assessors are rare. It is of course possible to apply the method to sets of data in which the second factor is something other than assessors (Harris, Strong & Sunde, 1968) but the main interest must lie in comparing the variation within and between assessors. Because the triangle test has mainly been used in small-scale investigations most easily calculable coefficients will be of no more than local interest. Occasionally, however, the method has been used on a larger scale, though such experiments are often complicated either by a multiplicity of sensory differences ('treatments', in the terminology of experimental design) or irregular attendance of the assessors. One such data set has been examined in detail, concerning the comparison of feral and farmed plaice (Hume, Farmer & Burt, 1972).

A typical data set

Twenty-eight tasting sessions were carried out over a period of 16 months to see whether plaice from a fish farm could be distinguished from plaice which has been trawled at sea. The series of tests reflected seasonal effects, differences in condition of the fish (exercised or rested) and method of handling (gutted or ungutted between catching and storage).

There were no large differences between the proportion of correct responses for each treatment, but there were significant differences between those for each season. Using the binomial assumption and testing for the difference between the proportion correct for spring ($p = 0.59$) and summer ($p = 0.42$) the difference was significant at the 0.001 level (see Smith, 1981). The variation both in panel attendance, and in the proportion of correct results achieved by the individual assessors, can be seen in Table 2. There were actually 280 assessments

Table 2. Individual performances

Assessor No.	Total			March–May			June–July			October		
	Trials	Correct	p	Trials	Correct	p	Trials	Correct	p	Trials	Correct	p
1	22	10	0.45	8	5	0.62	12	5	0.42	2	0	0.00
2	21	10	0.48	10	4	0.40	6	5	0.83	5	1	0.20
3	20	8	0.40	9	6	0.67	6	1	0.17	5	1	0.20
4	17	7	0.41	6	3	0.50	6	3	0.50	5	1	0.20
5	17	5	0.29	9	4	0.44	3	1	0.33	5	0	0.00
6	16	11	0.69	5	2	0.40	6	4	0.67	5	5	1.00
7	14	9	0.64	9	6	0.67	3	2	0.67	2	1	0.50
8	12	9	0.75	9	6	0.67	—	—	—	3	3	1.00
9	11	8	0.73	—	—	—	6	4	0.67	5	4	0.80
10	11	7	0.64	9	6	0.67	—	—	—	2	1	0.50
11	11	6	0.55	8	6	0.75	3	0	0.00	—	—	—
12	8	6	0.75	6	4	0.67	—	—	—	2	2	1.00
13	8	1	0.12	5	1	0.20	3	0	0.00	—	—	—
14	7	4	0.57	1	1	1.00	6	3	0.50	—	—	—
15	6	5	0.83	—	—	—	6	5	0.83	—	—	—
16	6	2	0.33	—	—	—	6	2	0.33	—	—	—
17	6	2	0.33	—	—	—	6	2	0.33	—	—	—
18	5	5	1.00	—	—	—	—	—	—	5	5	1.00
19	5	4	0.80	2	2	1.00	—	—	—	3	2	0.67
20	5	3	0.60	2	1	0.50	—	—	—	3	2	0.67
21	3	2	0.67	—	—	—	3	2	0.67	—	—	—
22	3	2	0.67	—	—	—	3	2	0.67	—	—	—
23	3	2	0.67	—	—	—	3	2	0.67	—	—	—
24	3	1	0.33	—	—	—	3	1	0.33	—	—	—
25	3	0	0.00	—	—	—	3	0	0.00	—	—	—
26	3	0	0.00	—	—	—	3	0	0.00	—	—	—
27	3	0	0.00	—	—	—	3	0	0.00	—	—	—
28	1	1	1.00	1	1	1.00	—	—	—	—	—	—
29	1	1	1.00	1	1	1.00	—	—	—	—	—	—
30	1	0	0.00	1	0	0.00	—	—	—	—	—	—
Total	252	131	0.52	101	59	0.58	99	44	0.44	52	28	0.54

Table 3. Results from six sets of three trials

Date	Length of storage time (days)	Panel size	Number of assessors with each number of correct responses				p	Variance		Estimated values	
			0	1	2	3		Actual	Expected*	α	β
Mar '70	1	11	1	4	4	2	0.55	0.095	0.083	22.0	18.4
June '70	1	8	1	3	4	0	0.46	0.062	0.083	—	—
June '70	5	11	2	3	4	2	0.52	0.119	0.083	2.9	2.8
July '70	1	11	5	4	2	0	0.24	0.069	0.061	19.9	62.3
July '70	3	10	2	2	5	1	0.50	0.105	0.083	7.0	7.0
Oct '69	3	11	2	2	4	3	0.58	0.135	0.081	1.7	1.3

*Variance of an estimate of probability p based on $n(=3)$ trials. The expected variance (according to the binomial model) is $p(1-p)/n$, while the actual variance is the variance between the p's obtained from the individual judges. The expected variance for each set of trials has to be estimated using p, the proportion of correct responses from all the judges in that set, and based on the binomial assumption of equal and independent chance of success.

made (103 in March–May, 120 in June–July and 57 in October) but for only 252 was it possible for us to identify the individual assessors. There is therefore a small discrepancy between the proportion given above and those at the foot of Table 2. Thus although Hume *et al.* (1972) contains a large amount of data, the constantly changing panel makes it difficult to make properly valid tests of the variances. It is possible to extract six sets each of three trials carried out at the same time with the same panel, and the results are shown in Table 3. For all but one of the sets, the actual variance is slightly greater than would be expected under the binomial assumption.

When the judges have not all taken part in the same number of tests, the expected variances for each judge will depend on his number of tests. It may be reasonable to consider the results for a group of judges with different n to be from a mixture of populations, so that the expected variance is the mean of the individual variances. As an estimate of the combined probability, the weighted mean of the individual judge's probabilities has been used. This idea may be applied firstly to all the combined results, ignoring those where the judge could not be identified, and then, since the season appears to have made a difference to discrimination, spring, summer and autumn results are treated separately (Table 4). For the pooled data, and for the summer and autumn data, the actual variance is greater than the theoretical variance, but for the March–May data they are reversed, though not very different. It may perhaps be that as discrimination was easier with spring-caught fish (Hume *et al.*, 1972, p. 31, say that the flavours of wild stocks are known to vary seasonally; perhaps this does not apply to farmed fish) there is less of a random element in selection of the odd sample.

For those sets in Tables 3 and 4 for which the actual variance was greater than

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Table 4. Actual and expected variances obtained for data at different seasons

Set	No. of assessors (m)	No. of 'tastings'	p	Actual variance	Expected variance	Estimated values α	β
All data	30	252	0.52	0.089	0.063	9.3	8.4
March–May	18	101	0.58	0.078	0.090	—	—
June–July	21	99	0.44	0.085	0.062	6.1	7.8
October	14	52	0.54	0.144	0.078	0.9	0.8

the expected, estimates were obtained of α and β by maximizing the log-likelihood function using the method of simplex optimization (Nelder & Mead, 1965). This method was considered more appropriate to use than the ones in Skellam (1948), Chatfield and Goodhardt (1970) or Wierenga (1974) because of the differing numbers of trials for each assessor and the smaller number of assessors. The presence of a dash instead of a value indicates a set for which the actual variance was not greater than expected. The relation between the values of α and β depends only on the estimated value of p, as the mean of the beta distribution with parameters α and β is $\alpha/(\alpha+\beta)$. Apart from that consideration, low values of α and β correspond to high variances, as can be seen by considering Tables 3 and 4, while high values of α and β indicate sets whose variances are quite close to those which would be expected under the binomial model.

Thus judges probably differ in their ability to distinguish farmed plaice from feral plaice, but it is difficult to interpret this in terms of a higher than expected variance, possibly because of irregular attendance at tasting sessions. The overall result, obtained by dividing the total number of successes by the number of trials, is a biased estimate in the sense that it is more influenced by frequent attenders, who may or may not be the most discriminating. The expected variance is also clearly similarly affected.

It is standard practice (Amerine, Pangborn & Roessler, 1965) in the sensory assessment of food quality, to choose the most consistently discriminating subjects as members of taste panels as far as practically available. The problem is that, in such an extensive investigation as that quoted here, the identity of the most discriminating members is not known until after the event. Different conclusions could well have been drawn from panels composed of different subjects and it is clear that much more remains to be discovered about differing sensory sensitivity.

Possible interactions

This two-factor extension of the triangle test permits a new interpretation of one of the difficulties that workers have experienced in its use – its propensity to

influence by psychological bias. Early in its history, there were reports of results being influenced by the coding of the odd sample, and it is now generally accepted (B.S.I., 1980) that care must be taken to include all possible combinations of codings in an experiment, though this is sometimes inconvenient. The existence of a bias due to other factors, such as whether the 'stronger' of two stimuli to be compared is made the odd sample or the pair, has been reported (Wasserman & Talley, 1969; Griffiths, 1979). Whatever the origin or nature of such biases, they can be interpreted in the present context as interactions in the statistical sense. If we consider the results of a number of triangle tests done with many assessors all using the same stimuli as being analysable by a two-factor analysis of variance (analagous to that of Crowder, 1978) we should have a variance between assessors, and one within. Ideally the variance within assessors would be comparatively constant and the intention of the preceding section is basically to see whether the variance between assessors is greater. The purpose of this paragraph is simply to point out that in the present interpretation the reported biases constitute an interaction term, in that they render the conditions of the experiment inconstant from trial to trial (Altham, 1978). Another type of bias, that does not fall into this category, may occur when a preference test is conducted concurrently with the triangular test. Peryam (1958) reports that responses tend to favour whichever of the two stimuli constituted the pair, but the existence of the phenomenon has been questioned by Basker (1977).

It is however clear that a sufficiently large and homogeneous body of data, on which to test these ideas empirically, is not available. This may be because the triangle test is not particularly appropriate for such an extended investigation. In practice, the method is often used with one assessor n times to study his own performance perhaps as part of a selection procedure, and with n assessors once to study product differences. However, various combinations of n tasters m times, with m varying between assessors, have been reported in the food science literature, but always on the assumption of a binomial model. But it may also be that we have not found a suitable set of data because its existence has not been publicized. However the evidence from the one large set of data that we have examined points to a higher variance between assessors under certain conditions than would be expected if the binomial distribution held.

It should perhaps be emphasized that the two-factor model discussed in this paper is not intended to replace the accepted binomial one, but to include it in a wider application of more general interest. The investigator who has used the triangular test would still be well-advised initially to test the null hypothesis of no discrimination using published binomial tables. If the null hypothesis has to be accepted, that is an end of the matter. (An example is that of Cunningham & Lillich, 1975, where, after correction of the misprint, no assessor gives a significant result.) When the null hypothesis is rejected, however, either for some or all assessors – a situation that frequently arises to judge by the literature – the two-factor method might be expected to help further investigation. It will clearly be some time before we can expect values of α and β that have any parametric significance.

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Scoring methods used in the sensory analysis of foods and beverages at Long Ashton Research Station

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Summary

The development of a scientific programme of research into sensory quality of fruits and beverages at Long Ashton Research Station has necessitated improvements in the evaluation methods used. It has not only meant the development of precisely defined vocabularies for describing products and methods for obtaining data from consumers, but also means of scoring the intensity of attributes. This paper describes the various scoring methods used at Long Ashton, ranging from nominal to interval and ratio, and their applicability to particular problems.

Introduction

Since the foundation of Long Ashton Research Station in 1903, the Food and Beverages Division (until recently known as the Cider and Fruit Juices Section and Home Food Preservation Section) has been closely concerned with the production, utilization and analysis of fruits, fruit juice products and their fermented beverages. Over recent years, as the change in name implies, the products examined have increased to cover a much wider spectrum of foods. With all products destined for consumption, the only way to judge their success or failure is to have them examined by human assessors. As a consequence, sensory assessment has always played an important part in the Division's research programmes. Various approaches have been used over the past 20 years, that chosen for a particular exercise depending on the state of knowledge at the time, the type of information required and the degree of sophistication given in training panelists.

Prior to 1970, sensory assessment of a product at Long Ashton Research Station was performed largely by discussion between six to ten experts who recorded their joint opinion. A typical assessment of a cider is shown in Fig. 1.

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Code No.	Bottle No.	Identity	Aroma	Flavour
XII/4A/66	43	Stenbridge Jersey. Normal	Golden, pleasant. Generally mild bittersweet	Fruity, pleasant, appley. Reasonable bitter aftertaste

Figure 1. Assessment of ciders at Long Ashton Research Station, before 1970.

Although all people present had a chance to voice their opinion, the record generally reflected the opinion of the most dominant members of the panel. Scoring and the use of scales formed a minimal part of the procedures.

Such approaches were incompatible with expanding interest in defining the meaning of sensory quality in products and with attempts to understand the vast amount of analytical data which was becoming available through the use of such techniques as gas chromatography, gas chromatography-mass spectrometry and, more recently, high performance liquid chromatography (Williams, 1974). A more precise definition of the attributes present in a product was needed, together with some means of measuring their magnitude and acceptability. The first requirement led to the compilation of precisely defined vocabularies for particular products (Williams, 1975; Williams & Carter, 1977), and the third to involvement in measuring consumer acceptance. This paper deals with the development of the accompanying scoring aspects.

Scoring methods

Stevens (1960) distinguished four basic types of scoring systems – nominal, ordinal, interval and ratio; virtually all types have been used at Long Ashton over the past 10 years.

Nominal and ordinal scales

Once a vocabulary had been developed, the discussion system was improved by asking people merely to score presence or absence of the attribute, using a questionnaire of the format shown in Fig. 2.

The order of the attributes is of no relevance from the point of view of scoring and, although no actual numbers are used, it is analogous to the nominal scale of Stevens. This procedure proved very useful in training and for extracting descriptive comments from large untrained consumer populations.

Scoring systems of this nature have also been used with trained panelists. By having several attributes which possess an underlying continuum but are impossible to define more precisely, they approach the ordinal-type scale. An

Please indicate which of the following adjectives are appropriate to describe the following samples:

	Sample No.			
Fruity				
Fuselly				
Phenolic				
Sulphurous				

Figure 2. Score sheet for assessing presence or absence of attribute in cider.

example is that used for describing colour in ciders (Fig. 3). Ranking a series of products with respect to the amount of a particular attribute also gives more information than simply scoring the presence or absence of an attribute.

Please tick the appropriate box

green

straw

yellow gold amber brown

pink

Figure 3. Cider colour assessment.

Interval scales

Ranking of samples requires all samples, on which one wants information, to be present at the same time and, of course, gives little indication of the magnitude of the difference between samples. To get estimates of relative amounts of attributes in each product, it is essential to resort to magnitude or interval scaling, either using a series of numbers to indicate magnitude or a line against which to measure an attribute's intensity. In such cases it is usual to fix the ends of the scale with appropriate descriptions and instruct panelists that equal intervals on the scale represent equal increments of intensity of the attribute. Examples of such scales used at Long Ashton are illustrated in Figs 4 and 5. In these, the ends of the scales are fixed by a complete absence of the

Please taste jams in order shown. Assess each one for 'fruitness' by taste only, marking how far along the line you rate it

Jam No. [ ] Not fruity ———————— Very fruity

Jam No. [ ] Not fruity ———————— Very fruity

Jam No. [ ] Not fruity ———————— Very fruity

Figure 4. Line scale for assessing intensity of fruitiness in jam.

0 = No crispness
1
2
3
4
5 = Very crisp

Figure 5. Early scale for assessing crispness in apples.

attribute and 'very fruity' or 'very crisp'. An alternative phrase for setting the upper end of the scale is the maximum amount of the attribute the panelist has ever experienced.

Ratio scales

In another variation, ratio scaling, the numbers used or length of a scale can be left to the individual. Panelists choose an arbitrary point on a mental scale and give it a number for the first sample assessed. Remaining samples are then assessed with respect to this sample. For example, if one is scoring 'appleyness' in cider and gives this attribute a score of 50 in the first cider, the second cider will receive a score of 100 if it is found to be twice as 'appley'. With experienced panelists, because they are aware of the range of intensity an attribute can possess, ratio scaling has not shown any advantage over simple interval scales. However, this is not so in dealing with the consumer who has no such preconceived ideas before starting an assessment (see p. 172).

Assessing against a standard

Measurement of difference or similarities, either between two samples or between the sample and standard, either mental, defined verbally or by chemical or physical means, using interval scales similar to those discussed above, have also been used (Williams, Carter & Langron, 1979). Examples are given in Figs 6 and 7.

0 = No similarity
1
2
3
4
5 = Identical

Figure 6. Scale used for assessing similarity of wine descriptions.

NAME .... DATE ....

SESSION NO. .... GROUP ....

Please taste sample ... first

	Bitterness		Astringency	
	☐	Much more	☐	
	☐	Slightly more	☐	
Sample ... has	☐	The same	☐	than sample ...
	☐	Slightly less	☐	
	☐	Much less	☐	

Please state if you find any other major taste difference:

Figure 7. Assessment sheet for evaluating bitterness and astringency in ciders.

The use of any scale, however, depends very much on the meaning attached to the ends and, of course, sensitivity to a particular attribute. This obviously can produce considerable variation between tasters. Experience at Long Ashton has shown that greater consistency is obtained when measuring the difference between scores given to two sets of samples than in that given to one sample (Williams, 1978). In sensory profiling of cider a control is introduced, which is assessed along with the sample. The experimental cider can then be evaluated as a difference from the control or as an absolute score by adding this difference to a predetermined score for the control.

Assessment with a standard which can be given a pre-established score is preferred. This is possible in the case of some beverages, but with other food products, e.g. apples, it is less easy. The use of standards different from the product being assessed, such as n-butanol as an odour intensity standard as advocated by Dravnieks (1977) may well prove of use in this context but has not been investigated.

Evaluations of cider have been conducted in which the panelists were given scores for a control before they commenced assessment of the unknown. Difficulties arose, however, as people do not have the same sensitivities to different attributes. The mean score for a particular attribute in the control may still be below a given panelist's threshold. If he or she cannot detect this attribute in the unknown, there is a quandary as to how it should be scored. Should the attribute in the unknown be given a score equal to or lower than the control in which it also could not be detected? In consequence, the choice of scores given to the control is now left to the decision of the individual, but in the statistical analysis attention is only paid to the difference.

Even when having two samples, and particularly when assessing one sample in isolation, panelists in general are not happy in using scales merely with defined ends; they prefer to have verbal cues as well, to assist in the interpretation of intermediate points. We have reverted to a cross between a category scale and pure magnitude estimation. The scale can be viewed either as an ordinal-category scale with the categories chosen to be equal intervals of magnitude apart or, alternatively, as an interval scale with guidelines as to the interpretation of points. Examples for cider and apples are given in Figs 8 and 9 where a six-point scale has proved adequate for most objective assessments. After long experience panelists become more discriminating, and half points have been introduced extending scales to eleven points.

0 = No odour
1 = Very weak odour
2 = Weak odour
3 = Moderate odour
4 = Strong odour
5 = Very stong odour

Figure 8. Scale for assessing intensity of odour characters in cider.

0 = Like ripe bananas/strawberries
1 = Like cooked carrots
2 = Like peeled cucumber
3 = Like unpeeled fresh cucumber
4 = Like raw green cabbage
5 = Like celery

Figure 9. Verbal cues for interpreting crispness scale when assessing apples.

Assessment of hedonic data

Assessment of hedonic information is less amenable to interval scoring than objective data. To say something is twice as pleasant as something else has little meaning. At Long Ashton, however, the same sort of scales have been used for hedonic assessments as for objective assessments, but inherently they must be more ordinal than interval in nature: Fig. 10 illustrates a typical ten-point hedonic scale. This scale differs both in length and constitution from the more conventional nine-point hedonic scale (Peryam, 1964), in order to increase discrimination over the acceptability point and positive aspects of quality (the quality range in which more of the products examined fall). Fewer subdivisions are considered necessary for negative quality, as people seem to be much less discriminating when judging unacceptable products.

1 = Very bad (most unacceptable)
2 = Bad
3 = Poor (unacceptable)
4 = Poor (just unacceptable)
5 = Indifferent (just acceptable)
6 = Fair
7 = Pleasant
8 = Good
9 = Very good
10 = Excellent

Figure 10. Ten-point hedonic scale.

Although people can be trained to assess objectively, they need not necessarily agree with respect to the acceptability of the products. Reliable hedonic information can only be obtained by examining statistically valid samples of the populations whose opinion is to be determined. This, in general, means eliciting responses from the public who are not trained in sensory assessment and who, unless one is prepared to spend a great deal of time, cannot assess a product objectively in the same way as a trained panel. In the first instance consumers have difficulty in describing many flavour sensations adequately so that the interviewer is uncertain what they are talking about, and even with attributes they can comprehend and about which they can communicate, their internal standard is basically what they like and it is against this that they assess. In a

monadic situation they can therefore only be asked to score relatively to this ideal, using a one-ended scale for general attributes such as overall acceptability or the acceptability of a particular general character such as appearance, flavour and texture (Fig. 11), or a two-ended scale when something more specific is being assessed (Fig. 12) and participants can be asked to relate the levels of an objective attribute to acceptance. Again, as can be seen from these examples, verbal cues to assist participants are preferred to scales with merely the ends fixed.

Is the flavour of this apple:

(1) Unacceptable to me	☐
(2) Indifferent to me	☐
(3) Fair to me	☐
(4) Desirable to me	☐
(5) Very desirable to me	☐

Figure 11. Example of one-ended scale for assessing general sensory characters.

Is the apple:

Much too sharp for me	☐
Somewhat too sharp for me	☐
About the right sharpness for me	☐
Not quite sharp enough for me	☐
Nothing like sharp enough for me	☐

Figure 12. Examples of two-ended scale for assessing specific sensory characters

With children, visual cues as opposed to verbal cues have obvious advantages (Fig. 13) provided they mark their reaction to the product rather than to the face they like.

Influence of scales used on results obtained

As well as people having different criteria for making judgements, these criteria can be influenced by the state of mind of the individual at the time of

1 =

2 =

3 =

4 =

5 =

Figure 13. Smiling face scale.

making the judgement. This in turn can be influenced by the adjectives and phrases used to determine the ends of the scales.

Three sets of scales for the opposing terms, 'roughness' and 'smoothness' are laid out in Fig. 14. Ignoring psychological problems associated with reversing a scale, one would expect, if people were interpreting the scales correctly, that the two scales on the top of the figure would add up to the bottom one. If a user is satisfied with putting one point to mark the position of a product on the lower 'smoothness/roughness' scale he or she should score positive on only one of the other scales. Inevitably products are scored as having both 'roughness' and 'smoothness'. What is happening is that criteria (if that is the right word) are changing. In case 1, the participant is directed to rough aspects in the product and hence his or her zero point is lowered with respect to the centre zero in the bottom scale. In case 2, emphasis is directed towards smooth aspects and the zero again lowered. Hence one product appears to have both rough and smooth characters simultaneously. Similar exercises can be performed with

No roughness — Extremely rough

No smoothness — Extremely smooth

Extremely smooth — Extremely rough

Figure 14. Unidirectional and bidirectional scales for roughness and smoothness.

'pleasantness' and 'unpleasantness', the product being both pleasant and unpleasant depending on the state of mind at the time of the assessment.

Obtaining objective data from the consumer

Objective scaled information can be obtained from the consumer, but it is essential to present them with a series of samples to compare, because such populations have limited knowledge of scaling procedures and vary in the range of samples they have experienced prior to assessing any given samples. Also, because of their limited experience, ratio scaling as opposed to interval scaling is preferred.

Experience with consumers using ratio scales has shown that they can understand the concept readily. When using this technique in surveys prospective participants were introduced to the concept of ratio scaling using a training exercise based on shapes. They were asked to give a number to the first of five shapes indicating its size. They then examined the second and gave this a number indicating its size in relation to the first, the exercise being repeated for all five shapes. Having completed this they were asked to give the number corresponding to the ideal size of some specified food, such as an apple, as well as the number corresponding to the largest example they had ever come across. They would then be asked to use the same approach to assess five or so samples of the product being tested, giving scores for attributes such as redness, intensity of colour, sweetness and acidity, as well as for the amounts of these attributes in their ideal product and the maximum amount of these attributes they had ever come across in any similar product.

Interpreting results from scored data

The data obtained from any scoring system must be subjected to statistical analysis. Examination of population distributions within each category and evaluation of the significance of any difference by comparing results with probability tables is the usual approach adopted for simple category data (Roessler *et al.*, 1978). The significance of rank data may be tested by comparing the rank sums for each sample with rank total tables or by comparing the distribution with Fisher Z distribution tables (Kendall, 1962; Kahan *et al.*, 1973).

With most scored data in which there is an underlying continuum, however, one may use an analysis of variance coupled with F tables to determine the significance of difference. Univariate techniques are used when dealing with one attribute, but multivariate approaches often provide a much clearer picture when handling several attributes. Techniques, such as principal component, principal co-ordinate and discriminant analysis (Clapperton & Piggott, 1979; Frijters, 1976; Pangborn, 1981; Williams, 1981; Williams *et al.*, (in press)) can also be applied to determine underlying dimensions, but for determination of

the significance of any difference it is necessary to resort to multivariate analysis of variance or canonical variate analysis (Vuataz, 1976/77). Multivariate techniques, in general, allow one to examine the spatial distribution of scores given to attributes, selecting orthogonal axes through this space which satisfy certain criteria, i.e. accounting for maximum variation in the case of principal component or co-ordinate analysis, or maximum discrimination in the case of discriminant or canonical variate analysis. The contribution of the attributes to the axes serves to define them. When dealing with situations when several not necessarily independent attributes are scored, such techniques enable one to discover underlying relationships which are often not readily apparent from examining several univariate analyses of the same data. The following example taken from a consumer survey on apples, illustrates the type of information which can be obtained by the use of multivariate techniques (Williams & Langron, unpublished data).

Six apples, four Cox's Orange Pippin and two Suntan, each given a different combination of controlled atmosphere (CA) (1% and 2% oxygen) and post-CA storage (11 and 17°C) were evaluated with respect to overall acceptability and the acceptability of their appearance, aroma, flavour and texture as well as for the more specific attributes, sharpness, sweetness, juiciness and crispness. Principal component analysis of the mean scores for each attribute over each set of apples followed by plotting the first three principal component scores (the sum of weighted scores for each attribute for each apple set) showed underlying continua which distinguished the apples. Figure 15, showing the plot of the first two principal component scores, typifies the information obtained. Visual

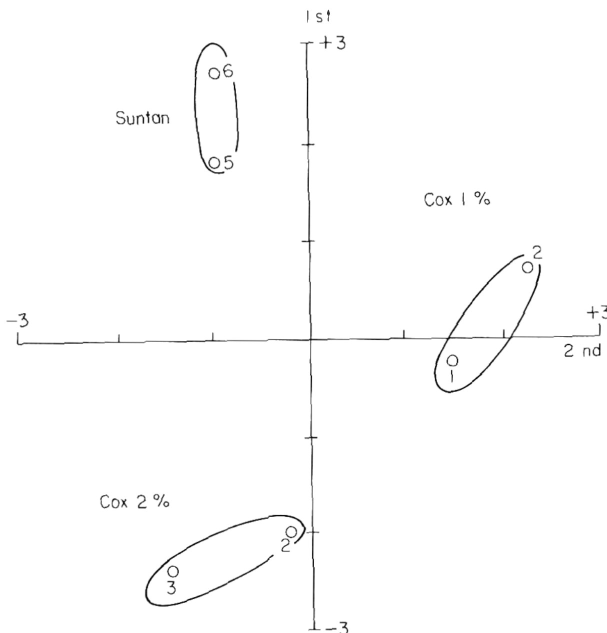

Figure 15. Plots of principal component scores for apples as assessed by the consumer.

inspection of the arrangement of the samples shows each axis to tie in with one of the three sets of treatments investigated, i.e. apple cultivar, CA storage conditions and post-CA storage conditions.

By comparing these treatment variables to the actual composition of the principal components (those attributes contributing to high and low scoring in particular) it is possible to determine the effect of the three treatments on the acceptability of the various sensory attributes evaluated. Hence it could be deduced that Suntan was considered crisper and sharper and to have a better appearance than Cox. One per cent oxygen CA produces a crisper, better textured apple, but one having a poorer flavour and aroma than fruit stored under 2% oxygen. Post-CA storage at 11°C produced a juicier, better flavoured and textured apple than at 17°C.

Conclusion

Descriptive sensory analysis provides information on a food or beverage which is much more comprehensive and useful than straight forward difference testing. By coupling this with a scoring system it provides the research worker with a powerful tool for understanding sensory quality, the results from which, unlike the early descriptive procedures, are amenable to statistical analysis.

Problems exist in the use of scales, as has been pointed out in this article. The perception of attributes themselves can also change during an assessment either because the product itself changes or because of adaption by the assessor. Apart from certain multidimensional scaling techniques, methods generally used for the analysis of scaled data rely on the information collected being metric and assume an underlying Gaussian distribution. In many cases sensory data are not metric and are skew in nature. Scoring systems do have problems but without them a great deal of useful information on the sensory properties of foods and beverages would be lost.

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Economic study of tomato paste production

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Summary

Statistics of tomato production and utilization have been reported to show the recent development of the tomato industry in the EEC countries. In particular, the profitability of tomato paste production has been evaluated and then analysed to assess the influence of the operating costs, EEC payments and market price of tomato paste. The economic balance for a plant capacity of 14 tons of raw tomatoes per hr and 2880 operational hr per year shows profitability of 22% in 1979 and 16% in 1980 owing to inflation and decrease in product demand. The annual cost of raw materials is not only more than 75% of production costs, but is also double the total capital invested. For this reason the tomato industry is compelled to resort to high-interest, short-term loans which further reduce net earnings. Mechanization of crop harvesting, automation of sorting, optimization of evaporation and packaging units and flexibility of plants are needed to maintain interest in tomato paste production in industrialized areas.

Introduction

The recent evolution of tomato paste production has seen the growth of large-scale and capital-intensive enterprises, even though a large number of production units are still of small or medium size.

More effective co-operation between the farmer and the processor and between the agricultural engineer and the food engineer has also exerted a deep influence on food industrial activities.

In this paper statistics of tomato production and utilization in Italy and in the EEC countries have been used to characterize the economics of the tomato

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paste industry by carrying out a profitability analysis. As production costs are affected by the size of the operation, our analysis has been referred to a plant of medium size (working capacity 14 tons of raw tomatoes per hr).

Production and utilization of tomatoes

The tomato is a plant species of the genus *Lycopersicon* belonging to the plant family *Solanaceae.* Its fruit consists of an outer peel; an intermediate part containing pale straw-coloured juice, insoluble pigments (lycopene, carotene, xanthophyll and chlorophyll), pectic substances and pectic enzymes; and a central placenta containing many small, oval seeds. The composition of tomatoes and tomato products is affected by such factors as growing, seasonal and climatic conditions, maturity, varieties, etc. Table 1 gives an indication of the range of physical and chemical composition of raw tomatoes (Guastalla, 1968). The sugars in tomatoes are practically all reducing sugars, i.e. glucose (0.88–1.25%), fructose (1.08–1.48%), etc. (Lamb, 1977). The pH of raw tomatoes is 4.2–4.6 and is mainly due to citric and malic acids (Lamb, 1977). Although the nutritional value of tomato is rather low, the large amount of tomatoes consumed in any meal of the day, raw or cooked, makes it quite valuable in standard and special diets.

The development of many new tomato varieties in order to improve yield, crack resistance, quality of fruit, etc. makes it difficult to consider all the varieties currently in use. The harvesting season depends on the climatic conditions: in Italy, it lasts from July to the end of September.

The leading countries in terms of acreage are the U.S.A., Italy, Mexico, Egypt and Brazil. Table 2 shows tomato production in the EEC and other countries from 1978 to 1980: Italian production, being the highest in Europe, ranged from 57 to 67% of that of the U.S.A. in 1979 and 1980.

The evolution of tomato production, acreage and yield per hectare in Italy from 1970 to 1980 is shown in Table 3.

Table 1. Physical and chemical composition of tomatoes (Guastalla, 1968)

Component	(%)
Juice	94–96
Seeds	1–1.5*
Peel and fibres	1.5–2.5*
Water	95
Carbohydrates	2.8–4.1
Protein (N × 6.25)	0.6–0.8
Acid (expressed as citric acid)	0.3–0.5
Ash	0.4–0.5

*Moisture content 66%

Table 2. Crop estimates (1000 tons) for tomatoes as reported in OECD. report (1979)

Country	1978	1979	1980*
Belgium	114.8	117.5	—
Denmark	18.3	17.0	16.5
France	802.1	824.7	826.7
Germany	28.0	29.3	25.8
Ireland	28.8	28.0	26.0
Italy	3850.0	5130.0	4660.0
Netherlands	371.6	405.1	380.0
United Kingdom	129.0	139.0	—
Spain	2223.0	2049.8	2056.0
Greece	1718.0	1750.0	1750.0
Portugal	950.0	970.0	—
Austria	28.0	37.0	35.0
Turkey	3300.0	3500.0	—
Canada	477.7	466.5	—
U.S.A.	6780.6	7699.1	—
Japan	887.8	941.1	—

*Estimate as at 6.5.80

Table 3. Tomato production, acreage and yield per hectare in Italy from 1970 to 1980 (IRVAM, 1980)

Year	Production (1000 tons)	Acreage (ha)	Yield (t/ha)
1970	3617.9	129 967	27.84
1971	3423.6	120 190	28.48
1972	3050.4	111 026	27.47
1973	3310.4	109 988	30.10
1974	3637.4	116 999	31.09
1975	3511.96	113 178	31.03
1976	2968.65	98 938	30.01
1977	3299.91	107 317	30.75
1978	3820.5	112 970	33.82
1979	5132.1	132 002	38.88
1980*	4810.0	125 930	38.20

*Estimates from IRVAM

Table 4. Supply balance sheet for fresh tomatoes (1000 tons) in the EEC countries in 1976/77 and 1977/78, as reported in Eurostat (1981)

	Germany		France		Italy		U.K.		EEC	
Utilization (1000 tons)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
(a) Sales by professional producers	30	29	573	573	2517	2711	187	180	3875	4036
(b) Imports	339	343	188	185	2	2	130	149	364	378
Intra EEC	227	240	57	49	—	—	36	51	—	—
(c) Resources = uses (a+b)	369	372	761	758	2519	2713	317	329	4239	4414
(d) Exports	1	—	5	8	21	24	5	8	54	53
Intra EEC	1	—	3	4	16	20	4	8	—	—
(e) Total domestic uses (c−d)	368	372	756	750	2498	2689	312	321	4185	4361
Losses	37	37	82	79	204	—	—	—	339	128
Animal feed	—	—	—	—	1	—	—	—	4	2
Processing	—	—	218	225	1518	1897	—	—	1737	2122
Human consumption	331	335	456	446	775	792	312	321	2017	2109

(1) From 1 April 1976 to 31 March 1977
(2) From 1 April 1977 to 31 March 1978

In 1976–77 and 1977–78, the last years for which statistics are available, the general aspects of the tomato sector in the main EEC countries and in the EEC as a whole are summarized in Tables 4 and 5, which report the supply balance sheets for fresh tomatoes and processed tomatoes respectively (Eurostat, 1981). From these tables it is possible to derive that the imports of fresh tomatoes are about 9% of total EEC uses, while those of processed tomatoes are approximately 30% of total EEC consumption.

Table 5. EEC supply balance sheet for processed tomatoes expressed in fresh product weight (1000 tons) in 1976/77 and 1977/78, as reported in Eurostat (1981)

	Germany		France		Italy		U.K.		EEC	
Utilization (1000 tons)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
(a) Usable production	—	—	218	225	1518	1897	—	—	1737	2122
(b) Imports	627	556	322	364	100	129	569	494	864	830
Intra EEC	459	464	184	201	2	5	243	228	—	—
(c) Resources = uses (a+b)	627	556	540	589	1618	2026	569	494	2601	2952
(d) Exports	22	20	37	47	1062	1155	—	—	36	155
Intra EEC	19	12	8	9	618	581	—	—	—	—
(e) Final stocks	—	—	—	—	60	—	—	—	—	—
(f) Change in stocks	—	—	—	—	−290	−10	—	—	−290	−10
(g) Total domestic uses	605	536	503	542	846	881	569	494	2855	2807
Losses	—	—	—	—	—	—	—	—	—	—
Human consumption	605	536	503	542	846	881	569	494	2855	2807

(1) From 1 April 1976 to 31 March 1977
(2) From 1 April 1977 to 31 March 1978

The tomato industry can be described by two different types of culture, that is, glasshouse or greenhouse and open field. All greenhouse-grown tomatoes are marketed fresh, while a high proportion of the tomatoes grown in open fields is processed. Table 6 deals with the utilization of tomatoes grown in open fields in Italy and shows that more than two-thirds of the crop are processed. About 45% of fresh tomatoes are canned as peeled tomatoes, 53% as tomato paste at 28–30% natural tomato soluble solids (NTSS) and the remainder as chopped peeled tomatoes and tomato juice (INCA, 1979).

In all probability the EEC Common Agricultural Policy (CAP), involving a system of production grants, may have been particularly significant in encouraging the recent expansion (126% increase from 1977 to 1980) in tomato concentrate production in Italy, although in the same period a remarkable 20% increase in tomato yield per hectare was obtained (Table 3). Certainly, the introduction of common policies for all agricultural products has been

Table 6. Utilization of tomato in Italy from 1974 to 1980

Utilization (1000 tons)	1974*	1975†	1976†	1977‡	1978§	1979§§	1980**§§
Industrial processing	1850	1480	1240	1550	2300	3730	3500
Fresh market	940	1011.4	942	1000	828	895	918
Market surplus	5.2	124.6	10	14	13	163	70
Waste and losses							
Production and self-consumption	325	330	371	300	198	262.1	240
Distribution	114	105	75	86	50	64.5	62
Export	15.8	19	15	20	19	17.5	20
Total	3250	3070	2653	2970	3408	5132.1	4810

*IRVAM (1975)
†IRVAM (1977)
‡IRVAM (1978)
§IRVAM (1979)
§§IRVAM (1980)
**Estimates at 31st July, 1980

responsible for the operation of a scheme of contractual systems between the growers and the processing industry, thus representing a stabilizing element in the EEC market against the large variations of world market prices for raw materials. In fact, the competitive position of EEC producers and manufacturers can be greatly affected by the importation of processed fruits and vegetables from non-EEC countries.

For this reason, the CAP introduced a support system for a large number of products (such as stewed or frozen fruits with or without sugar addition, dried fruits, citrus peels, pectic substances, fruit purées, fruit pastes, fruit juice and grape juices with a sugar addition greater than 30%) by establishing no internal trade barriers between the member states and common external tariffs against the non-EEC producers (EEC regulatory n. 516, 1977). Furthermore, the importations of products containing sugar substitutes like glucose and/or glucose syrups, which are less expensive than sucrose were submitted to import tariffs to maintain the competitiveness of the European industry (EEC regulatory n. 516, 1977). On the other hand, the exportation of products requiring sugar addition to non-EEC countries was supported by the granting of aids, proportional to the addition of sucrose, glucose, or glucose syrups (EEC regulatory n. 516, 1977), while that of the other products mentioned above was helped by grants covering the difference between the EEC and world market prices.

As far as the production of tomato products is concerned, the contracts regulating intra-European trade between tomato suppliers and tomato manufacturers for each type of product in 1979–80 are summarized in Table 7 (EEC regulatory n. 1346, 1980).

Table 7. Contracts regulating the intra-EEC trade between growers and manufacturers, as reported in IRVAM (1980): minimal raw tomato price to be paid to growers and rewards to be given to processors according to the type of final product

	Payments to			
	Producer (L./kg of fresh tomatoes)		Manufacturer (L./kg of packed product)	
Final product	(1979)	1980)	(1979)	(1980)
Peeled tomato				
type 'Roma'	110.44	127.70	148.31	147.39
type 'S. Marzano'	146.42	169.38	186.17	185.01
Chopped peeled tomato	91.25	105.59	72.68	72.25
Tomato juice				
3.5—5% NTSS	91.25	105.59	69.33	68.89
5 —7% NTSS	91.25	105.59	106.67	105.94
7 —8% NTSS	87.58	101.31	146.84	145.88
8 —10% NTSS	87.58	101.31	167.81	166.72
10 —12% NTSS	87.58	101.31	199.28	197.98
Flakes	110.44	127.70	1562.77	1675.09
Frozen peeled tomato	110.44	127.70	148.31	147.39
Tomato paste 28–30% NTSS	87.58	101.31	400.87	429.54

In general, every year a European committee lays down the minimum selling prices for fresh tomatoes to be transformed into tomato products and the aids to the processors. The former are evaluated by taking into account the average market prices during the previous harvesting season and the increase in the cultivation costs, while the latter depend on the mean processing costs in the EEC and the average CIF (Cost Insurance Freight) selling prices for each tomato product imported from Third Countries (EEC regulatory n. 516, 1977).

Finally, the contracts mentioned above also guarantee a certain income to farmers who have not sold their crop by withdrawing the surplus for other uses, such as industrial utilization or animal feeding. For instance, in 1979 about 168 000 tons of fresh tomatoes were withdrawn by the government association (AIMA).

A typical tomato paste production process

Of the various technological lines actually used to process fresh tomatoes the economics of tomato paste production has been studied because of its high

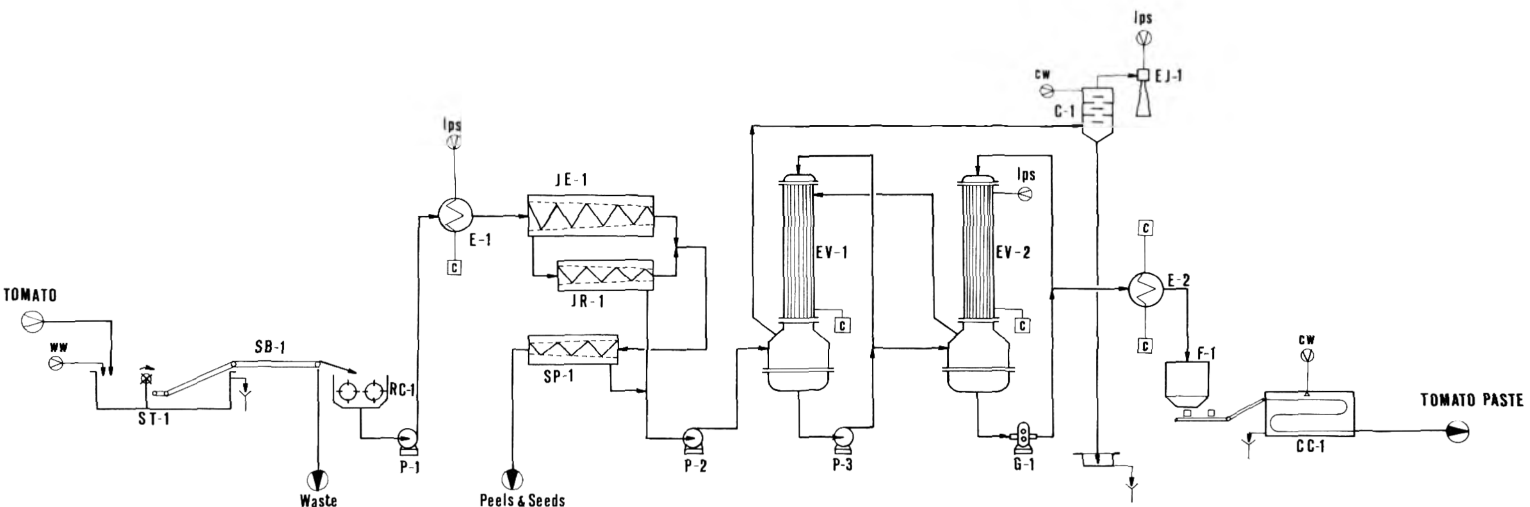

Figure 1. Flow diagram for tomato paste process.
Equipment identification Items: C, Condenser; CC, Container cooler; E, Heat exchanger; EJ, Ejector; EV, Long-tube falling-film evaporator; F, Filler; G, Rotary gear; JE, Juice extractor; JR, Juice refiner; P, Centrifugal pump; RC, Rotary comb chopper; SB, Sorting belt; SP, Screw press; ST, Soaking tank.
Utility Identification Items: c, Condensate; cw, Cooling water; lps, Low pressure steam; ww, Washing water.

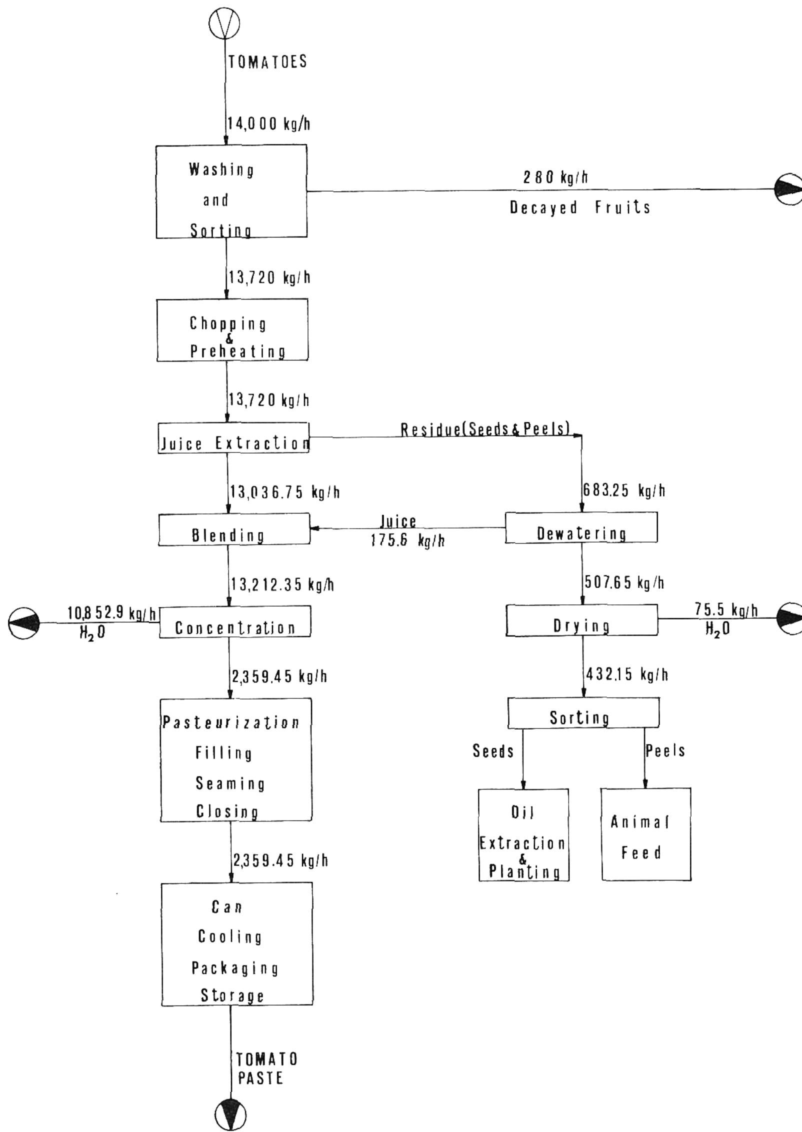

Figure 2. Material balance flow sheet for the flow scheme of Fig. 1.

output (INCA, 1979), by considering a medium size plant with a working capacity of 14 tons of fresh tomatoes per hr and operating for 120 days per year.

The process flow diagram and material balance flow sheet are shown in the Figs 1 and 2, respectively. The process may be described as follows.

Washing and sorting

Fruit is generally brought to the concentration plant in bulk form or polyethylene tubs by trucks. Storage should be no longer than 48 hr to limit

bacterial contamination which modifies the chemical and nutritional characteristics.

Two soaking tanks are commonly used to remove all the adhering dirt; then the washed tomatoes are moved to the sorting belts by a conveyor.

Under optimal conditions of lighting and belt design (area, width and speed of the belt of about 0.07 m^2 per kg of tomatoes, 0.5 m and 5 m/min respectively) seasonal workers are usually employed to inspect 400–500 kg/hr per person (Guastalla, 1968). Decayed fruits are picked out by hand and discarded. Washing water is chlorinated (5–10 p.p.m.) to maintain sterility during operation. In our specific case, 1.43 m^3 of water was found to be sufficient for each ton of fresh tomatoes (Leoni, 1978); sorting required three sorting belts working in parallel and thirty men per shift.

Chopping and pre-heating

Washed tomatoes are first chopped into small pieces by a machine consisting of a rotating, cogged drum and a stationary comb, and then pre-heated to 90–95°C for 1–2 min ('hot-break' method) or to 60°C ('cold-break' method) to allow easier peeling (Guastalla, 1968; Tressler & Joslyn, 1961). The 'cold-break' treatment is actually more widely used because the 'hot-break' method inhibits pectic enzymes, giving highly coloured, thick and viscous juices and heavy fouling in heaters and evaporators (Morgan, 1959). Moreover, seed recovery for future planting is also possible (Fig. 2), although the commercial value of the seeds is rather low.

Extraction

Chopped tomatoes are pressed through various screens, the openings of which decrease from 1.5 to 0.4 mm. The residue is dewatered by a continuous screw press to improve the extraction yield and then sent to final disposal in a controlled discharge area. Other alternatives, such as seed recovery for oil extraction or future planting, and peel utilization as animal feed or fuel, involve a drying operation and are usually regarded as being uneconomic for a number of reasons (seasonal operation, high operating costs of drying, low commercial value of final products, etc.).

Concentration

This operation is carried out at sub-atmospheric pressures by using falling-film and scraped-film evaporators according to the viscosity of the final product. In this study tomato juice was concentrated from 5 to 28% NTSS by using counter-current two-stage falling-film evaporators. Material and energy balances solved by trial and error are shown in Table 8. Physical data (boiling-point rise, density and specific heat of tomato products) were derived from Liverotti (1980), and heat transfer coefficients from manufacturers' catalogues.

Table 8. Material and energy balances for the evaporation unit of the tomato paste process shown in Fig. 1

Parameter	First effect	Second effect	Unit
Internal pressure	55	196	mmHg abs.
Juice temperature	42	69	°C
Boiling-point rise	0.7	1.7	°C
Heat-transfer coefficient	2900	1980	$W/m^2 . °C$
Temperature difference	29	35	°C
Heat-transfer surface	52	60	m^2
Input liquid flow-rate	13212	7927	kg/h
Input NTSS	5	8.3	%
Output liquid flow-rate	7927	2359	kg/h
Output NTSS	8.3	28	%

Pasteurization

Continuous pasteurization at 90–92°C has been found to allow safe storage by preventing any spoilage by lactobacilli and avoiding any local superheating of the product (Guastalla, 1968; Tressler & Joslyn, 1961).

Filling and closing

Pasteurized juice is automatically filled into thoroughly cleaned and steam-treated lacquered tin cans; the cans are then seamed and cooled. Insufficient cooling is detrimental to flavour and often causes labelling difficulties.

Storage

Tomato products may be temporarily stored in 5–20 kg cans, 115–225 kg barrels (Cultrera & Giannone, 1965) or sterile tanks in an inert gas atmosphere (Menoret & Gautheret, 1962). The first two methods involve high packaging costs if further processing is required. The rate of deterioration increases rapidly with increase of temperature. Canned tomato products stored at 12–24°C retain their normal flavour and appearance for about 18 months if air humidity is kept low to avoid condensation and can rusting (Guastalla, 1968).

Waste disposal

Although the liquid wastes from processing and washing operations have a BOD ranging from 600 to 1000 p.p.m. (Degrémont, 1972), the small volumes arising during the seasonal operation of the tomato industry involve limited environmental contamination. Therefore, after screening to remove suspended solids wastewaters are usually disposed of as irrigation water or piped into existing sewers (Tressler & Joslyn, 1961), but this procedure may not be acceptable in future.

Cost estimates

The economic balance of the plant has been calculated as outlined below.

The capital investment C_i has been derived by using Chilton's method (1960), as summarized in Table 9. In particular, capital investment for the major items of equipment (Fig. 1) has been derived from recent data from manufacturers, while the minor items, such as decayed fruit and pallet conveyors, screw press, etc., have been assumed to represent 5% of the 'total purchased equipment cost' C_e.

Table 9. Capital investment estimate for the tomato paste process shown in Fig. 1: working capacity 14 tons of fresh tomatoes per hours; output level about 6800 tons of tomato paste at 28% NTSS

Unit		Investment cost (in millions L.)
Washing—extraction		100
Concentration		350
Pasteurization		20
Packaging		35
Cooling		100
Steam production		50
Pumps		3
Minor items		35
Total purchased equipment C_e		693
Ancillary process equipment and installations		
Piping and valves	$0.20\ C_e$	
Instruments and controls	$0.10\ C_e$	
Electrical	$0.15\ C_e$	
Equipment installation	$0.30\ C_e$	
Painting, insulation	$0.02\ C_e$	
	$0.77\ C_e$	534
Civil works and services	$0.55\ C_e$	381
Utility installation	$0.20\ C_e$	139
Total direct cost $C_d = 2.52\ C_e$		1747
Engineering	$0.10\ C_d$	175
Contractor fees	$0.05\ C_d$	87
Contingencies	$0.15\ C_d$	262
Total capital investment $C_i = 1.30C_d$		2271

A summary of all the items contributing to operating costs C_o is set out in Table 10.

Table 10. Production costs for the tomato paste process shown in Fig. 1 for Italy in 1979 ($1 = 1000 Italian Liras)

Operating costs	Cost (in millions L.)
Investment-related costs	
Depreciation (10 years, 10% interest rate)	370
Maintenance (3% C_d)	52
Subtotal	422
Utilities	
Electricity: L.25/kWhr × 139 kWhr × 2880 hr	10
Cooling water: L.20/m^3 × 158 m^3/hr × 2880 hr	9
Well water: L.80/m^3 × 20 m^3/hr × 2880 hr	5
Fuel: L.165/kg × 558 kg/hr × 2880 hr	265
Subtotal	289
Raw materials	
Tomato: L.96·58/kg × 14 000 kg/hr × 2880 hr	3894
Lacquered tin cans: L.80/kg × 2359·45 kg/hr × 2880 hr	544
Chlorine:	2
Subtotal	4440
Labour	
Seasonal worker: L. 4500/hr × 90 SW × 960 hr/SW	389
Seasonal skilled worker: L. 14 × 10^6/year × 9 SSW × 1/3 year/SSW	42
Permanent skilled worker: L. 16 × 10^6/year × 4 PSW × 1 year/PSW	64
Administrative Worker: L. 16 × 10^6/year × 4 AW × 1 year/AW	64
Supervisor: L. 20 × 10^6/year × 1 S × 1 year/S	20
Subtotal	579
Total	5730

The economic balance of the concentration plant can be written as follows,

$$C_o + P = Q_P(c_P + c_{EEC}) \tag{1}$$

where Q_P and c_P are respectively the overall quantity and selling price of tomato paste, and c_{EEC} the EEC payments per kg of packed product. The plant profitability P is expressed as a percentage p of the 'total capital employed' C_T, which is made up of C_i and the fraction n of the operating costs related to 3 months of turnover:

$$C_T = C_i + nC_o \tag{2}$$

By combining equations (1) and (2) we have

$$p = \frac{Q_P(c_P + c_{EEC}) - C_o}{C_i + nC_o} \tag{3}$$

For 1979 the profit from the operation and the return on capital p have been calculated as shown in Table 11. A profitability of about 22% is usually considered satisfactory for a venture of this nature, depending on the risk relative to alternative utilization of capital within a company.

Table 11. Profitability analysis for the tomato paste process shown in Fig. 1 with reference to the operating costs shown in Table 10

Areas of profitability	10^6 L.	%
Gross profit		
Sales: L. 560/kg×2359.5/β kg/h×2880 hr	4181	
EEC payments: L. 400.87/kg×2359.5/β kg/h×2880 hr	2992	
Subtotal (a)	7173	
Operating costs (b)	5730	
Profit from operations (a−b)	1443	
Total capital employed (C_T)		
Original fixed capital invested	2271	
Turnover: 0.75×5730×10^6 L.	4297·5	
Total	6568·5	
Profitability (a−b)/C_T		21·97

β = filling degree of 5-kg container—0.91

To determine how p is influenced by several parameters, such as tomato transport, package and labour costs, the marked price of the packed product and EEC payments, equation (3) has first been modified by subsituting all the terms of the operating costs and then differentiated with respect to each parameter x_i at $x_{j\neq i}$ = const. Each partial derivative, $\delta p/\delta x_i$, has been finally used to determine the relative variation Δp of p at different degrees of variation of each factor as follows,

$$\Delta p = \sum_i \left(\frac{\delta p}{\delta x_i}\right)_{x_{j\neq i}} x_{io} \left(\frac{\Delta x_i}{x_{io}}\right) \qquad (4)$$

where x_{io} is the basic value of the generic parameter x_i. All the data useful for this analysis of sensitivity are presented in Table 12. A 10% variation of raw tomato and tomato paste market prices and EEC payments varies the return on capital by 29 and 21% respectively, while the same variation of the various other factors yields a mean effect on p less than 5%.

These results make the consideration of alternative investment extremely difficult, as the differences are of the same order of magnitude as the profit or loss. In the past year, owing to the effect of inflation, raw tomato costs increased by 15.7%, product prices by 11%, the EEC subsidies by 7.2%, transport costs

Table 12. Sensitivity analysis of profitability referred to a +10% relative variation of each parameter contributing to the economic balance of the tomato paste process shown in Fig. 1.

Parameter x_i	Basic value x_{io}	$\left(\frac{\delta p}{\delta x_{io}}\right)_{x_{j \neq i}}$	$\left(\frac{\Delta p}{p_o}\right)^*$
Raw tomato cost	87.58 L./kg	-7.150×10^{-3}	−28.5%
Transport cost	9.00 L./kg	-7.150×10^{-3}	−2.9%
Tomato paste package cost	80.00 L./kg	-1.205×10^{-3}	−4.4%
Labour cost	579.00 10^6 L.	-1.773×10^{-10}	−4.6%
Tomato paste market price	560.00 L/kg	1.137×10^{-3}	+28.9%
EEC payments	400.87 L/kg†	1.137×10^{-3}	+20.7%

*Referred to a +10% relative variation ($\Delta x_i/x_{io}$) of each parameter x_i
†Packed tomato paste at 28% NTSS

by 33%, package costs by 25% and labour costs by 15%, thus yielding a lower return on capital (16.3% instead of 22%). If tax, duty, insurance, advertising investment and interest allowed are deducted from gross profit, profitability is further lowered.

The annual contribution of raw material costs (Table 10) is more than 75% of the overall operating costs and about twice the total capital invested. Therefore, tomato manufacturers may have to resort to high-interest, short-term loans to obtain working capital, thus drastically reducing net earnings.

Moreover, not only low, raw tomato prices but also low production costs have to be considered to understand why tomato products of first quality from Greece, Spain and Portugal are not so expensive as those from Italy. In fact, even if the EEC payments for tomato manufacturers were to reduce raw material costs from L. 87.58/kg to L. 29.02/kg the selling price (L. 560/kg) of 28% NTSS tomato paste, for instance, would still be higher than that (L. 450–500/kg) of a similar product from Portugal. This can be easily explained by taking into account the higher labour costs of the EEC industry in comparison with Third Countries like Spain, Greece, Portugal, Turkey, etc.

Therefore, it is virtually certain that the present CAP support system for the tomato processing sector should adopt drastic changes in its mode of operation in order to allow the EEC manufacturers to cope with increased levels of competition from non-EEC countries.

In fact, one of the main disadvantages of the CAP system is that the obligation for the EEC processors to purchase tomatoes at prices much higher than their competitors in the non-EEC countries lowers their competitiveness on EEC and export markets.

In a short-term period this might be guaranteed by increasing the import tariffs for tomato products from Third Countries or granting greater aids to the manufacturers, while a new market intervention activity should be introduced to favour a long-term reorganization of the EEC tomato sector, as its profitability cannot be improved only by optimizing tomato processing

methods. In fact, the incidence of the actual processing equipment on the total capital invested is rather less than 31% (Table 9) and in the near future this proportion is likely to be reduced further following the installation of wastewater treatment plants to fulfil anti-pollution regulations.

In more specific terms, to support continuing interest in these processes in industrialized areas the following changes are necessary:

(1) The industry will need to develop new 'crack-resistant' varieties of tomato in order to favour the mechanization of crop harvesting, thus obtaining a substantial reduction in manpower and raw tomato costs.

(2) Automatic sorting will be required, although this has proved to be one of the most difficult operations to mechanize. However, we are certain to see a major drive in this area in the future, more than two-thirds of labour costs being associated with manual sorting.

(3) For the concentration of tomato juice 2-effect or 3-effect falling-film evaporators are most commonly used. However, dewatering costs for a water removal of about 10 ton/hr (Fig. 2) might be further reduced by increasing the number of effects up to 4 or 5 in accordance with Thijssen and van Oyen (1977). Further energy might be saved by expanding live steam through an exaust-steam turbine-engine to drive circulation pumps (Angeletti, 1979).

(4) The adoption of multi-purpose plants capable of treating different kind of fruits (such as tomato, grape, apple, orange, lemon, etc.) would increase the annual working period of the industry, thus reducing the contribution of amortization and permanent worker costs to the overall operating costs.

(5) Low interest government loans will also help industry to deal with the problem of delayed EEC payments and the burden of interest.

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An objective index for the evaluation of the ripening of salted anchovy

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Summary

Salted anchovies were conditioned in order to allow ripening in brine. A sensory panel procedure was developed to systematically follow the process.

This sensory evaluation was carried out for up to 328 days, and the results indicated 10 months as the minimum time required to obtain an adequately cured product.

Total ester index was determined at the successive stages of anchovy ripening. There was a close correlation between total ester index and sensory score between 100 days and 328 days of ripening. This relationship to storage time and to sensory assessment supports the use of total ester index as an objective method to follow and assess the later stages of this little known process.

Introduction

Salting and curing of anchovies is a traditional process used by Mediterranean fishermen to obtain a product with a tender consistency and specific pleasant aroma and taste as a result of enzymic activity on the fish flesh.

The reproduction of *Engraulis anchoíta* takes place all year round, but there are two principal periods of spawning, the main one taking place during October–November and the second during May–June (Bellisio, López & Torno, 1979).

From experience it has been observed that the desired ripening reaction takes place only in *E. anchoíta* caught during the October-November period and this fish is suitable as raw material for semi-preserves.

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In spite of the antiquity of the process, there is a marked lack of reports on the curing of anchovies and most of the knowledge about the process is empirical (Cheftel, 1965).

The minimum time described for the ending of maturation is 6 months for anchovies in Spain (Kemp, 1973), and 8 months for *E. anchoíta* (Mattos *et al.*, 1977). After these periods of time, an adequately cured product is suitable for the preparation of several subproducts, such as pickled fish, salted fish, fish sauce, or anchovy paste, all of them with the same characteristics as those obtained using European anchovies.

In spite of the importance of the ripening process, visual inspection and tasting are still the usual methods of following the process (Thackaberry, 1979).

Chemical changes have been described during the salting and ripening of Atlantic herring (Plorina & Leonova, 1970) Baltic herring (Podeszewski, Stodolnik & Otto, 1975) Caspian herring (Konnova, 1966) Siberian cisco fish (Sedova & Emel'yanova, 1976) and anchovies (Baldrati *et al.*, 1977; Establier & Gutierrez, 1972; Lee & Choe, 1974). Some of those changes could be used as maturation indices (Sedova & Emel'yanova, 1976; Mattos *et al.*, 1977). However, a systematic attempt to find a reliable and objective parameter to follow and assess the different stages of fish ripening in brine has not been made. The objective of this work was to undertake an investigation on the applicability of the total ester determination as an objective method of following the anchovy maturation process, compared with the sensory assessment. A systematic arrangement of sensory scores based on flavour, colour, odour, flesh consistency and flesh adherence to backbone was also developed in order to follow the ripening of salted anchovy.

Materials and methods

Anchovy salting, packing and ripening in brine

Anchovies (*E. anchoíta*) were caught on the Argentinian platform in the south-eastern Atlantic Ocean from *ca.* 37.4°S to *ca.* 38.8°S during spring, when they arrived at the coast for spawning. Fish samples were obtained from commercial vessels and were immediately immersed at *rigor mortis* stage in saturated brine in plastic boxes after their arrival at the laboratory. After 24 hr of brining, the fish were manually beheaded and partially gutted in the same operation, and then returned to strong salt solution in order to wash them thoroughly. The washed anchovies were packed in 10-kg tins, in layers with two fish in each layer being at right angles to each other. There was close contact between the different layers of fish, and dry salt was not added, except at the bottom and the top of packed fish. The average fish content was 7.3 kg. One hundred and twenty grams of dry salt was put into the bottom of each can and a further 120 g was also scattered, when packing was completed, on top of the last layer of fish.

A round piece of wood was placed on the top layer of salt and pressed to a maximum of 80 g/cm^2 by means of heavy weights on top of the wooden disk. Due to the applied pressure and to the osmotic effect of the salt, extruded liquid containing biological material in brine was produced, which completely covered the fish.

The cans were stored for ripening in a cellar at 18–22°C under the above mentioned pressure for a period up to 400 days.

Sensory assessments

At regular intervals, anchovies were taken from different cans in order to obtain representative samples. The anchovies were washed with running water to remove extruded liquid and then were organoleptically evaluated by a panel of at least three people experienced in judging fish quality. The assessors were previously trained in the fish processing industry according to the current methods used to assess the degree of ripening of salted anchovy. Based upon personal communications with fish processors, a scoring method to follow the anchovy ripening process was developed. This is shown in Table 1. A scale of

Table 1. Organoleptic panel.

	Sensory score				
Factor	0	2	4	6	8
Flavour (disregarding salt)	Raw fish	Neutral	Slightly ham-like	Ham-like cured meat	Rancid off-flavours
Flesh colour	Natural fresh fish	Natural around borders, deep red in the middle, pink in between	Light pinkish meat, deep red or pink in the middle	Uniformity in the pink tone distribution	Dark red, black, red blots and/or black dots
Odour	Fresh fish	Neutral (smells like brine)	Smooth agreeable odour to volatile esters	Smells of agreeable volatile esters. 'Characteristic anchovy odour'	Rancid, acid, ammoniacal or sulphurous off-odours
Flesh consistency	High elasticity, damp	Less elasticity, less damp	Slight elasticity, more compact, does not feel damp	No elasticity, firm and resistant to finger pressure	Flimsy
Flesh adherence to backbone	Very adherent, does not separate	Very adherent, does not separate easily	Adherent, it separates (incomplete filleting)	Very little adherence, it separates neatly (adequate filleting)	Flesh gets torn in the filleting process

points from 0 to 8 is proposed to replace the ambiguous 'unripe' or 'green', 'ripe' and 'over-ripe' terms which are traditionally used by the expert tasters of the fish processing industry. The odd numbers were reserved to intermediate stages. Number 8 corresponds to deteriorated or over-ripe anchovy. A fish is assigned a score for each factor according to the descriptions in the Table. The average of the five considered factors was taken as the score for the fish. The final score given to the sample was the average of at least eighteen specimens, evaluated in groups of six, by three or more assessors.

Total ester index

The method used was a modification of that given in the Official Methods of Analysis of the AOAC (1970, method No. 9.125). Ten grams of anchovy paste obtained from fillets were ground in cooled, distilled water in a Virtiss homogenizer and made up to 100 ml. Aliquots of 20 ml were taken after adjustment to volume for the determination of ester indices. Since the measurements were done in aqueous homogenates instead of purified lipid extracts, the values obtained are representative of total ester indices, including the saponification indices. Results were expressed in gKOH on a dry weight (CDW) basis. Dry weight was obtained after dessication of 20 g of anchovy paste in a oven at 100±5°C up to a constant weight. The residues were ground in a mortar to a fine meal and representative 2 g samples were quickly taken and transferred to beakers. Approximately 150 ml of distilled water were added to each beaker and the solution boiled for 5 min. After cooling, the suspension was filtered through glass wool to a 250 ml volumetric flask. Aliquots of 10 ml were taken for NaCl determinations according to Mohr's method as given in the Official Methods of Analysis of the AOAC (1970, method No. 50.028). Corrected dry weight was calculated by subtracting the salt content from the weight obtained by drying.

Statistical analysis

The experimental curves were mathematically expressed as empirical models. The functional form

$$\tan[(y-a)\,z] = mx+b$$

was chosen for the sensory assessments *vs* days and for the total ester index *vs* days curves. Parameters a and z were chosen by a trial and error method. Parameters m and b were estimated by the regression analysis procedure from least squares estimates (Himmelblau, 1970).

Statistical analysis carried out by a model such as $y = A+Be^{-kx}$ had no special advantages on the tangent transform system (data not shown). Moreover, the proposed functional form can be used to analyse data by means of a small personal programmable calculator instead of requiring a computer when an exponential model is used.

Results and discussion

Visual inspection and tasting are still the usual methods of following anchovy-ripening in brine (Thackaberry, 1979). However, there are no references on the use of a sensory panel to follow the maturation of salted anchovy. Assessment of the changes in the sensory characteristics of anchovies during ripening in brine was made using the scoring system described in Table 1. This procedure, based on the evaluation characteristics empirically used by the producers, allows for the systematic assessment of the ripening process.

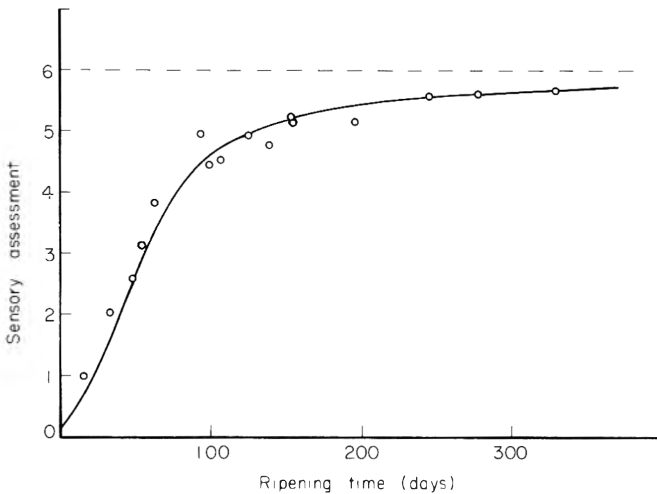

Figure 1. Sensory assessment during ripening time of anchovy. Each point is representative of at least eighteen samples, and is the average of the sensory characteristics evaluated according to Table 1. Results are representative of experiments performed during 1979 and 1980.

Figure 1 shows the sensory scores obtained during ripening of anchovy in brine. The line

$$\tan [24(y-2.25)] = 0.027x-1.191 \qquad (r = 0.982) \qquad (1)$$

where y is the sensory score and x is the ripening time (days), fits the experimental data presented in Fig. 1. Although the most pronounced sensory changes took place within the first 100 days of ripening, the anchovy were not yet ripe. It can be observed that the curve tends toward an asymptotic value after 10 months, which can be considered to be the time normally required to obtain a fully maturated anchovy. A maturation period of approximately 8 months has been reported for *E. anchoíta* (Mattos *et al.*, 1977). The differences from the results presented here could be due to the wide temperature range (11-30°C) described by these authors, the biological condition of the anchovies used or to differences in the technological process used, such as the salt content, pressure employed, and others.

The total ester indices obtained during the ripening of anchovy are presented in Fig. 2. Starting from 90 days of anchovy ripening, the total ester index determinations plotted against time showed a hyperbolic shape (Fig. 2) which had similar asymptotic characteristics to that of the organoleptic assessments *vs.* time (Fig. 1). The line

$$\tan[22.5(y-5.85)] = 0.008x - 1.396 \qquad (r = 0.914) \qquad (2)$$

where y is the total ester index determination (gKOH/100 gCDW) and x is the ripening time (days), fits the experimental data presented in Fig. 2. Despite the good correlation obtained for the data shown in Fig. 2, results obtained during the first 90 days of ripening varied between 3.19 and 9.30 gKOH/100 gCDW. This wide variation rules out the use of the total ester index determination as a method to follow the first stages of the anchovy ripening process. However, it is necessary to wait a further 200 days in order to obtain a fully ripe anchovy despite the fact that the sensory score during that period changed more slowly than during the first 100 days of ripening (Fig. 1). In contrast, the total ester index showed a systematic change after only 90 days of anchovy ripening and in such a way, could be used to follow both of the later third stages of the process (Fig. 2).

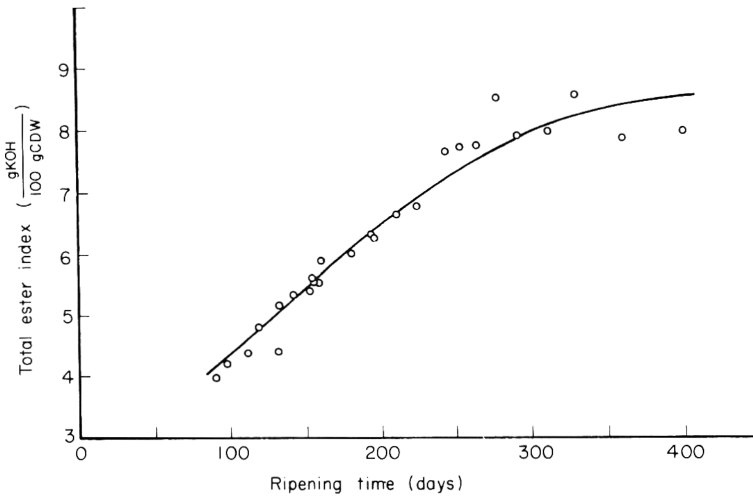

Figure 2. Total ester index during ripening time of anchovy. The points presented are the average of at least three individual determinations and results are representative of experiments performed during 1977, 78, 79 and 80.

Figure 3 shows the relationship between the total ester index plotted on a logarithm scale and the sensory score during anchovy ripening. In the same figure the points calculated from equations (1) and (2), and the experimentally obtained values are plotted respectively. A linear correlation can be observed,

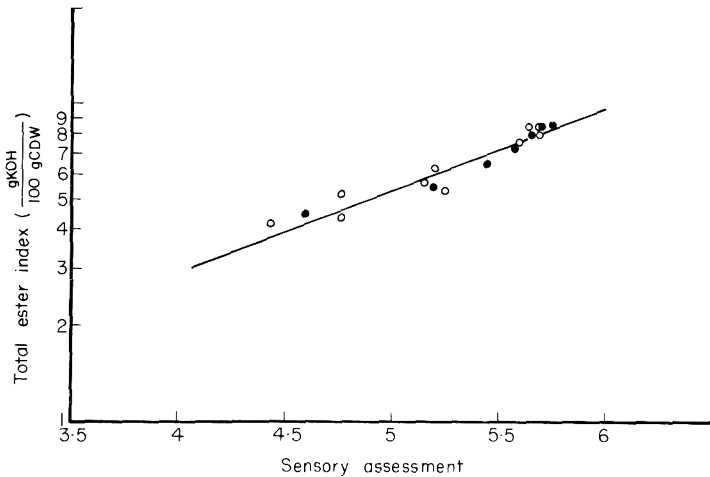

Figure 3. Total ester index *vs* sensory assessment. ●, calculated from equations (1) and (2) given in the text; O, experimental results obtained during 1979 and 1980.

and as much as the resulting equation and the correlation coefficient are the same in both circumstances:

$$y = 0.3136.\ e^{0.5706x} \qquad (r = 0.968)$$

where y is the total ester index determination (gKOH/100 gCDW) and x the organoleptic score.

These data support the total ester index determination as a valid proposition of an objective method to follow and assess the process of anchovy ripening in brine.

Acknowledgments

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Isohalic sorption isotherms

II. Use in the prediction of storage life of dried salted fish

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Summary

Isohalic sorption isotherms of cod (*Gadus morrhua*) are used, in conjunction with growth data for dun mould (*Wallemia sebi*), to predict the shelf life of dried salted fish in tropical climates. Measured shelf-lives of several fish species processed and stored by traditional methods are in agreement with the predictions.

Introduction

Isohalic sorption isotherms for salted fish can be calculated from the known physical properties of sodium chloride solutions and measured sorption characteristics of the unsalted fish; good agreement between calculated and measured moisture and salt contents has been found for salted cod fillets equilibrated to different water activities, a_w's (Doe *et al.*, 1982).

Fish processed in tropical climates by brining in salt solutions, or by dry-salting, and subsequent drying is liable during storage to deteriorate through mould growth, in particular the dun mould (*Wallemia sebi,* syn. *Sporendonema epizoum*) (Troller & Christian, 1978; Liston, 1980). This paper puts forward a method of calculating safe drying rates which prevent mould growth during drying and predicts storage times for dried fish under tropical conditions. A storage trial involving seven different tropical fish species was conducted at the Institute of Fish Technology in Sri Lanka by one of the authors (R.G.P.) in December 1979 through to July 1980.

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Materials and methods

Fish species

Tilapia (*Tilapia mossambica*), sprats (*Stolephorus* spp.), half beaks (*Hemirhamphus* spp.), walaya (*Wallago attu*), leather skin (*Chorinemus lysan*), skate (*Rajae* spp.) and sardines (*Sardinella* spp.) were used in the storage trials.

Processing

Tilapia were processed either split along the backbone and scored, or gutted, skinned and salted whole. Salt was rubbed into the split fish. Both processes used 1 part salt to 3 parts fish and the salted fish were left in drums for 24 hr. The fish were washed and then sun-dried on the ground for 3 days. The split sample was further sun-dried for 1½ days after purchase. Walaya were gutted, roughly scored and salt rubbed into the flesh (3 : 1 fish to salt). They were then brined, washed then sun-dried like tilapia. Sprats were salted whole in a cement tank (2 : 1 fish to salt) for 2–3 hr, then washed and sun-dried on coconut leaf mats for 2–3 days. Half beaks were processed by placing whole fish (114 kg) together with salt (67 kg) and enough sea water to form a salt slurry in a cement tank for 2–2½ hr, then sun-dried on coconut leaf mats for 2 days. Leather skins and skates were split, scored and placed in a cement tank with alternate layers of salt (10:3 fish to salt). After 12 hr the fish were washed in sea water then sun-dried on palm leaves placed on the ground for 3 days.

Commercial samples of smoked walaya and sardines were also purchased for storage trials. Walaya were split down the backbone and smoked without salting on racks 1·5 m above a wood fire in a coconut thatched shed for 3 days. The sardines were gutted and scaled, then salted in 60% saturated brine for 4–5 hr. Fish were drained then hot-smoked in a natural draught concrete kiln for 7–8 hr.

All samples were processed within 7 days of catching.

Proximate analysis

Moisture content, crude fat and crude protein were determined before storage. Moisture contents were found by drying 2–5 g samples in a convection oven for 24 hr at 105°C. Crude fat was determined on previously dried samples by the Soxhlet method using petroleum ether, b.p. 40–60°C. The standard macro-Kjeldahl technique was used to determine the nitrogen content; this was converted to crude protein content by multiplying by 6.25.

Initial quality of samples

The quality of the fish species after processing varied from top quality (whole tilapia) to poor (skate, walaya). Texture in all species tended to be soft while

odour was good except for skate which had a strong ammoniacal smell. There was no evidence of mould, pinking or beetles in any of the samples although split tilapia and half beaks had some sand adhering to the flesh and there was much sand adhering to the sprats. Colour varied from light yellow-brown (tilapia) to silver-grey (half beaks). There was some darkening of the flesh around the backbone in the leather skin while the skate was discoloured and showed signs of rancidity. Whole tilapia were also slightly rancid.

The smoked walaya samples had a bright appearance with good colour but were moist in parts. The smoked sardines had a pleasant odour and appearance but were rather soft and fragile and many of the fish therefore were partly damaged.

Proximate analysis for each sample was determined by the standard methods (AOAC, 1965).

Storage conditions

The seven samples of dried salted fish and the two samples of smoked fish were stored under conditions similar to those found in commercial dry-fish stores in Sri Lanka. At weekly intervals the quality of the samples was assessed visually with particular attention being paid to the presence or absence of mould, pinking, beetles, rancidity and putrefaction. Salt and moisture contents were determined at regular intervals during the trial. Relative humidity and temperature in the storage rooms were measured using a recording hygrometer and/or a sling psychrometer.

The split tilapia, sprats and half beaks were stored in a reasonably well-ventilated room with a simple asbestos roof. During the storage period (December 1979–July 1980) there was a wide variation in relative humidity between night and day and also from day to day; the extremes were 57 and 90%. The range in temperature was 29–33°C. Leather skin, skate, whole tilapia and walaya were stored in a poorly-ventilated, enclosed room with a false ceiling and asbestos roof. During the storage period (March 1980–June 1980) the relative humidity varied between 75 and 90% and the temperature from 30–33°C.

Water activity from isohalic sorption isotherms

There are several methods for measuring a_w but none is suited to routine measurements under field conditions. Drawbacks in the methods include the use of expensive, sophisticated equipment, complexity, long duration and inaccuracy (Troller & Christian, 1978). The determination of the salt and moisture contents of fish samples, on the other hand, is fairly straight forward but, on their own, these values do not give an indication of storage life. Isohalic sorption isotherms provide a means of calculating the a_w of a particular sample from measured salt and moisture contents. It has not been possible for the authors to determine isohalic sorption isotherms for the fish species used in the storage trials. Instead, the isotherms determined for cod (*G. morrhua*) (Doe *et al.*, 1982) were used.

Results

The proximate analyses of the processed fish samples before storage are given in Table 1. Table 2 is a summary of the results of the storage trials; the samples in the well-ventilated room were monitored for 26 weeks while the samples in the poorly-ventilated room were discarded, because of spoilage, after 8–13 weeks.

The values of a_w shown in Table 2 were calculated from the isohalic sorption isotherms determined for cod; Fig. 1 shows these a_w values plotted against time for each sample. The large variations of a_w with storage time for each species reflect the variations in air temperature and humidity in the store rooms. The

Table 1. Proximate analyses of processed fish used in storage trials. (% dry matter basis)

	Protein	Fat	Ash	Salt
Split tilapia	52.9	14.1	33.2	30.2
Whole tilapia	62.1	9.1	28.9	21.9
Sprats	56.2	5.6	39.3	16.2
Half beaks	62.6	10.1	27.4	18.2
Leather skin	60.3	1.9	37.8	33.1
Skate	57.9	1.3	40.8	35.3
Walaya (salted)	50.2	20.0	29.8	24.1
Walaya (smoked)	71.4	18.7	10.3	0.3
Sardines	66.0	11.1	22.9	16.5

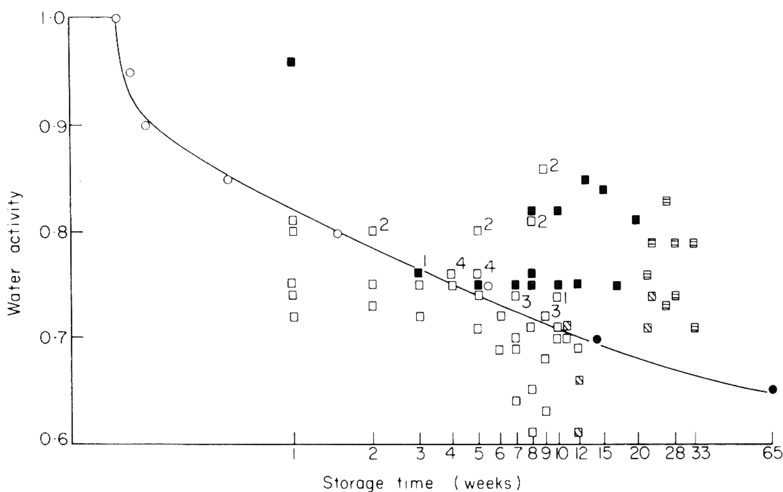

Figure 1. Times for visible colonies of dun mould *Wallemia sebi* at constant water activity with water activities of stored fish calculated from measured salt, moisture and fat contents and the isohalic sorption isotherms for salted cod.
Times to visible mould: O, Pitt & Hocking; ●, Shewan.
Results of storage trials: □, No mould or beetles; ■, Mould; ⧅, Beetles; ⊟, Mould and beetles.
1 = Smoked walaya; 2 = Smoked sardines; 3 = Split tilapia; 4 = Whole tilapia.

Table 2. Mass of water and salt in unit mass of wet fish

Storage Time (weeks)	Tilapia (split) M_w	M_s	a_w	Tilapia (whole) M_w	M_s	a_w	Sprats M_w	M_s	a_w	Half beaks M_w	M_s	a_w
1	0.359	0.215	0.75	0.398	0.121	0.80	0.233	0.110	0.72	0.309	0.130	0.74
3	0.328	0.225	0.75				0.241	0.109	0.72	0.238	0.143	0.73
4				0.381	0.134	0.76						
5	0.273	0.205	0.74	0.403	0.133	0.76	0.225	0.109	0.71	0.217	0.127	0.71
6	0.191	0.212	0.72				0.181	0.115	0.69	0.159	0.126	0.66
7	0.250	0.199	0.74	0.418	0.116	0.82	0.185	0.117	0.69	0.186	0.132	0.70
8	0.178	0.210	0.71				0.153	0.123	0.65	0.151	0.134	0.66
9	0.167	0.266	0.72				0.143	0.128	0.63	0.151	0.140	0.66
10	0.167	0.230	0.71	0.441	0.123	0.82	0.191	0.183	0.70	0.174	0.123	0.69
11	0.167	0.202	0.71				0.204	0.115	0.70	0.175	0.150	0.69
12	0.120	0.256	0.66	0.405	0.150	0.75	0.184	0.119	0.69	0.155	0.130	0.66
13												
15												
17				0.381	0.151	0.75						
20												
22	0.220	0.208	0.72				0.307	0.104	0.76	0.281	0.114	0.74
23	0.297	0.147	0.74				0.320	0.092	0.79	0.290	0.109	0.74
26	0.227	0.169	0.73				0.363	0.091	0.83	0.340	0.089	0.82
28	0.302	0.163	0.74				0.351	0.108	0.79	0.320	0.102	0.76
33	0.199	0.216	0.72				0.372	0.118	0.79	0.369	0.113	0.80

Storage Time (weeks)	Leatherskin M_w	M_s	a_w	Skate M_w	M_s	a_w	Walaya (salted) M_w	M_s	a_w	Walaya (smoked) M_w	M_s	a_w	Sardines M_w	M_s	a_w
1	0.399	0.207	0.75	0.473	0.206	0.75	0.346	0.149	0.75	0.280	0.002	0.96	0.362	0.106	0.81
3	0.375	0.230	0.75	0.417	0.204	0.75				0.120	0.002	0.76	0.365	0.111	0.80
4							0.352	0.151	0.75						
5	0.401	0.205	0.75	0.396	0.232	0.75	0.328	0.118	0.75	0.120	0.003	0.76	0.300	0.082	0.80
6										0.062	0.004	0.31			
7	0.390	0.210	0.75	0.412	0.231	0.75	0.419	0.146	0.76	0.103	0.013	0.64	0.260	0.087	0.75
8	0.447	0.197	0.75	0.418	0.221	0.75				0.094	0.005	0.61	0.353	0.101	0.81
9										0.102	0.005	0.68	0.365	0.083	0.86
10	0.464	0.177	0.75	0.464	0.177	0.75				0.117	0.004	0.74			
11										0.108	0.003	0.71			
12										0.097	0.006	0.61			
13													0.371	0.086	0.85
15													0.371	0.091	0.84
17															
20													0.375	0.109	0.81

Note: a_w according to Isohalic Sorption Isotherm for dried salted cod (Doe *et al.*, 1982).
Values of M_b calculated from $M_b = 1.0 - M_w - M_s$.

Table 3. The effect of water activity on the time for growth of visible colonies of the dun mould *W. sebi*

Water activity, a_w	0.95	0.90	0.85	0.80	0.75	0.70	0.65
Time to 1 mm diam. colony (days)	1.68*	1.92*	3.92*	10.2*	38*	100†	450†

*(Pitt & Hocking, pers. comm.).
†(Shewan, 1953).

curve fitted through the data is the growth times from Table 3, for visible colonies of *W. sebi.* Most of the samples with an a_w above the curve had mould; the samples above the curve which did not develop mould were the smoked samples (walaya and sardines) and the split tilapia which were infested with beetles after 3 weeks storage. The only other mould-free sample above the curve is the whole tilapia which after 28 days storage had discoloration typical of rancid samples and some surface dampness; it became mouldy during the next 7 days at the same a_w.

In all cases the fish samples with an a_w lying below the curve in Fig. 1 had no mould.

Discussion

The isohalic sorption isotherms were obtained from measurements on thin slices of fish under equilibrium conditions. However, it must be recognized that there are significant spatial variations in moisture and salt contents in dried salted fish; the salt and moisture content data in Table 2 are mean values for whole or split fish. Mould growth is a surface phenomenon. In most cases the surface of dried fish is drier than the interior; a_w values determined from the isohalic sorption isotherms using mean moisture and salt contents will be higher than the actual a_w at the surface and hence err on the safe side so far as mould growth is concerned. However under conditions of rehydration at high humidities, or when condensation occurs within packaging due to a rapid fall in temperature, the reverse can apply; this underlines the desirability of controlling the temperature and humidity in storehouses. Another effect is the tendency of salt to diffuse to the surface of dried salted fish (Young *et al.*, 1973). This causes an increase in the salt content at the surface and thus lowers the a_w at the surface below that predicted from the mean salt content of the whole fish.

The effect of fat content on sorption behaviour is known. Fat has an indirect effect as it does not contain much water but adds to the total mass of the fish (Leistner, 1976; Iglesias & Chirife, 1977). If moisture content is expressed on a wet weight basis, the sorption isotherms will vary with the fat content, but by expressing moisture and salt contents on a fat-free dry matter basis the sorption isotherms are not fat-dependent.

Unpackaged dried salted fish will absorb moisture from the atmosphere when the relative humidity of the air in contact with the fish exceeds the a_w of the fish.

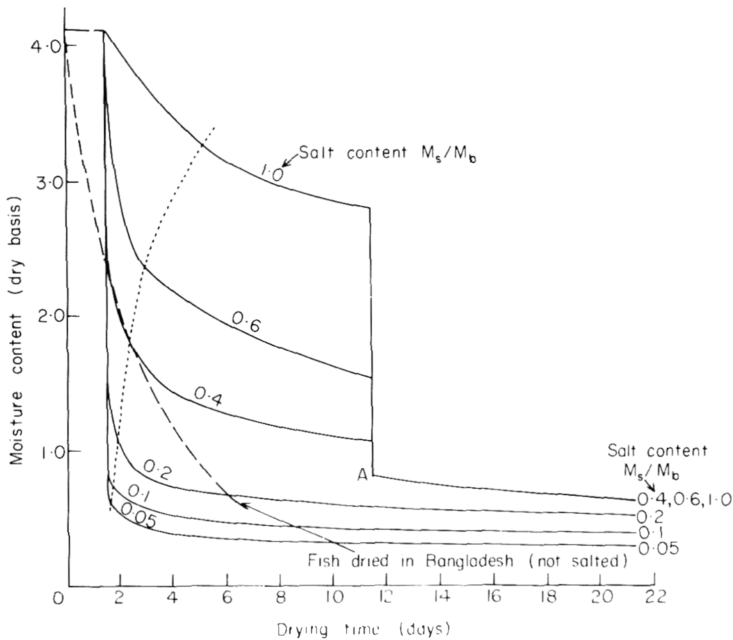

Figure 2. Curves showing maximum limits of moisture content at different times for no mould to appear during drying together with a drying curve recorded for fish in Bangladesh. .... = most likely first contact between actual and safe drying times; - - - = Fish dried in Bangladesh (not salted).

The isohalic sorption isotherm can be used to predict the storage life when adverse storage conditions cause an increase in the moisture content of the fish. For example, using the isohalic sorption isotherms for cod, presented as Fig. 2 in the previous paper (Doe *et al.*, 1982), an increase in moisture content from 20 to 30% dry basis (i.e. an increase of M_w/M_b from 0.2 to 0.3) would increase the a_w of unsalted fish from 0.69 to 0.83; fish with a salt solids content of 10% ($M_s/M_b = 0.1$) would have an a_w of 0.73. All fish with a higher salt content would have an a_w of 0.69. Similarly an increase from 30 to 40% moisture content would change the a_w from 0.73 to 0.81 in fish with 10% salt content and from 0.69 to 0.72 in fish with 20% salt content. All fish with salt contents above 25% would have an a_w of 0.74.

Safe drying and storage times for fish subject to microbial spoilage can be calculated if the response of the relevant micro-organisms to a_w is known. The red obligate halophiles (*Halobacterium* and *Halococcus*), which will grow at an a_w of 0.75, are best prevented by hygiene – the most effective disinfectant for the drying premises being fresh water (Lupin, 1978).

Conclusions

The results of storage trials of several tropical fish species are in broad agreement with storage life predictions derived by using the isohalic sorption isotherm for cod together with growth times for dun mould.

It must be acknowledged that the storage life of dried salted fish is affected by many factors that have not been taken into account in this study and probably cannot be taken into acount. Further experimental work is necessary to establish the differences between isohalic sorption isotherms of different fish species.

A method for predicting whether or not mould will develop during drying and storage is presented in the Appendix. There are no existing experimental data on which to test the accuracy of this method.

One specific conclusion that can be drawn in respect of storage life of salted fish with a moisture content in excess of 50% (dry basis) is that it is unlikely to exceed 28 days, since the a_w at this and higher moisture contents will be greater than 0.75 regardless of salt content. Hence storage life in excess of 28 days can only be achieved by reducing the moisture content below 50%.

Acknowledgments

This work was initiated at the 2nd Session of the Indo-Pacific Fisheries Council Working Party in Colombo, Sri Lanka, 1976. The authors would like to thank the Director and staff of the Institute of Fish Technology, Sri Lanka where all the storage trials were carried out and the staff of the Applied Food Science and the Food Safety and Nutritional Quality Groups of CSIRO, Division of Food Research, North Ryde for help and advice.

Appendix

The prediction of safe drying rates. The growth times in Table 3 are for constant values of a_w. During drying the moisture content and hence the a_w falls progressively. Fish dried to a particular a_w, say 0.8, over a period of 10.2 days would have been at a higher a_w for all of this period and hence would have spoiled. In order to ensure that spoilage does not occur during drying it is necessary that the integral of the a_w with respect to time must be less than the product of the a_w's and times given in Table 3, namely

$$\int_0^t a_w \, dt \leq a_w^x \, . \, t \text{ for all } t \tag{A1}$$

where a_w is the water activity of the fish during drying and a_w^x is the value of the (constant) water activity at which growth will occur after a time t as given in Table 3.

In order to find a mathematical expression for a_w^x a curve was fitted to the data in Table 3. The curve had two distinct parts

$$a_w^x = 1.0, \text{ for } 0 \leq t \leq 1.5 \tag{A2}$$

Thus there is no growth of mould for 1½ days when the water activity is 1.0.

$$a_w^x = 0.63 + 0.37 \exp[-0.381(t-1.5)^{1/3}], \text{ for } t \geq 1{\cdot}5 \qquad \text{(A3)}$$

Besides being a good fit to the data of Table 3, this curve has a number of features which tally with the known behaviour of mould growth. The curve provides that there is no mould growth at $a_w < 0.63$; there is an incubation period of 1.5 days at an a_w of 1.0 before moulds are visible.

The formula for a_w^x given by equation A3 with $t > 1.5$ enables a functional form of a_w to be found by differentiating equation A1, thus

$$a_w \leq \frac{d}{dt}(a_w^x\, t) \qquad \text{(A4)}$$

giving

$$a_w \leq a_w^x - 0.047(t-1.5)^{1/3} \exp[-0.381(t-1.5)^{1/3}] \qquad \text{(A5)}$$

By applying the isohalic sorption isotherms for cod (Doe *et al.*, 1982) to the a_w time relation of equation A5, the series of curves of moisture content against time for different salt contents, shown in Fig. 2, were developed. The significance of these curves is that they represent for each salt content the maximum drying times before mould growth is visible. Fish dried so that its drying curve (moisture content against time) intersects the particular curve in Fig. 2 corresponding to the salt content of the fish will spoil through mould growth.

Point A on Fig. 2 represents the point on the isohalic sorption isotherms where the a_w is 0.75. Fish which is not dried to this point (moisture content M_w/M_b = 0.8) within 11.5 days will spoil through mould growth; an increase in the salt content will not prevent this spoilage as point A is common to all the curves of salt content above M_s/M_b = 0.4. Thus there is nothing to be gained by increasing the salt content of the fish if the drying rate is too slow.

Plotted on Fig. 2 is a drying curve for a large fish (Golden Conger Eel, *Muraenesox talabonoides*) dried by the traditional sun-drying method in Bangladesh (Doe *et al.*, 1977). It can be seen that the drying curve just intersects the safe drying curve for a salt content M_s/M_b = 0.4, indicating that the fish would need this salt content to prevent mould growth during drying. Also shown in Fig. 2 are the critical points (joined by a dotted line) on the theoretical safe drying curve for each salt content. These are the points where under reasonably constant drying conditions a typical drying curve for fish with the given salt content would be likely to just touch but not intersect the safe drying curve; the fish would therefore just be protected from mould growth during the whole of the drying period. The moisture contents and drying times given by these critical points can thus be used as a rough guide to determine the drying rates necessary to avoid mould growth. For example, if a fish with a salt content M_s/M_b = 0.4 is dried at a rate such that the moisture content falls to M_w/M_b = 1.7 (63% moisture content wet basis) or below within 2½ days (i.e. the critical point on the safe drying curve) it is unlikely that mould will occur during the drying

Table 4. Rough guide to drying times necessary to prevent mould growth during drying in cod dried under reasonably constant conditions

Salt content (M_s/M_b)	Time (days)	Moisture content(%) Dry basis (%)	Moisture content(%) Wet basis (%)
1.0	5.3	3.25	77
0.8	4	2.85	74
0.6	3	2.4	71
0.4	2.5	1.8	64
0.2	2	1.05	51
0.1	1.8	0.7	41
0.05 (unbrined)	1.7	0.6	38

period. The corresponding times and moisture contents for other salt contents are given in Table 4.

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The application of technology to the processing of dry-salted fish in peninsular Malaysia: comparison of sun-dried and oven-dried fish

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Summary

Investigation of processing of dry-salted fish is described. The use of a satisfactory ratio of salt to fish, optimization of salting time and the use of mechanical driers to reduce drying time, insect infestation and microbial spoilage were investigated. Results indicated that oven-dried fish salted at 10% and 20% levels were most acceptable. The corresponding sun-dried samples had an undesirable odour, flavour and texture due to their higher moisture contents. At the 30% and 40% salt levels for both processes, the differences were less pronounced, but the samples that were sun-dried were more acceptable mainly as a result of a better appearance.

Introduction

The technique of salting and drying fish has been used for a long time in Malaysia. Fish provides approximately 49% of the total animal protein consumed and 12% of the total protein intake. About 5–10% of the total catch landed in the 1970s were salted and dried. On a finished product basis this amounts to 77% of the total edible output of processed fish (Abdullah & Idrus, 1978). Dry-salted fish forms a relatively cheap source of high-quality protein especially for those residing in the rural areas.

The techniques involved in salting and drying in Malaysia are rather simple. Except for the concrete vats for salting and the bamboo or wooden platforms for sun drying, no other equipment is used. The fish are usually washed before salting and for big fish like red snapper (*Lutianus* spp.) and cat fish (*Tachysurus*

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Kanagurta), the gills and gut contents are removed, whilst smaller fish such as herring and scad are not eviscerated. After evisceration, the fish may or may not be split along the dorsal line. The eviscerated fish is usually not cleaned before salting. The amount of salt used and the salting and drying times depend on the size of the fish. (Siaw & Yu, 1978).

Salting is carried out in concrete vats by arranging the fish in alternate layers with coarse rock salt. In order to prevent the fish from floating, weights are placed on the surface. The vats are housed within sheds to avoid excessive sun and rain and the length of salting times ranges from 1 to 5 days. When salting is complete the fish are removed and washed in the sea or brackish water to remove adhering salt. Sometimes the fish are soaked for 10–30 min to remove excess salt.

Sun-drying is the common practice and no mechanical driers or any other forms of accelerated drying methods are used. The fish are spread out on bamboo platforms or on the ground and are arranged so as to facilitate drying. Bigger fish are split open and flattened out and may be hung by the tail to dry for a day before being transferred to the drying platforms. In some areas along the east coast, pepper is rubbed into the gut cavity and cut surface of the fish to prevent spoilage. This procedure is only carried out for fish that will fetch a good price. On the west coast, powdered alum is used instead. Insect infestation becomes more serious in damp weather.

During drying the fish are turned over once or twice daily. Processors detect the stage of drying usually by pressing the fish with the hand, by judging the colour and appearance of the skin and by the condition of the eye. If the eye is clear and not watery, then the fish is considered sufficiently dried. In the last stage of processing, the dried fish are packed into cardboard boxes which may be lined with polythene sheets before dispatch to the wholesaler. Storage conditions are poor and no attempts are made to maintain the quality of the fish during transit.

The lack of technology associated with the production of dry-salted fish in Malaysia means that the products are very often of poor quality. In addition the poor quality of the salt used often imparts a rough, whitish crust to the surface of the fish, undesirable brown discolouration and gives rise to unpleasant bitter flavours and tough texture (F.A.O. Fisheries Circular 336, 1976). Bacteria which contribute to spoilage in dry-salted fish cannot survive below a water activity (a_w) of 0.75 (Beatty & Fougere, 1957). Malaysian dry-salted fish have 0.75–0.86 a_w (Siaw & Yu, 1978). In a hot, damp tropical climate (average temperature 26–28°C, with a relative humidity reaching 96–97%) dry-salted fish are highly susceptible to spoilage by either micro-organisms or infestation by flies. During the drying stage, no precautions are taken to prevent insect infestation so that flies lay eggs and subsequently the larvae tunnel into the flesh causing putrefaction and extensive damage.

Failure to recognize that poor fish make poor products of any kind is a common error among fish processors in Malaysia. Usually, fish considered to be unsuitable for distribution as fresh fish are diverted to the salting and drying process.

This study has been undertaken in an attempt to upgrade the quality of dry-salted fish produced in Malaysia and in particular to determine a satisfactory ratio of salt to fish used, to optimize salting time and to make use of mechanical driers in order to reduce drying time, insect infestation and spoilage due to moulds and bacteria.

Materials and methods

Raw materials

The species *Johnius soldado,* locally known as 'gelama', was used, as it is the most commonly available dry-salted fish. The fish (average thickness 4 cm, average weight 60–65 gm) were purchased fresh from the market and used for salting on the same day. Fine table salt was used.

Chemical analysis

Only the fish fillet which had been finely ground was used for analysis. Crude protein (NX6.25) was determined by the Kjeldahl method (Pearson, 1970). Crude fat was measured by the Soxhlet method using petroleum ether and sodium chloride (chloride ions) by the precipitation method (Pearson, 1970). Salt analysis was carried out using the modified method of Kam, Bauntlett and Smith (1964). Moisture content was determined using the oven method of Pearson (1970).

Salting

The fish were descaled, eviscerated and washed in clean water. They were then placed in alternate layers with salt in a salting vat (61 cm × 41 cm × 15 cm) and then covered with a plastic sheet and stored at room temperature (24°C). Salt ratio's of 10, 20, 30 and 40% (w/w) were used. At predetermined intervals samples were taken for analysis of salt and moisture.

Drying

For sun-drying, the fishes were dried to constant weight by suspending by the tails from hooks inside a drying chamber, consisting of racks supported by a metal frame covered with wire mesh. The fish were kept at a distance 15 cm apart in a staggered formation to ensure efficient circulation of air. The average temperature of the atmosphere during the drying period was 26.7°C and the relative humidity was 95.9%. Average wind speed was 1 m/sec. The oven-dried fishes were dried in a forced-air cabinet drier (Apex, U.K.) at 45°C using an air speed of 2.5 m/sec. The fish were placed on their sides on wire mesh and were turned over frequently for more uniform drying. The dried fishes were kept in sealed polyethylene bags until ready for sensory evaluation.

Sensory evaluation

A total of thirteen experienced panelists were chosen as assessors. The fish were cut into cubes (1.5 cm × 1.5 cm × 1 cm) and fried in vegetable oil at 200°C for 2–3 min before tasting. The panel was asked to assess the appearance, texture, flavour, presence of undesirable odour and overall acceptability of the samples using a hedonic rating scale of a maximum of five points for a favourable response. All samples were identified only by random numbers. The results were analysed using the Least Significant Difference method.

Results

Salting

Fig. 1 shows that samples with lower salt content (10 and 20%) took 24 hr to reach a constant salt level. Those in the 30 and 40% salt ranges took over 36 hr. The results also indicate that the maximum level of salt is attained in samples where 30% salt was used, and that 30 and 40% treatments result in equal salt levels in the product. Moisture losses were highest for the 30 and 40% salt samples (Fig. 2). All samples showed increasing moisture loss until a constant level was reached.

Drying

One of the problems encountered during sun-drying was the fluctuation in weather conditions. Generally, the sun-dried samples took much longer to dry, taking almost 40 hr of sunshine to reach a constant moisture level (Figs 3 and 4). On an average day only 5.75 hr of sunshine was available for drying. The average temperature during the drying period was 26.7°C. By using a forced-air

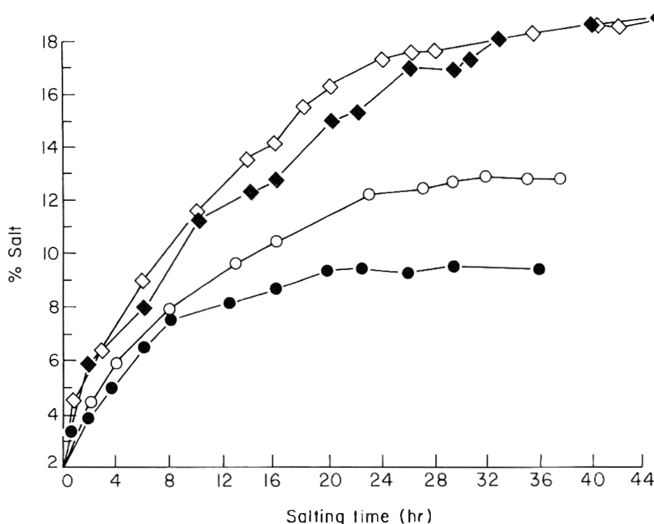

Figure 1. Effect of different salt levels and salting time on the salt content of *Johnius P. Soldade.* ◆ , 40% salt; ◇, 30% salt; O, 20% salt; ●, 10% salt.

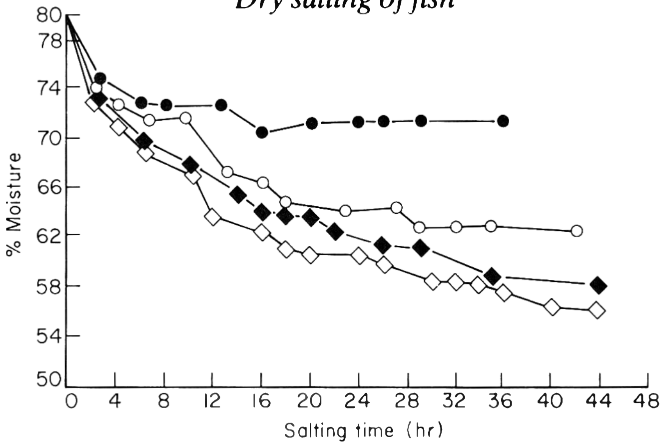

Figure 2. Effect of different salt levels and salting time on the percentage moisture of *Johnius P. Soldade.* ◇, 40% salt; ◆, 30% salt; O, 20% salt; ●, 10% salt.

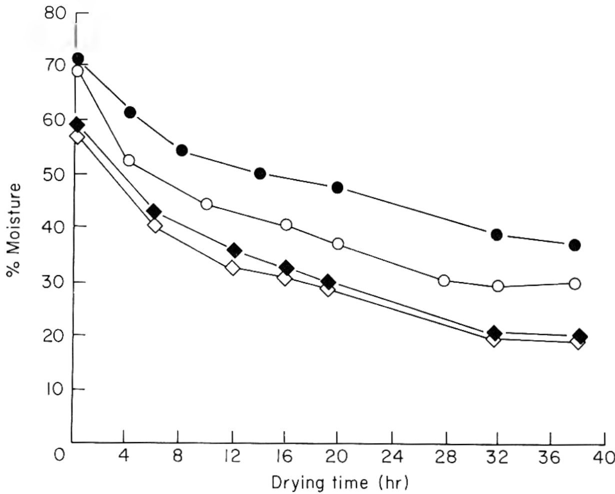

Figure 3. Effect of sun-drying at different salt levels on the moisture content of *Johnius P. Soldade.* ◇, 40% salt; ◆, 30% salt, O, 20% salt; ●, 10% salt.

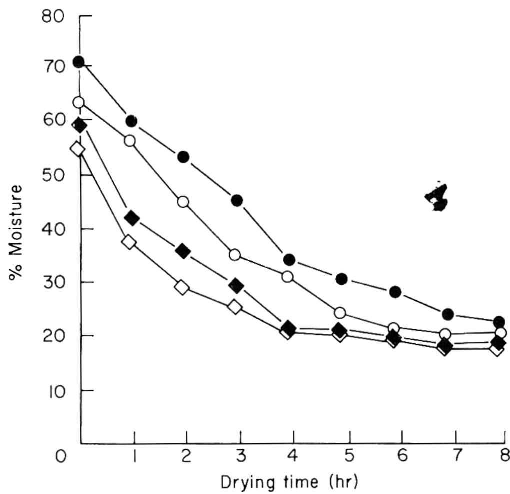

Figure 4. Effect of oven-drying at different salt levels on the moisture content of *Johnius P. Soldade.* ◇, 40% salt; ◆, 30% salt; O, 20% salt; ●, 10% salt.

dehydrator, the moisture content in the fish could be reduced to 18–21% in 8 hr (Fig. 4).

Sensory evaluation

From Table 1, the 20% salted, oven-dried sample was judged to be the most acceptable, followed by the 10% salted, oven-dried samples. Of the oven-dried samples the two containing greater than 20% salt were less acceptable. These two samples (30 and 40% salt treated) had total scores which were significantly different compared to the 20% salted sample (Table 2). Table 1 also shows that

Table 1. Statistical analysis of the scores by Friedman's two way analysis of variance by rank

Salt content (%)	Appearance	Texture	Flavour	Undesirable odour	Acceptability	Total score
Oven-dried						
10	42	38	36	50	44	210
20	44	46	47	48	47	232
30	36	36	34	48	36	190
40	31	33	32	45	32	173
Sun-dried						
10	34	28	29	31	29	151
20	36	24	33	34	39	166
30	44	33	41	45	43	206
40	40	33	34	46	37	190
χ_r^2	14.113*	14.182*	14.244*	19.192**	17.647*	19.637**

*P = 5%
**P = 1%

Table 2. Inter-sample comparison of total scores by the least square significant difference (LSD) method

Salt content (%)	Sun-dried 10	Sun-dried 20	Sun-dried 30	Sun-dried 40	Oven-dried 10	Oven-dried 20	Oven-dried 30	Oven-dried 40
Sun-dried								
10	0	1.15	4.32†	3.0	4.53†	6.23†	3.0*	1.69
20		0	3.08†	1.85	3.38*	5.08†	1.85	0.54
30			0	1.23	0.3	2.0	1.23	2.54
40				0	1.53	3.23*	0	1.31
Oven-dried								
10					0	1.7	1.53	2.84
20						0	3.23*	4.84
30							0	1.31
40								0

†LSD at 1% level = 3.647
*LSD at 5% level = 2.755

of the sun-dried samples the 30% salted fish was the most acceptable, followed by the 40% salted sample. Samples salted at 10 and 20% were the least acceptable. On a salt basis, samples salted at the same salt level contained similar levels of salt irrespective of the method of drying (Table 3). However, their moisture contents varied widely. The oven-dried fishes were more consistent as regards moisture content, whereas sun-dried samples generally had higher moisture contents. This is especially evident for the two samples with lower salt contents. Texture scores were also lowest for these two samples.

Table 3. Moisture and salt contents of dry-salted fish

Salt level (%)	Moisture content (%)	Salt content (%) of fish
Oven-dried		
10	20.95	10.52
20	20.00	12.02
30	18.00	20.59
40	19.50	20.69
Sun-dried		
10	38.00	9.70
20	29.00	12.60
30	24.00	19.20
40	24.00	19.80

Discussion

Oven-dried samples salted at 10 and 20% were the most popular (Table 1). Those oven-dried at higher salt concentrations were less acceptable mainly due to the salt level causing an unattractive appearance and undesirable texture and flavour. However, variation in salt content of dry-salted fish can be accepted provided that moisture levels are kept low. This is exemplified by the sun-dried samples at the 10 and 20% salt levels, where, although the salt levels are low, moisture levels are high compared to the corresponding oven-dried samples (Table 3). The high moisture levels in these two sun-dried samples favour the development of undesirable characteristics such as bad odour and flavour both of which are reflected in the scores for the samples (Table 1). In comparison, sun-dried samples with high salt content did not have bad odour or flavour due to inhibition of microbial growth. The texture of the 10 and 20% sun-dried fish was also adversely affected by their higher moisture contents.

At the 30 and 40% salt level for both processes, samples that were sun-dried were more acceptable (Table 3) mainly as a result of better appearance and a lesser dehydrating effect since the sun-dried samples contained more moisture.

The high relative humidity in Malaysia (average of 95.9% during the experimental period) does not allow natural drying to proceed to a satisfactory low moisture level. In addition, the average temperature during the drying period was only 26.7°C and the average number of hours of sunshine per day was 5.75. All these factors lengthen the drying time considerably, so that spoilage and insect infestation becomes common. All the sun-dried samples have a higher final moisture content that the oven-dried samples. The samples with lower salt contents contain more moisture as the presence of a higher concentration of salt in the 30 and 40% salted samples aids in increased water removal.

In Malaysia, the frequent rain and varied, humid climate limit application of the conventional sun-drying method as the product is subject to the vagaries of the weather. Dependence on sun-drying may result in excessive spoilage as the time required for drying is too long. The results show that the use of controlled environment would be desirable to process for a uniform dry-salted product.

Attempts to improve quality could begin with the use of better quality salt and more controlled salting concentrations and times. Naturally any form of a mechanized drying would involve higher initial investment costs. It is suggested that fairly simple pieces of equipment be employed to make the product better and therefore more marketable. For example, fairly cheap tunnel dryers have been used successfully in some tropical countries (Waterman, 1976).

Although the sun and the wind are not easily controllable, the source of energy is undeniably cheap. As such current research has been geared towards more efficient utilization of solar energy, it is not possible presently to predict costs of using solar driers and the possibilities of the simplest types should be examined instead of elaborate istallations.

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Effects of water blanching on pea seeds

II. Changes in vitamin C content

J. D. SELMAN* AND E. J. ROLFE

Summary

Laboratory scale experiments were conducted to elucidate the main mechanisms responsible for changes in the proportions of ascorbic acid (AA) and dehydroascorbic acid(DHA) in peas during water blanching. Studies utilized hand-harvested peas from specially grown Dark Skinned Perfection (DSP), Swan and Swift cultivars. The influence of pea size/maturity, blanch time and damage to the pea were studied over the temperature range 35–97°C.

With increasing DSP pea size/maturity, the proportion of AA oxidized increased, and the proportion of AA leached into the water decreased when blanching between 45 and 65°C. Maximum AA oxidation occurred at 60°C and leaching became the prime mode of loss above 70°C. Leaching of AA from DSP peas increased almost linearly from 40 to 97°C.

Damaging peas by bruising and slitting the testa. induced enhanced AA oxidation below 60°C and allowed immediate leaching of vitamin C largely as DHA even at the lowest blanch temperatures. Results suggested that the oxygen content of the tissues was a factor limiting the amount of AA oxidation. Cultivars Swift and Swan contained higher proportions of DHA particularly in the testa tissues, and calculations indicated that greater proportions of vitamin C were leached as DHA. A negligible proportion of AA was oxidized and some 28% of the initial AA was leached into the water when undamaged DSP peas were blanched at 97°C for 1 min. Bruised plus slit peas lost significantly more AA than undamaged peas when blanched at 97°C. Further evidence indicated that the micropyle serves as a major pathway for leaching losses.

Introduction

Several workers have shown that a more complete picture of the effects of cooking and storage of various vegetables may be obtained by studying both the

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reduced and oxidized forms of vitamin C and its degradation product 2,3-diketogulonic acid (DKA), e.g. Dietrich *et al.* (1957). Peas contain relatively large amounts of vitamin C, but few of the studies of the effects of preservation operations on the content of vitamin C have included both forms of the vitamin (Lee, 1958; Lynch, Mitchell & Casimer, 1959) and many results are unsatisfactory in various respects (Selman, 1978).

This work investigates some of the effects of blanching peas in water under carefully controlled conditions on the net changes in the ascorbic acid (AA) and dehydroascorbic acid (DHA) contents, and also the effect of simulated harvesting damage to the pea on those changes. This paper follows related studies on the variation of the vitamin C content of peas during maturation (Selman & Rolfe, 1979a) and the effects of blanching on the losses of water and solutes from peas of the DSP cultivar (Selman & Rolfe, 1979b).

Materials and methods

Plant material

Hand-harvested samples of peas were carefully selected from specially grown wrinkled-seeded varieties of Dark Skinned Perfection (DSP), Swan and Swift peas in 1974 as described previously (Selman & Rolfe, 1979a). The peas were graded into three sizes, to give a selection of maturity, according to the smallest diameter: (1) undersize, less than 8.75 mm, (2) standard size 8.75–10.20 mm (optimum maturity for freezing) and (3) oversize, greater than 10.20 mm. The graded peas were divided into samples of fixed number (40) and weight of peas (*c.* 20 g). Data for the mean pea weight, percentage dry solids, percentage alcohol insoluble solids and initial total vitamin C content (AA+DHA) for the grades and cultivars are shown in Table 1.

Table 1. Data for mean pea weight, percentage dry solids content, maturity index and initial total vitamin C content of the pea sizes and cultivars studied.

Pea sample	Mean pea weight (g)	Dry solids (%) ($\sigma = 0.41$)	Maturity index: Alcohol insoluble solids (%) ($\sigma = 0.22$)	Maturity index: Equivalent Tenderometer reading	Initial total Vitamin C content (mg/100 g fresh weight) ($\sigma = 1.9$)
DSP standard size*	0.50	21.3	11.1	104	35.6
DSP undersize	0.31	19.1	8.7	83	45.8
DSP oversize	0.69	25.7	17.7	157	21.0
Swan standard size	0.41	21.9	12.9	—	42.8
Swift standard size	0.38	21.9	11.9	—	42.9

*DSP – Dark Skinned Perfection.
σ = standard deviation of 10 replicate samples.

Analytical procedures

The methods used to determine the dry solids content, maturity index and total vitamin C content of whole and separated peas, together with typical intersample variations, have been described previously (Selman & Rolfe, 1979a). The vitamin C content of the blanch water was estimated using 25 ml aliquots of the prepared blanch water solution. The initial contents of AA and DHA in the whole pea were determined before blanching. After blanching, the residual AA and DHA in the pea, and the AA and DHA then present in the blanch water were determined. The resulting DKA residing in the pea plus blanch water was calculated by difference, i.e. no estimate was made of the proportions of DHA hydrolyzed to DKA in the pea tissues and blanch water.

Blanching

The standard method described by Selman and Rolfe (1979b) was used. Again, a post-blanch cooling operation was excluded. After the required blanch time, the peas were sieved out over a funnel and the blanch water collected in an ice-cooled 100 ml volumetric flask. This solution was made up to the mark with ice-cooled 3% metaphosphoric-8% acetic acid solution to stabilize the vitamin C prior to estimation. The peas were cooled directly in 50 ml of this metaphosphoric-acetic acid solution ready for vitamin C extraction.

A summary of the apparent net changes in the AA and DHA contents in the peas and blanch water as a result of blanching is shown in Fig. 1.

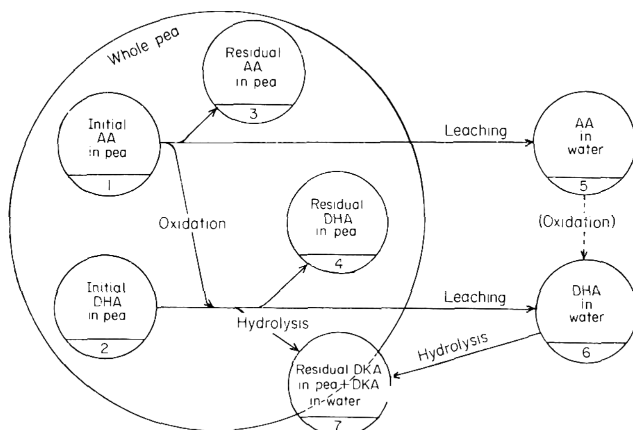

Figure 1. Net changes in the ascorbic acid (AA) and dehydroascorbic acid (DHA) contents of whole peas after blanching in water. Basic reaction: $\text{AA} \overset{\text{oxidation}}{\rightleftharpoons} \text{DHA} \overset{\text{hydrolysis}}{\longrightarrow}$ 2,3-diketogulonic acid (DKA) and further breakdown products. Where initial total vitamin C (AA+DHA) in pea is taken to be 100%, then initial total vitamin C in pea before blanching $1+2 \equiv 3+4+5+6+7$ in pea and water after blanching. The proportion of DHA hydrolyzed to DKA (and further breakdown products) was calculated thus: $7 \equiv (1+2)-(1+4+5+6)$ and the proportion of AA oxidized to DHA was similarly calculated thus: $1-(3+5) \equiv (4+6+7)-2$. Where appropriate these numbers are indicated on subsequent figures.

Harvesting damage

Three sorts of damage were chosen. Standard size DSP pea samples were damaged to produce slit, bruised or bruised plus slit peas as described previously (Selman & Rolfe, 1979b).

Experiments and results

Temperature of blanching

Samples of standard size peas were blanched at different temperatures for 10 min and the resulting AA contents of the peas and blanch water were determined. A uniform time of 10 min was chosen to induce measurable changes especially at the lower temperatures. The AA oxidized was calculated by difference and the results for DSP peas harvested in 1974 are shown in Fig. 2. This pattern was very similar to that exhibited by DSP peas harvested in 1973, but then the maximum oxidation occurred when blanching at 50°C (Birch *et al.*, 1974). The rate of heat transfer into the pea will be critical as there is always a finite time-lag, during which the temperature at the centre of the pea reaches that of the blanching medium. Assuming the rate of heat transfer was similar in all cases, the difference between the temperatures of maximum AA oxidation in 1973 and 1974 peas may have been due in part to the greater concentration of AA in the cotyledons of 1974 peas, particularly as the rate of temperature rise would have been slowest in the central cotyledon tissues of the pea (Selman &

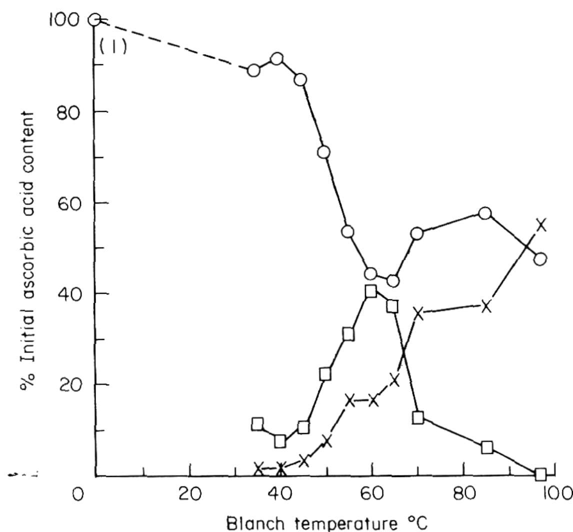

Figure 2. Standard size DSP peas. Proportions of ascorbic acid (AA) expressed as a percentage of the initial ascorbic acid content, remaining in the pea, oxidized, and lost into the water after 10 min at the given blanch temperature. (Means of two replicate samples); O, AA in pea (3); □, AA oxidized 1 − (3+5); ×, AA in water (5).

Rolfe, 1979a). In both cases the temperature of maximum AA oxidation was higher than the optimum temperature of 40°C observed for the extracted AA oxidase enzyme (Birch *et al.*, 1974), and suggested that some factor other than temperature was limiting the rate of AA oxidation.

The oxidation of AA requires the presence of oxygen and the access of the oxidase enzymes to the AA in the cells. The effect of raising temperature will be to promote initially the activity of the enzymes up to an optimum, followed by denaturation of the enzymes. Also the cell membranes will be disorganized and both oxygen dissolved in solution together with intercellular oxygen will tend to be driven out of the tissues. As at least some AA oxidase is known to be present in or associated with the cell wall, the extent of tissue disorganization will be an important influencing factor.

Membrane disorganization occurs at around 45–50°C, and the temperature of maximum AA oxidation occurs above this at around 55–65°C. Leaching of AA increased almost linearly with time over the temperature range 45–97°C, although between 55–65°C there is some indication that AA oxidation is occurring so rapidly that less AA is available to be leached. Negligible losses of AA were recorded below 40°C where cells were still intact.

The amount of AA oxidized was considerably less than would have been expected from the activity of the extracted AA oxidase enzyme. It was calculated approximately that the activity of the AA oxidase in a pea macerate was about fifty to sixty times greater than the activity observed in the whole pea. Other workers have found that the full potential of many oxidase enzymes is not realized *in vivo* (Mapson, 1958). Such a phenomenon may be due to the spatial separation of the enzyme and substrate, and the presence of substances modifying the action of enzymes such as hydrogen peroxide. The slow diffusion of gases through the pea testa may well influence the activity of oxidase enzymes by restricting the availability of oxygen in the tissues.

(1) Effect of pea size and maturity. Samples of the three defined size grades of DSP peas were blanched for 10 min at temperatures between 35–70°C. This temperature range was chosen because the main changes influencing the proportions and locations of the AA, DHA and DKA appeared to occur below 70°C; for example AA oxidase is largely inactivated at temperatures above 70°C (Birch *et al.*, 1974). The AA and DHA contents of the peas and blanch water were determined after blanching. The DHA hydrolyzed to DKA was calculated by difference. Results for standard size, undersize and oversize peas are shown in Figs 3, 4 and 5 respectively.

Standard size. Figure 3 shows that blanching up to 45°C resulted only in small losses of vitamin C. From 45–65°C the vitamin C content fell continuously. AA was lost by both oxidation and leaching, oxidation being the major means up to 65°C. At higher temperatures resulting in more rapid enzyme inactivation and cell disorganization, leaching of AA became the main mode of loss. Subject to experimental error, the pattern of changes indicate that AA was oxidized to DHA within the tissues at a rate that was higher than the rate of leaching in the range 45–65°C. Above 65°C, the oxidase enzymes were inactivated so that more

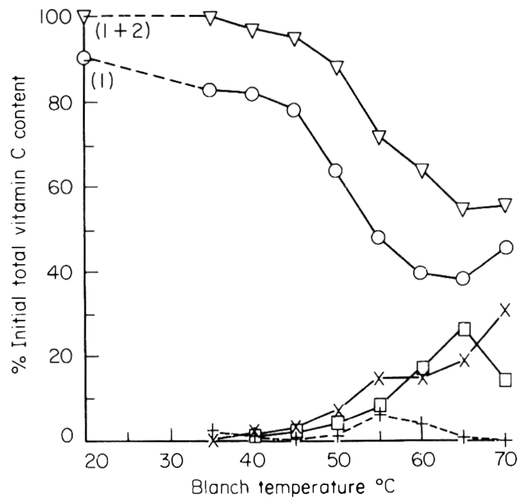

Figure 3. Standard size DSP peas. Proportions of ascorbic acid (AA) and dehydroascorbic acid (DHA) expressed as a percentage of the initial total vitamin C content, remaining in the pea, lost into the water and the DHA hydrolyzed, after 10 min at the given blanch temperature. (Means of two replicate samples); ▽, total vitamin C in pea (3+4); O, AA in pea (3); □, DHA hydrolyzed (7); ×, AA in water (5); +, DHA in water (6).

AA was available to be leached out, the rate of leaching also increasing at higher temperatures.

Only small amounts of DHA were detected in the blanch water in the temperature range 50–60°C. The net content of DHA remaining in the pea was approximately constant between 45–60°C, and was influenced by the rate of AA oxidation, the rate of thermal destruction of DHA and the rate of leaching of DHA. The thermal half-life of DHA at pH 6 has been shown to decrease from 35 min at 40°C to 2 min at 70°C (Huelin, 1949).

Undersize. To simplify discussion, comparison of the effects arising during blanching of the various categories of peas is made at 45°C, before full disorganization of the cell membranes, and at 65°C before full inactivation of AA oxidase. From Table 1 it is seen that, per unit weight, undersize peas contained more vitamin C than standard size peas. It follows from Fig. 4 that although greater amounts of AA were oxidized in the undersize peas, the percentage oxidized was similar to that of standard size peas at 45 and 65°C being about 11 and 31% of the initial total vitamin C content respectively.

Loss of AA into the water increased more rapidly with increasing temperature when compared to the loss from standard size peas. At 45°C the losses were 3 and 5% respectively and at 65°C, 19 and 26% from the standard size and undersize peas respectively. The DHA content of undersize peas was initially some 7% higher than that of standard size peas, and at 45°C this differential was maintained being 17 and 25% respectively. At 65°C, the proportion of vitamin C

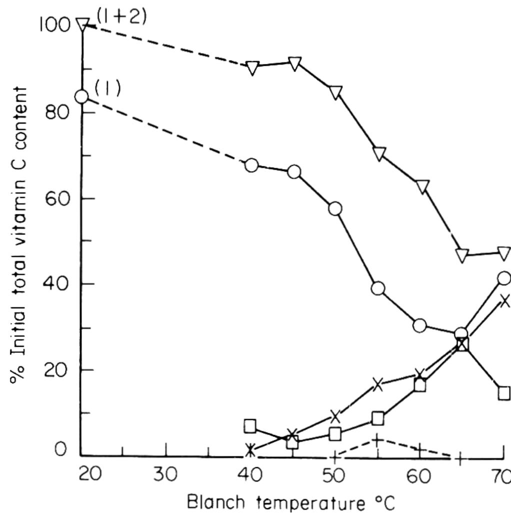

Figure 4. Undersize DSP peas. Proportions of ascorbic acid (AA) and dehydroascorbic acid (DHA) expressed as a percentage of the initial total vitamin C content, remaining in the pea, lost into the water and the DHA hydrolyzed, after 10 min at the given blanch temperature. (Means of two replicate samples); symbols as in Fig. 3.

remaining as DHA was about 16 and 19% respectively, with similar proportions of DHA hydrolyzed (26% in both) as would be expected from the thermal half-life characteristics of DHA.

The more rapid loss of AA from the undersize peas into the water may have been influenced largely by the higher concentrations of AA in the testa, a greater testa/cotyledon ratio and greater surface/volume ratio in the undersize peas. The cell walls may be more permeable to solutes in these botanically less mature peas, the smaller pea diameter also allowing shorter diffusion paths for both heat transfer and leaching of solutes. Thus it might be expected, where a relatively greater proportion of the tissues were disorganized in a given time, that the rate of leaching and hence the amount of AA leached would be greater. Similarly there might be greater AA oxidase activity initially followed by the inactivation of a relatively greater proportion of the AA oxidase present, resulting in a more limited amount of oxidation in total.

Only small quantities of DHA were detected in the water of both standard and undersize peas. This would be expected as the greater proportion of DHA was located in the testa tissue (Selman & Rolfe, 1979a) where the rate of temperature rise would be greatest.

Oversize. Figure 5 indicates some notable differences in the pattern of changes observed with the other pea sizes. At 45°C only 1% of the initial vitamin C remained in the water as AA compared to 3 and 5% for standard and undersize peas respectively. At 65°C only 7% remained in the water as AA compared to 19 and 26% for standard and undersize peas respectively. This smaller leaching loss may have arisen from the smaller testa/cotyledon ratio,

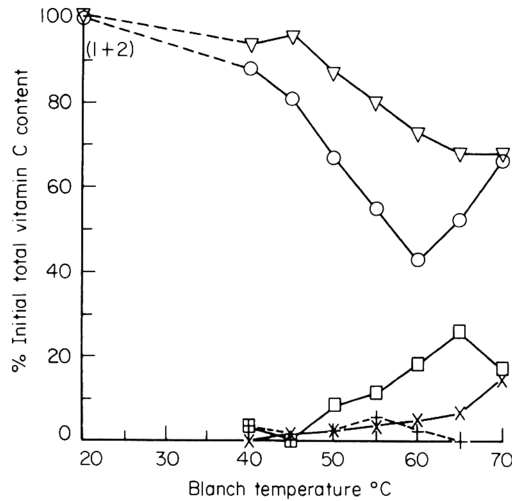

Figure 5. Oversize DSP peas. Proportions of ascorbic acid (AA) and dehydroascorbic acid (DHA) expressed as a percentage of the initial total vitamin C content, remaining in the pea, lost into the water and the DHA hydrolyzed, after 10 min at the given blanch temperature. (Means of two replicate samples): symbols as in Fig. 3.

lower vitamin C concentration and the less permeable cell walls of these botanically more mature peas. However it appears that the slower rate of temperature rise at the centre of these larger, more dense peas may have promoted more AA oxidation. At this maturity, significant quantities of starch are present in the cotyledons, and although pea starch contains a low proportion of amylopectin, some swelling does occur during gelatinization. It is possible that the imbibition and removal of water from the cells may limit the movement of solutes in the tissues and further reduce solute losses from the undamaged peas. Thus more AA would remain in the tissues and therefore be available to be oxidized. At 45°C, 22% of the initial total vitamin C content had been oxidized, compared to 10 and 12% for the standard and undersize peas. At 65°C, 45% of the initial total vitamin C had been oxidized, compared to 33 and 29% for standard and undersize peas respectively.

Table 1 indicates that, per unit weight, the total vitamin C content of the oversize peas was less than either that of the standard or undersize peas. It has previously been shown that the total vitamin C content of peas decreased with maturity and that this arises largely from the decrease of DHA in the testa tissues (Selman & Rolfe, 1979a). During blanching, the amount of DHA present in the oversize peas increased from a negligible value up to a maximum of 30% at 60°C, then decreased to a negligible value again at 70°C. In all three sizes of peas, relatively small amounts of DHA were detected in the blanch water. Maximum presence of DHA in the water was seen at 55°C in all three cases. Above 65°C, no DHA was detectable due to the enhanced rate of hydrolysis to DKA at these temperatures.

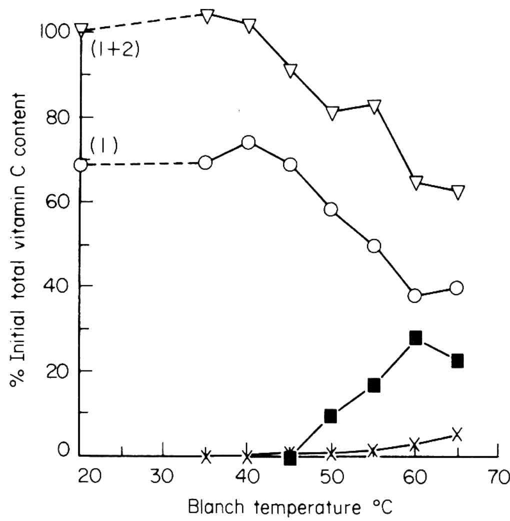

Figure 6. Standard-size Swan peas. Proportions of ascorbic acid (AA) expressed as a percentage of the initial total vitamin C content, remaining in the pea, lost into the water and oxidized, after 10 min at the given blanch temperature. (Means of two replicate samples); ▽, total vitamin C in pea (3+4); O, AA in pea (3); ■, AA oxidized 1−(3+5); ×, AA in water (5).

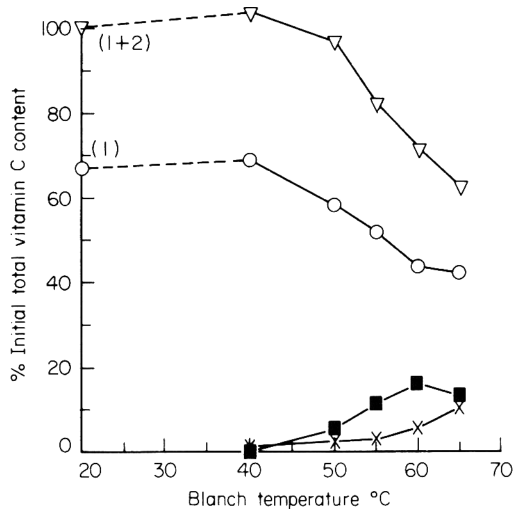

Figure 7. Standard-size Swift peas. Proportions of ascorbic acid (AA) expressed as a percentage of the initial total vitamin C content, remaining in the pea, lost into the water and oxidized, after 10 min at the given blanch temperature. (Means of two replicate samples); symbols as in Fig. 6.

(2) Effect of pea cultivar. Similar experiments were conducted using standard size samples of cultivars Swan and Swift (Table 1). Samples of these peas were blanched for 10 min at temperatures between 35 and 65°C. The AA and DHA contents of the peas and the AA content of the blanch water were determined after blanching. The AA oxidized was calculated by difference and the results for Swan and Swift are shown in Figs 6 and 7 respectively.

The initial DHA contents of both Swan and Swift peas were considerably higher than that of DSP peas, particularly in the testa tissues (Selman & Rolfe, 1979a). Although the general pattern of changes in vitamin C components was essentially similar to those observed in DSP peas, relatively small amounts of AA were lost into the water over the whole temperature range. At 45°C, almost negligible leaching of AA occurred from both Swift and Swan peas. At 65°C, 5 and 11% of the initial total vitamin C remained in the water as AA in the cases of Swan and Swift respectively; 23 and 13% had been oxidized respectively.

The proportion of vitamin C leached into the water as DHA was not measured in the cases of Swift and Swan peas due to time constraints, but an estimate of this amount was calculated from the data obtained for DSP peas in the following way. The proportion of DHA hydrolyzed at each blanch temperature had been calculated for DSP peas as indicated in Figs 3, 4 and 5 (for method see Fig. 1), and from the thermal half-life characteristics of DHA it was assumed that similar proportions of DHA would have been hydrolyzed at each temperature in both Swift and Swan peas. As an example, for standard size, undersize and oversize DSP peas blanched at 65°C the proportions of DHA hydrolyzed were 25.9, 26.6 and 26.2% respectively, giving an average of 26.2%. It was then assumed that 26.2% DHA had also been hydrolyzed in both Swift and Swan peas at 65°C, and thus the proportion of vitamin C leached into the water as DHA could be calculated by difference (for method see Fig. 1).

Such calculations suggested a negligible loss of vitamin C as DHA by leaching into the water from Swift peas at 45 and 65°C, this being similar to the results for DSP peas. For Swan peas, the calculations suggested a 5% loss of vitamin C as DHA into the water at 45 and 65°C, with a maximum of 14% at 60°C. Such enhanced losses relative to Swift and DSP peas may have been due to the higher concentration of DHA present in the testa of Swan peas (Selman & Rolfe, 1979a).

(3) Effect of damage. Experiments were conducted to show how the observed net changes in vitamin C compounds differed when peas were first damaged and then blanched. Samples of standard size DSP peas were damaged to produce samples of bruised and bruised plus slit peas. These were blanched for 10 min between 35 and 65°C and the AA and DHA contents of the peas and blanch water were determined after blanching. The DHA hydrolyzed was calculated by difference. Results for bruised and bruised plus slit peas are shown in Figs 8 and 9 respectively.

In both cases damage induced oxidation of AA, and the ruptured tissues allowed immediate leaching of vitamin C. At 45°C, AA oxidation was 18 and 23% greater in the bruised and bruised plus slit peas respectively, when compared to undamaged standard size peas. Also, in both cases, less than 1% of the initial vitamin C was detected as AA in the blanch water at 45°C. However 14 and 18% of the initial vitamin C was detected as DHA in the water, from bruised and bruised plus slit peas respectively, compared to 0.3% from undamaged standard size peas. Within the limits of experimental error, this suggested that the presence of a slit in the testa enhanced the amount of AA

oxidation, possibly by allowing some ingress of oxygen into the tissues just prior to blanching, and also allowed increased leaching of solutes through the slit during blanching. At 65°C, the amount of AA oxidation in both cases was almost identical to that observed in undamaged standard size peas. The AA remaining in the water in both cases was some 4% less than for undamaged standard size peas.

The main physical effect of bruising peas by the standard method was to rupture the crushed parenchyma cells of the testa. The results shown in Figs 8 and 9 suggest that below 50°C bruising serves to disorganize the cell membranes mechanically, promoting AA oxidation and leaching of vitamin C largely as DHA, thus producing a net effect similar to that produced by heat at temperatures above 50°C. However at 65°C the pattern of changes in the vitamin C compounds was almost the same as that observed in undamaged standard size peas, due to the more rapid rates of heat transfer at the higher temperatures.

The maximum amount of AA oxidation observed in the bruised plus slit peas was about 5% greater than that occurring in bruised peas, again indicating the possibility that some oxygen had diffused into the tissues through the slit in the testa prior to blanching. However, the maximum amount of oxidation that occurred in bruised peas was the same as that in the undamaged standard peas, indicating some factor limiting the amount of AA oxidation. Such a factor may well be the rate of heat transfer and consequently enzyme inactivation, but the fact that 5% more oxidation occurred in the bruised plus slit peas suggests at least the possibility that a limiting factor could be the oxygen concentration in the pea tissues.

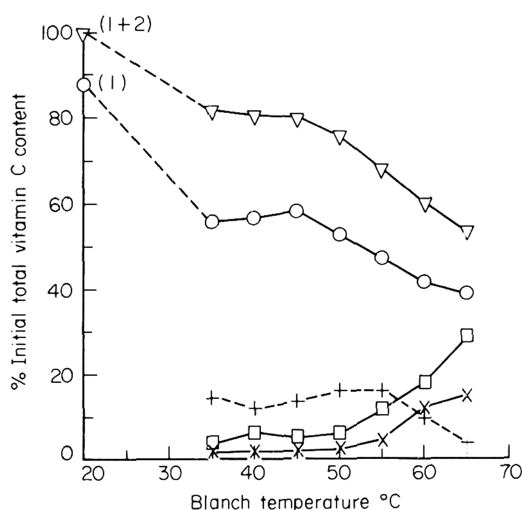

Figure 8. Standard-size bruised DSP peas. Proportions of ascorbic acid (AA) and dehydroascorbic acid (DHA) expressed as a percentage of the initial total vitamin C content, remaining in the pea, lost into the water and the DHA hydrolyzed, after 10 min at the given blanch temperature. (Means of two replicate samples); symbols as in Fig. 3.

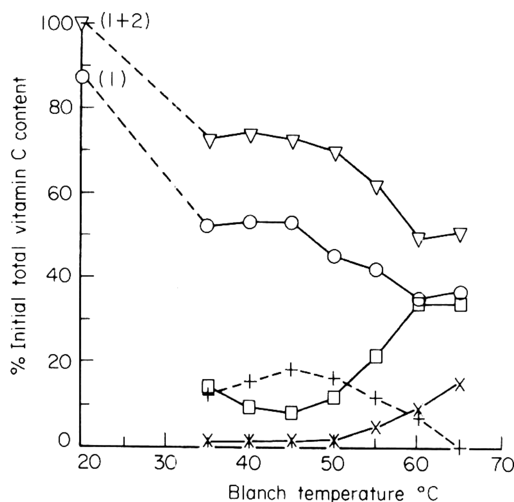

Figure 9. Standard-size bruised plus slit DSP peas. Proportions of ascorbic acid (AA) and dehydroascorbic acid (DHA) expressed as a percentage of the initial total vitamin C content, remaining in the pea, lost into the water and the DHA hydrolyzed, after 10 min at the given blanch temperature. (Means of two replicate samples); symbols as in Fig. 3.

Time of Blanching

Experiments were carried out to show the effect of blanch time on the AA content of DSP peas harvested in 1973.

(1) Effect of temperature. Commercially a blanch temperature of about 97°C is employed with a corresponding holding time of about 1 min prior to the freezing operation. It was previously shown that a temperature of 70°C was sufficient to completely disorganize the cell membranes in the whole pea and therefore the effect of a slower rate of heat penetration was also studied.

The AA contents of the pea and blanch water were determined after blanching and the AA oxidized calculated by difference. In a second set of experiments, the AA analyses were carried out on the cotyledons and testas which had been separated after blanching samples of whole peas for the same times. The results for these experiments at 97°C (0.25–3.0 min) and 70°C (2–25 min) have been reported previously, together with data for the equivalent decrease in peroxidase activity (Birch *et al.*, 1974). At the blanch temperature of 97°C the rate of AA loss was most rapid in the first minute, the amount of AA oxidized being negligible. At 70°C, the rate of AA loss into the water was most rapid in the first 2 min of blanching, the loss thereafter steadily increased with time. The pattern of loss occurred in both the cotyledons and testa, the loss from the testa being greater during the first 2 min. This might be expected considering that the AA concentration in the testa tissues was about twice that of the cotyledon tissues (Selman & Rolfe, 1979a). The slower rate of heat penetration

at 70°C also resulted in the oxidation of some 15% of the AA present during the first 2 min. The rate of loss of AA increased with blanch temperature.

These tests were repeated with DSP pea samples grown in 1974, but using only whole peas. Similar changes were observed although the higher concentration of AA in the cotyledons of 1974 peas resulted in slightly more AA oxidation and correspondingly less leaching of AA into the blanch water. The net result was a slightly greater retention of AA ($P = 0.05$) in the 1974 peas after blanching.

(2) Effect of damage. Standard size DSP peas were blanched at 97°C for 0.25–3 min (Birch *et al.*, 1974). The AA contents of the peas and blanch water were determined after blanching, the AA oxidized being calculated by difference. The experiment and analyses were then repeated using damaged peas of the three grades slit, bruised and bruised plus slit.

For each grade of damaged pea, the results for the AA remaining in the peas, lost into the water and oxidized at each of the six blanch-times, were compared with the results for whole undamaged peas using a statistical t-test.

The results for slit peas were not significantly different from those for the undamaged peas. The amount of AA remaining in bruised peas was not significantly different, however, more AA was oxidized ($P = 0.001$) and correspondingly less AA was lost into the water ($P = 0.01$). In bruised plus slit peas, the amount of AA oxidized was not significantly different, but less AA remained in the peas ($P = 0.01$) and less AA was lost into the water ($P = 0.01$); this loss of AA into the water was greater ($P = 0.001$) and the amount of AA oxidation was less ($P = 0.01$) than that from bruised peas. This suggested that the slit in the testa of the bruised pea allowed AA to leach out into the water before oxidation could take place.

Discussion and conclusion

Leaching of AA from undamaged peas was initiated at about 40°C due to heat induced disorganization of the cytoplasmic membranes of the cells. Leaching losses below 40°C were negligible. Leaching losses from standard size DSP peas increased almost linearly up to 97°C, but between 55 and 65°C there was some indication that oxidation of AA occurred rapidly enough to reduce the rate of loss of AA by leaching.

The size of DSP peas increased with increasing maturity over the maturity range studied and so the two parameters were inseparable. The results indicate that with increasing pea size and maturity, the proportion of AA oxidized increased and the proportion of AA leached into the water decreased, during blanching at temperatures between 45 and 65°C. Leaching of AA from undersize DSP peas increased more rapidly with increasing temperature compared to standard size DSP peas. This may have been due to higher initial concentrations of AA in the testa, greater testa/cotyledon ratio, greater surface/volume ratio and greater permeability of the cell walls of these botanically

immature peas. Leaching of AA from oversize peas was considerably reduced at all temperatures, arising from the lower initial vitamin C concentration in the tissues, smaller testa/cotyledon ratio, smaller surface/volume ratio and the less permeable cell walls of these more mature peas. The presence of significant quantities of starch (and protein) may have the effect of reducing the movement of water and soluble solids within the tissues.

Blanch temperature similarly influenced the leaching of AA from standard size Swan and Swift peas, although the percentage losses were low being of the order of those observed from oversize DSP peas. Cultivars Swan and Swift contained higher proportions of DHA particularly in the testa tissues, and calculations indicated that significant amounts of vitamin C were leached as DHA. The overall percentage retention of vitamin C was similar in both standard size Swan and DSP peas over the temperature range 40–65°C, but net retention was some 5% higher in Swift peas. In all experiments, only small quantities of DHA were detected in the blanch water due to the thermal instability of the compound.

Oxidation of AA during blanching of standard size DSP peas increased to a maximum at about 60°C. AA oxidase enzymes were largely inactivated at temperatures of 70°C and above so that leaching became the prime mechanism of vitamin C loss. There was some indication that the distribution of AA between cotyledons and testa influenced the amount of oxidation occurring. 1974 DSP-pea samples contained a greater concentration of AA in the cotyledons resulting in a greater amount of AA oxidation, whilst 1973 DSP-pea samples contained a greater concentration of AA in the testa tissues which resulted in conversely more leaching of AA. This is similar to the effect observed with DSP peas of different maturity; for example, in oversize DSP peas considerable AA oxidation occurred and little AA was leached out. Additional influencing factors in this case would be the increased pathway for heat transfer, smaller proportion of testa tissues and the less permeable cell walls of the testa tissues due to the developing secondary thickening as previously indicated.

Damaging peas by bruising and slitting induced enhanced oxidation of AA and allowed immediate leaching of vitamin C now largely as DHA, even at the lowest blanch temperatures, soluble material being observed to issue forth from the micropyle. The maximum amount of oxidation that occurred in bruised peas was the same as that occurring in undamaged peas, suggesting that some factor other than temperature and cell integrity was limiting the amount of AA oxidized. In fact the optimum activity of the AA oxidase enzymes occurred some 20°C higher than that of the extracted enzyme, and with considerably reduced activity. A possible reason for this arises from the pattern of results for the bruised plus slit peas. Some 5% more oxidation occurred in these peas, suggesting that the slit in the testa had allowed the ingress of some oxygen into the tissues just prior to blanching. It is possible that the content and availability of oxygen in the pea tissues may limit the amount of AA oxidation that can occur as previously discussed (Selman & Rolfe, 1979a). Simple experiments involving the artificial alteration of the oxygen content of the pea tissues, followed by

subsequent observations of the amount of AA oxidized as a result of damage or blanching, might help to elucidate this. At 60°C and above, the rate of heat penetration was such that the activity of enzymes was rapidly reduced, resulting in similar net total vitamin C retention to the undamaged peas.

Commercially, peas are blanched at about 97°C, and in the case of undamaged standard size DSP peas, about 28% of the initial AA was lost into the water during the first minute. The rate of loss of AA was greater from the testa tissues than cotyledons during the first 2 min as a result of the higher AA concentration in the testa tissues. The proportion of AA oxidized during blanching at 97°C was negligible. A comparative blanch at 70°C indicated a similar pattern of changes, but the slower rate of heat penetration resulted in the enzymic oxidation of some 15% of the initial AA content during the first 2 min. It may be significant that the trend of the rate of loss of AA into the water more closely resembled the rate of loss of moisture, rather than the rate of loss of total solutes from peas during blanching at the same temperatures. This observation arises from previous results that suggested that cell contents were, at least initially, lost from the testa tissues via the micropyle (Selman & Rolfe, 1979b). This links with the hypothesis that the vitamin C in the testa tissues is located largely in the watery parenchyma tissue that constitutes the inner layer of the testa.

DSP peas damaged by slitting the testa showed a retention of AA similar to that of undamaged peas when blanched for various times at 97°C. Again this suggests that the micropyle is a significant pathway for loss of cell contents by leaching, as the loss of total solutes was similarly unaffected by slitting the testa. Bruised peas exhibited similar net AA retention to undamaged peas although the bruising gave rise to more AA oxidation and proportionally less AA leaching. Bruised plus slit DSP peas, probably the commonest form of damage in reality, did lose significantly more AA during blanching at 97°C. Loss of AA into the water was greater, and the oxidation of AA less than that from peas that had only been bruised. This might be expected as leaching from the bruised tissues could occur through both the slit in the testa and the micropyle immediately on immersion of the peas in the blanch water, thus reducing the amount of AA available to the oxidized.

In summary, these results have demonstrated the main mechanisms of change of the vitamin C compounds in peas during blanching and the proportions in which some of these changes are likely to occur as influenced by such factors as pea size/maturity, cultivar, blanch temperature and time. Further comparative work on the blanching of undersize and oversize peas at commercial blanch temperatures would be useful. Harvesting damage may radically affect the vitamin C content of the final product on an industrial scale, and other pre-blanch handling operations will be important. For example, heat arising from metabolism has been reported to result in temperatures of around 30°C in peas when bulk stored experimentally for 12 hr (Holdsworth, 1969). Cooling systems may therefore be required to reduce and control the temperature when handling large quantities of peas in order to prevent undesirable changes such as AA oxidation.

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Functional properties of acetylated and succinylated sunflower protein isolate

M. KABIRULLAH AND R. B. H. WILLS

Summary

Sunflower protein isolate was acylated with acetic and succinic anhydride. Succinylation changed the electrophoretic mobility, decreased the lysine and chlorogenic acid content and bulk density, increased the aqueous solubility, shifted the isoelectric point and increased the absorption, emulsification and foaming properties. Acetylation improved the functional properties of the protein to a much lesser extent. Implication of these changes is discussed with reference to food applications.

Introduction

Sunflower seed (*Helianthus annus*) is the second largest source of vegetable oil and fourth largest source of plant protein (FAO, 1977). Sunflower protein has great potential as a source of human protein food because it is free of any known anti-nutritive factor (Clandinin, 1958) and is has a high digestibility (90%) and biological value (60–70) compared to most other oilseed proteins (Clandinin, 1958; FAO, 1970). Various workers have examined the functional properties of sunflower protein fractions and their findings have been recently reviewed by Sosulski (1979). He concluded that sunflower protein had some unique organoleptic and functional properties which would be of importance in some foods. The main problems in using sunflower protein in foods was seen to be the presence of chlorogenic acid which causes discolouration and the high globulin content (about 75% of the total protein) which has a low solubility in water at pH <8.

A number of methods have been examined to modify the structure of various novel proteins to improve their functional properties for specific food applications. One such approach has been to treat the protein with acetic and

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succinic anhydrides and studies have been reported for soybean protein (Franzen & Kinsella, 1976b), casein (Creamer, Roeper & Lahrey, 1971), cottonseed flour (Childs & Park, 1976), fish protein (Groninger, 1973), peanut protein (Sundar & Rao, 1978; Beuchat, 1977), wheat flour protein (Grant, 1973), egg protein (Gandi *et al.*, 1968) and leaf protein (Franzen & Kinsella, 1976a). In general, functional properties such as solubility and emulsification capacity were improved by acetylation or succinylation. Canella, Castriotta and Bernardi (1979) have recently reported on the effects of acetylation and succinylation on the properties of sunflower protein concentrate that had been treated with acid and ethanol to remove chlorogenic acid. They found that the acyl derivatives had increased solubility, emulsifying and foaming properties but decreased water absorption. However their results cannot be assumed to apply to sunflower protein generally as the acid-ethanol treatment would in itself cause the loss of certain functional properties (Canella, 1978). Also sunflower protein concentrates would differ in their physico-chemical and functional properties from other protein products such as sunflower protein isolates (Lin, Humbert & Sosulski, 1974) due to their different methods of extraction and preparation. The food applications of protein isolates and concentrates differ (Sosulski, 1979; Kinsella, 1976) and there are some foods such as snack foods and breakfast cereals where the presence of chlorogenic acid may not be a problem (Sosulski, 1979). Further studies on sunflower protein are therefore warranted and in this paper we report on the effect of acetylation and succinylation on the functional properties of sunflower protein isolate (SPI) that are important in food applications.

Materials and methods

Preparation of sunflower protein isolate (SPI)

Sunflower seed (cv. Hysun 21) meal prepared from dehulled and defatted seeds was suspended in water (1 : 10) and adjusted to pH 11 by the addition of 1 N NaOH. The mixture was stirred for 1 hr at 20°C, then centrifuged at 12 000 rev/min for 10 min at 10°C. The supernatant was filtered and adjusted to pH 4.5 with 1 N HCl (Gheyasuddin, Cater & Mattil, 1970). The resultant precipitate was separated by centrifugation, dialyzed against distilled water for 72 hr then freeze dried.

Acylation

The procedure of Riordan and Vallee (1971) was followed to acylate the free amino groups of SPI using acetic and succinic anhydride. The required amounts of anhydride were added slowly to a protein suspension (2.5% w/v) with constant stirring and concurrent addition of 2 N NaOH to maintain the pH at

7.5–8. After the reaction was complete, the proteins were dialyzed against distilled water and freeze-dried.

Analysis

The amount of free amino groups in the protein before and after acylation was determined spectrophotometrically following reaction with ninhydrin (Moore & Stein, 1954). The amino acid composition of the protein was analyzed by the method of Spackman, Stein and Moore (1958). The moisture, ash, fat, protein and fibre of the protein samples were determined according to methods 14.084, 14.085, 14.089, 14.086 and 14.087 (AOAC, 1980). Chlorogenic acid was measured at 330 nm according to the method of Dorrell (1976).

Polyacrylamide gel electrophoresis

Electrophoresis of the protein was carried out on a discontinuous gel (2.5–27%) (Pharmacia Fine Chemicals) in Tris-borate buffer (0.09 Tris, 0.08 M boric acid, Na_2 EDTA 0.93 g/l, pH 8.4) at 400 V for 1 hr. The gels were stained with Coomassie blue (0.1% w/v) dissolved in trichloroacetic acid (50% w/v) and destained with 7.5% acetic acid.

Functional properties

(1) *Protein solubility.* A 1% w/v protein suspension in water was adjusted to various pH levels with 2 N NaOH or 2 N HCl. The proteins were occasionally shaken over 1 hr at 20°C and then centrifuged at 12 000 rev/min for 15 min at 10°C. The clear supernatant solutions were analyzed for nitrogen content by Kjeldahl method 14.086 (AOAC, 1980).

(2) *Absorption of fat and water.* Absorption of fat and water by proteins was determined by a modification of the method described by Lin, Humbert and Sosulski (1974). Soybean oil or water (10 ml) were stirred for 1 min with a protein sample (1 g) in a graduated tube which was allowed to stand for 30 min, then centrifuged at 3500 rev/min for 20 min. The decrease in volume of free liquid indicated the amount of liquid absorbed by the protein.

(3) *Bulk density.* Bulk density of the protein was measured following the procedure of Wang and Kinsella (1976).

(4) *Emulsification properties.* Emulsifying activity (EA) and emulsion stability (ES) as described by Yasumatsu *et al.* (1972) and emulsifying capacity (EC) as adopted by Swift, Lockett and Fryar (1961) were taken as the indices of emulsification. EA was estimated by blending a protein suspension with soybean oil then centrifuging the emulsion and calculating the ratio of the height of the emulsified layer to the height of whole layer in centrifuge tube. ES was determined by heating the emulsion at 80°C for 30 min, cooling to 20°C then centrifuging and calculating as for EA. EC was determined by adding soybean

oil to a protein suspension during blending and noting the volume of oil added when the emulsion collapsed.

(5) *Interaction with muscle protein.* Meat (silverside) was homogenized at 10 000 rev/min for 2 min in a 3% w/v NaCl solution to produce a 1% w/v suspension of muscle protein. The pH of the slurry was adjusted to pH 6 using Na_2CO_3. SPI and meat slurry were mixed in various proportions from 0–100% and EA, ES and EC were determined as described.

(6) *Foaming properties.* Foam expansion and foam stability were assessed as indices of foaming using the procedure described by Puski (1975). This involved blending a protein suspension adjusted to the required pH level at 10 000 rev/min for 1 min at 20°C and determining the volume of foam that was present above the surface of the liquid. After standing for 30 min, the volume of foam remaining was recorded as foam stability. Egg albumin (Egg Marketing Board, Australia) was used as a standard protein for comparison.

Results

Extent of acylation

The degree of acylation of the free amino groups on sunflower proteins increased with the increasing amounts of anhydrides used in the reaction mixture (Fig. 1) with acetic anhydride being more reactive than succinic anhydride. The maximum level of acylation was about 90% of the total free amino groups of the proteins.

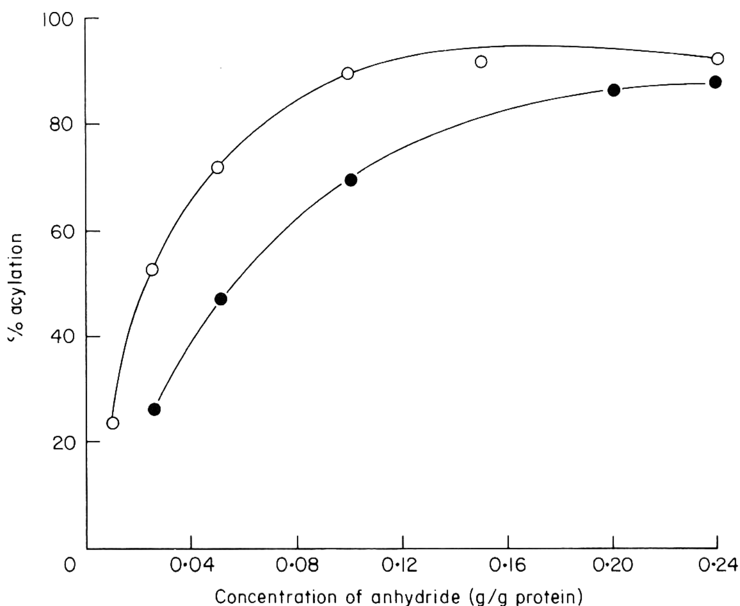

Figure 1. Effect of the concentration of acetic and succinic anhydride on the degree of acylation of sunflower protein isolate; o—o, acetylated; •—•, succinylated.

Composition

The proximate composition of the derivatized sunflower proteins are shown in Table 1. The protein content of the derivatized proteins showed a small decrease, with increasing acylation probably due to a dilution effect resulting from the addition of acyl groups to the structure. The chlorogenic acid content of the modified proteins was less than the untreated SPI but the amount present was similar in all modified proteins. There was, however, still sufficient chlorogenic acid or oxidation products present to cause the derivatized proteins to remain green.

Table 1. Proximate composition of acylated sunflower proteins (g/100 g dry sample)

Protein	Moisture	Protein	Ash	Chlorogenic acid
SPI	5.2	88.2	3.9	1.8
Acetylated (%):				
23	5.1	86.5	4.0	1.2
53	4.9	86.0	3.8	1.2
84	5.0	85.4	3.9	1.2
Succinylated (%):				
27	5.5	87.0	4.0	1.2
48	5.3	86.5	4.0	1.2
70	4.9	86.4	3.9	1.2
87	5.2	85.3	3.9	1.2

Values are the average of at least three determinations.

Table 2. Essential amino acid content of acetylated and succinylated sunflower protein (g/16 N)

		Acetylated %			Succinylated %			
Amino acid*	SPI	23	53	84	27	48	70	84
Lysine	3.2	2.8	2.6	2.6	2.9	2.9	2.8	2.8
Threonine	3.2	3.3	3.3	3.4	3.4	3.3	3.4	3.4
Half cysteine	1.2	1.1	1.0	1.0	1.0	1.1	1.0	1.0
Methionine	1.9	1.8	1.7	1.9	1.9	1.8	1.9	1.9
Valine	4.9	5.2	5.2	5.6	5.4	5.5	5.4	5.3
Isoleucine	4.2	4.4	4.4	4.4	4.4	4.5	4.4	4.4
Leucine	5.9	6.2	6.3	6.5	6.3	6.4	6.4	6.1
Phenylalanine	5.2	5.5	5.7	5.7	5.6	5.7	5.6	5.1

*Tryptophan was not analysed.
Values are the average of at least three determinations.

The essential amino acid (EAA) content of the derivatized proteins is shown in Table 2. Lysine content of the modified proteins was less than in the unmodified proteins but valine, leucine and phenylalanine contents were higher in the modified proteins.

Gel electrophoresis

Electrophoretic patterns of the acylated sunflower protein are presented in Figs 2 and 3. The major storage protein of sunflower, presumably globulin, is seen in a single band in the 3 cm region of the gel. This band is dissociated into fractions which moved further into the gel with increased degree of acylation. Some new peaks are seen in the 0–1 cm region of the slab with proteins acetylated to 23 and 53%. Similarly succinylated protein showed some new peaks in 0–2 cm region (Fig. 3).

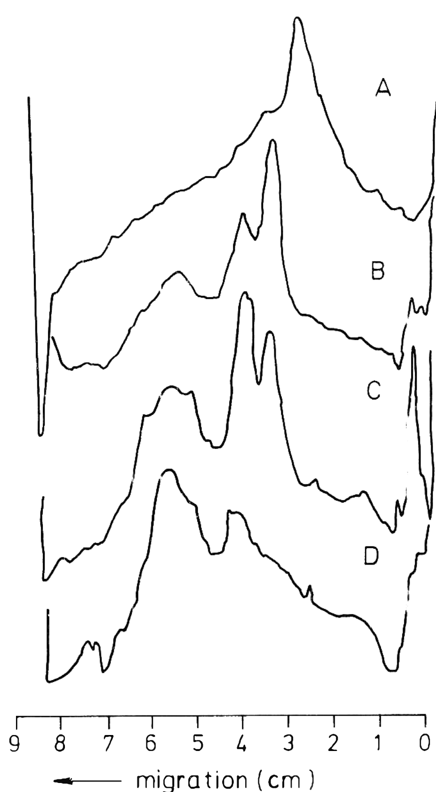

Figure 2. Electrophoretic mobility of acetylated sunflower protein isolate; A, B, C and D – sunflower protein isolate unmodified, acetylated to 23, 53 and 84% of the free amino groups respectively.

Functional properties

Acylation of proteins caused a reduction in bulk density with succinylation causing a relatively greater decrease than acetylation (Fig. 4). Succinylation at low levels markedly decreased the bulk density but further succinylation above 26% resulted in a less marked decrease. The effect of acetylation was more linear over the whole range.

Fat and water absorption was increased in the derivatized proteins (Table 3) with succinylation being more effective in increasing absorption. However the magnitude of the increases was not great.

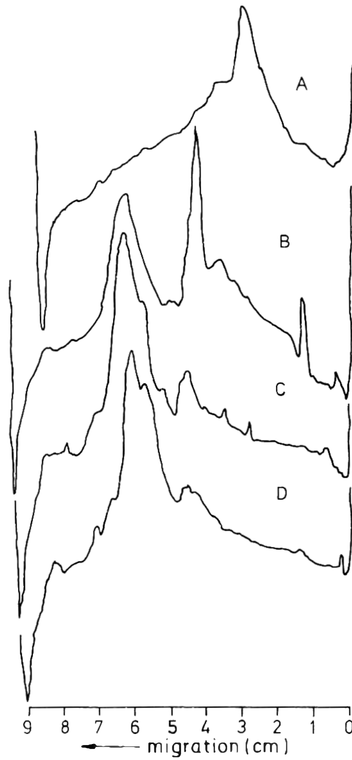

Figure 3. Electrophoretic mobility of succinylated sunflower protein isolate; A, B, C and D – sunflower protein isolate unmodified, succinylated to 26, 48 and 87% of the free amino groups respectively.

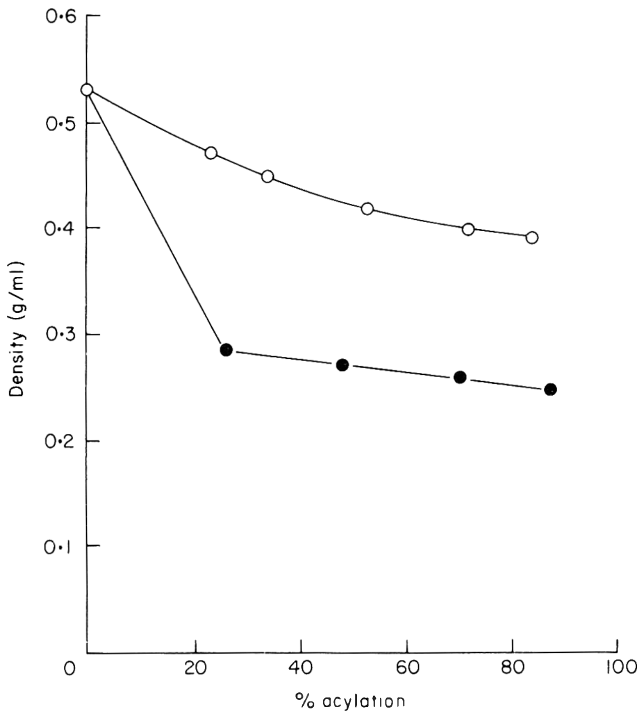

Figure 4. Bulk density of the acylated sunflower protein isolate; o—o, acetylated; ●—●, succinylated.

Table 3. Absorption of water and fat by acylated sunflower protein isolate

Protein	Water (ml/g sample)	Fat (ml/g sample)
SPI	5.31 ± 0.10[a]	4.22 ± 0.02[a]
Acetylated %:		
23	5.36 ± 0.07[a]	4.33 ± 0.04[a]
54	5.48 ± 0.05[a]	4.42 ± 0.01[ab]
85	5.94 ± 0.02[b]	4.97 ± 0.03[c]
Succinylated %:		
26	5.69 ± 0.04[b]	4.53 ± 0.06[b]
48	5.84 ± 0.01[b]	5.95 ± 0.01[d]
70	6.19 ± 0.07[c]	6.51 ± 0.04[e]
87	6.24 ± 0.01[c]	6.72 ± 0.08[d]

Values in columns with same letter are not significantly different at 5% level.

Protein solubility was appreciably changed by succinylation (Fig. 5). Succinylation shifted the isoelectric point (IEP) of the proteins from pH 4–5 to pH 3–4. In the pH range 1–4, the solubility of the proteins was less than that of SPI and in the pH range 4–8 the solubility was greater. Increasing the degree of succinylation tended to cause an increase in solubility with the effect being greater at pH 1–2 and pH 5–8. Acetylation did not greatly affect the protein solubility although there was a tendency for acetylation to decrease solubility at pH <4 and increase solubility at pH 6–8 (Fig. 5).

Emulsification properties

The effect of pH on the emulsifying capacity (EC), emulsion stability (ES) and emulsion activity (EA) of the acylated sunflower proteins is shown in Fig. 6. Succinylation increased the emulsifying properties of SPI appreciably at all pH. The greatest changes had occurred with succinylation of 27% and while further succinylation also increased the emulsifying properties the extent of these changes was not proportional to the degree of succinylation. Acetylation however had little effect on the emulsification properties although EA at pH 3–4 was increased.

The emulsification properties of protein acetylated to 84% and succinylated to 87% in the presence of sodium chloride and sucrose were determined and compared to the emulsification properties of untreated SPI (Table 4). At pH 4 the addition of salt increased the EC of all proteins but only increased the ES and EA of SPI. At pH 6, salt only increased the EC of SPI and acetylated protein and at pH 9 had no marked effect on any emulsification property. The emulsification properties of succinylated protein still remained greatly superior to SPI and acetylated protein under all conditions. The presence of sugar only

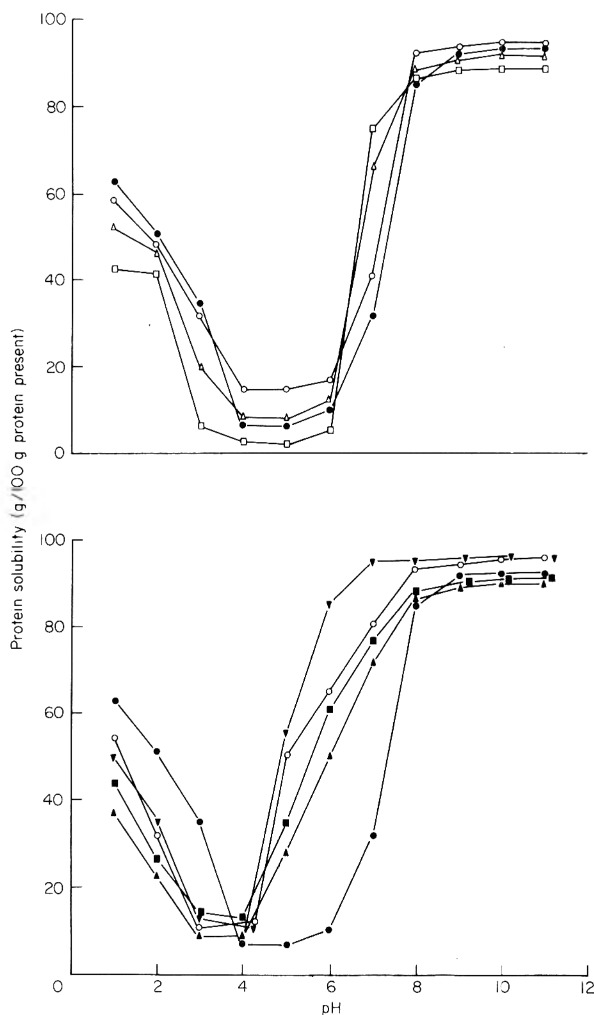

Figure 5. Effect of pH on the solubility of acylated sunflower protein isolate; ●—●, SPI; o—o, acetylated to 23%; △—△, 53%; □—□, 84%; ▲—▲, succinylated to 26%; ■—■, 48%; o—o, 70%; ▼—▼, 87%.

caused a small increase at pH 4 in the ES of SPI and acetylated protein and the EA of SPI.

The effect of substitution of meat protein with untreated SPI, SPI acetylated to 84% and succinylated to 87% on emulsification properties is shown in Table 5. Substitution with succinylated protein increased EC and maintained a similar level of ES and EA. Substitution with SPI and acetylated protein resulted in a decrease in EC, ES and EA.

Foaming properties

Acetylation and succinylation of SPI improved the initial foam volume and foam stability of untreated SPI at all pH from 4–10 (Fig. 7). The degree of

Figure 6. Effect of pH on the emulsification properties of acylated sunflower protein isolate; ●—●, SPI; ○—○, acetylated to 23%; △—△, 53%; □—□, 84%; ▲—▲, succinylated to 26%; ■—■, 48%; ⊙—⊙, 70%; ▼—▼ 87%.

Table 4. Effect of sodium chloride (1 M) and sucrose (25%) on the emulsification properties of acylated sunflower protein (10 mg/ml) at pH 4, 6 and 9

Emulsification property	Protein	pH 4			pH 6			pH 9		
		No additive	NaCl	Sucrose	No additive	NaCl	Sucrose	No additive	NaCl	Sucrose
EC										
(ml oil/g sample)	SPI	210	320	230	250	330	250	450	460	440
	acetylated to 84%	200	330	210	215	340	215	580	475	475
	succinylated to 87%	325	710	315	900	900	860	1200	1150	1180
ES										
(% of emulsion)	SPI	30	41	40	46	51	46	56	57	56
	acetylated to 84%	15	16	46	55	55	57	53	53	56
	succinylated to 87%	59	57	58	65	65	65	65	65	66
EA										
(% of emulsion)	SPI	0	40	40	45	50	44	55	55	53
	acetylated to 84%	48	47	47	43	43	44	53	53	55
	succinylated to 87%	43	42	45	57	58	59	60	61	62

Table 5. Effect of the replacement of meat protein by sunflower protein on emulsification properties

Emulsification property	Protein	Ratio sunflower protein: meat protein					
		0:100	20:80	40:60	60:40	80:20	100:0
EA (ml/g)	SPI	59	55	54	53	50	45
	acetylated to 84%	59	57	56	55	53	43
	succinylated to 87%	59	59	58	58	58	58
ES (%)	SPI	61	60	60	58	54	46
	acetylated to 84%	61	59	60	60	58	55
	succinylated to 87%	61	62	62	62	63	64
EC (%)	SPI	420	380	345	340	260	255
	acetylated to 84%	420	310	280	240	220	210
	succinylated to 87%	420	550	670	775	830	890

acylation did not appear to be directly related to the foaming properties and acetylation and succinylation were equally effective. The foaming properties of egg albumin were similar to SPI at pH 7–10 and therefore less than acylated SPI. At pH 6, the foaming properties of egg albumin increased and at pH 4, its foam volume was greater than, but the foam stability was similar to, the acylated proteins.

Foam expansion and stability on the different types of protein were examined in the presence of sodium chloride and sucrose (Table 6). The addition of sodium chloride markedly increased foam expansion of untreated SPI so that the foam volume was greater than that achieved with egg albumin. The foam expansion of egg albumin, acetylated and succinylated protein were increased slightly. Only small changes in foam stability of all proteins were effected by sodium chloride. The addition of sucrose substantially reduced foam expansion and its stability in all sunflower proteins but had no effect on foaming properties of egg albumin.

Discussion

Acylation of sunflower protein isolate with acetic and succinic anhydrides decreased the lysine and chlorogenic acid content, changed the electrophoretic mobility, decreased the bulk density, increased the aqueous solubility around the neutral pH, shifted the isoelectric point to more acidic pH and increased the absorption, emulsification and foaming properties. Most of these changes were more pronounced with succinylated protein than acetylated protein. The addition of salt increased the solubility of modified sunflower protein and increased emulsification and foaming properties while sucrose had little or no effect on foaming and emulsification. Some of these effects are in disagreement

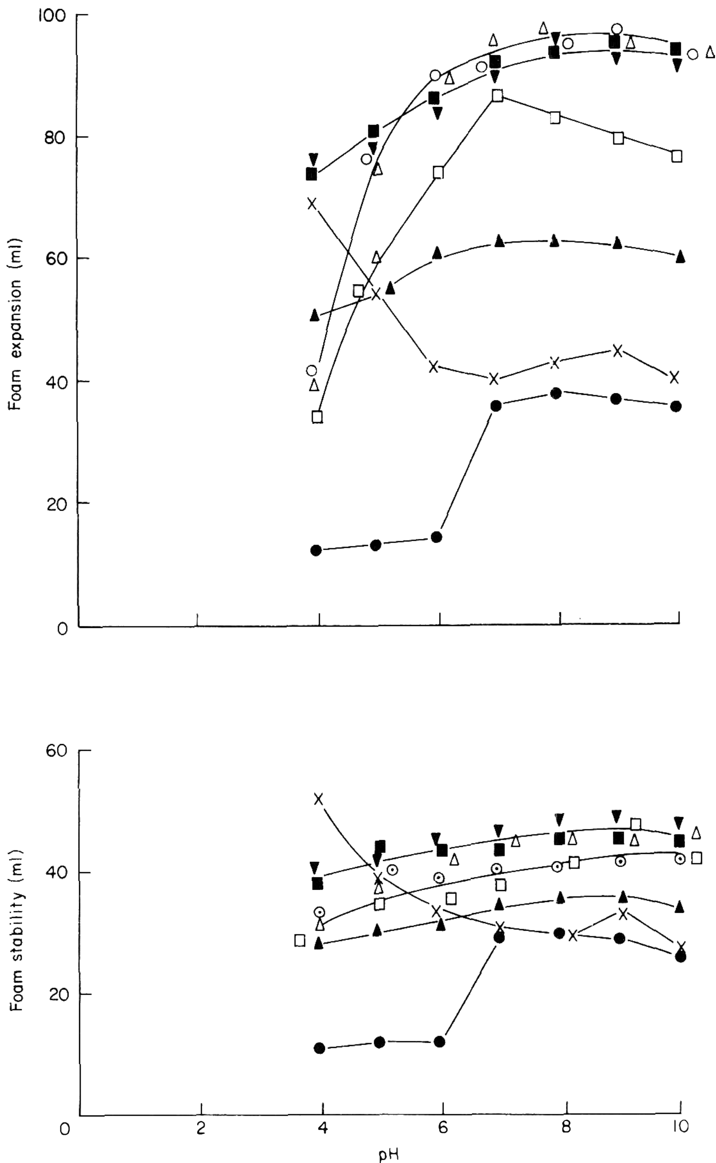

Figure 7. Effect of pH on the foaming properties of acylated sunflower protein isolate; ●—●, unmodified SPI; ×—×, egg albumin; o—o, SPI acetylated to 23%; △—△, 53%; □—□, 84%; ▲—▲, succinylated to 48%; ■—■, 70%'▼—▼, 84%.

with the observations of Canella, Castriotta and Bernardi (1979) on acylated sunflower protein concentrate, who reported a decrease in absorption properties and foam stability and higher bulk density. Further they reported improved solubility, emulsification properties and foam expansion of the protein but the extent of improvement was less than that observed in this study. The differences in effects could be due to the acid-alcohol treatment given by

Table 6. Effect of sodium chloride (1 M) and sucrose (25%) on the foaming properties of sunflower proteins and egg albumin (at 10 mg/ml) at pH 7

Protein	Foaming properties (ml)	No additive	+ NaCl	+ sucrose
Egg albumin	Expansion	40	50	43
	Stability	31	24	33
Native SPI	Expansion	36	75	11
	Stability	29	40	9
Acetylated to 84%	Expansion	88	98	34
	Stability	39	48	24
Succinylated to 87%	Expansion	91	112	50
	Stability	46	52	20

Canella, Castriotta and Bernardi (1979) in the preparation of sunflower protein concentrate which might have denatured the proteins (Canella, 1978). However similar effects of acylation to those obtained in this study have been observed with peanut (Beuchat, 1977), cottonseed (Childs & Park, 1976), leaf protein (Franzen & Kinsella, 1976a), soy protein (Franzen & Kinsella, 1976b).

The reduced chlorogenic acid content of the modified protein could be due to removal of some of the chlorogenic acid during acylation and dialysis but the colour of the modified proteins remained green-brown due probably to the presence of oxidation products of chlorogenic acid (Sabir, Sosulski & Finlayson, 1974). The decrease in lysine content may be due to degradation of lysine residues during acylation and the removal during dialysis (Franzen & Kinsella, 1976b). The increase in electrophoretic mobility of acylated sunflower proteins would be due to unfolding of the protein structure and dissociation of the protein into sub-units (Beuchat, 1977; Klotz & Keresztes-Nagy, 1963). Unfolding of the protein structure would have also enhanced the entrapment of liquids (Beuchat, 1977) resulting in the increase in absorption properties. Succinylation would have increased the negative charge on the protein molecules (Franzen & Kinsella, 1976b) and as a result would have allowed the solubility of the proteins to increase. The effect of acetylation on the solubility of the proteins was, however, not as pronounced. The increase in other functional properties following acylation such as emulsification and foaming would be mainly due to the change in charge, since they are dependent on the protein solubility (Yasumatsu *et al.*, 1972).

Succinylation appears to be an effective method of improving the functional properties of sunflower protein isolate and thus of widening its scope for food application. The markedly increased solubility in the neutral pH region could be advantageously utilized to prepare specific foods with high soluble ingredients, e.g. soups, snacks, sauces and fruit purées. The positive effect of succinylation

on emulsification is highly significant for its potential application in various meat emulsions such as wieners, sausage, and also in doughnuts, mayonnaise and salad dressings. Application of succinylated sunflower protein in meat emulsion increased the emulsion capacity of the mixture significantly and indicated its definite potential in such products. In addition, acylated sunflower proteins could be used in various fabricated food products requiring the formation of fine texture, voluminous and stable foam such as ice cream, confections, soufflés. Many authors have reported the problem of chlorogenic acid which imparts dark colour in food (Sosulski, 1979). But there are food products which are coloured and for which any slight colouration in the sunflower protein would not be a problem.

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Residual cyanide concentrations during the extraction of cassava starch

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Summary

Cassava starch is traditionally extracted on a small scale in many tropical countries. The process consists of wet-milling the washed roots, washing the starch from this milled pulp on vibrating trays or in mixing tanks, sedimenting the starch in wooden canals or concrete tanks and sun-drying the product. This process was analysed during six production runs in two factories. The distribution of cyanide followed a similar pattern in both factories. Most of the cyanogenic glucosides (bound cyanide) in the roots are converted to free cyanide during the milling operation. Between 40 and 70% of the total cyanide appears in the water used to wash the starch from the disintegrated tissue, and between 5 and 10% appears in the fibrous residue used in animal feed. This residue also contains between 12 and 23% of the starch present in the cassava. The eluted starch is allowed to sediment for 1–3 days, after which it contains less than 4% of the cyanide present in the raw material. The dried product contains less than 1% of the quantity of cyanide present in the raw material; the residual concentration is 1–5 p.p.m. The factors involved in the removal of the cyanide during starch extraction are discussed, and their importance to more efficient large-scale processes is indicated.

Introduction

The tropical root crop cassava (*Manihot esculenta* Crantz) provides a major source of calories for more than 300 million people (Nestel, 1973). Cassava is also used for animal feed, and industrial uses based on the production of starches (Phillips, 1974). Starch plays an important part in the formulation of a

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variety of foods; important starch sources include maize, potato, cassava, wheat, sorghum and rice. Cassava starch has potential advantages in a number of speciality food applications, which should maintain the increasing demand for this starch (Knight, 1974).

Cassava starch extraction outside the cassava producing countries is limited by the transport costs of this high moisture content raw material and by its perishable nature. Root storage can be extended to several weeks in field clamps or boxes, but starch content drops by about 10% in the first two weeks (Booth *et al.*, 1976). This limitation could be overcome by the use of dried cassava. For example, European starch factories could process some of the million tons of dried cassava chips imported annually for animal feed (Phillips, 1974); but Meuser *et al.* (1978) indicated that starch yields and quality are inferior to those from fresh cassava. In cassava producing countries, starch is extracted by a small-scale slow process within 3 days of harvest. de Buckle *et al.* (1978) have described starch extraction in rural areas of Colombia, indicating that the process is similar to that in other tropical countries. Two types of starch are produced in Colombia:

(1) sour starch, obtained by fermentation of the extracted starch, is used exclusively in the food industry especially in the traditional baked product known as pan de yuca;

(2) sweet starch which suffers less fermentation and is used primarily in the textile, paper and adhesive industries.

No data was presented about the cyanide contents of these starches. In Costa Rica, the starch for both food and industrial uses is produced in a similar fashion to that described by de Buckle *et al.* (1978) for sweet starch.

Cassava contains the cyanogenic glucosides linamarin and lotaustralin (referred to as bound cyanide), which are hydrolyzed to hydrogen cyanide by the endogenous enzyme linamarase (EC 3.2.1.21) when the cellular structure of the plant is damaged (Conn, 1969). Simple processing such as drying, soaking or boiling in water is unlikely to remove all the cyanide (Cooke & Maduagwu, 1978), the presence of which is responsible for the chronic toxicity associated with the continued ingestion of cassava products (Montgomery, 1969). The cassava used in Costa Rica is harvested at plant ages between 8 to 20 months, but an earlier study (Cooke & De La Cruz, 1981) has shown that the root cyanide concentration is similar in this age range. Recent medical studies (Ermans *et al.*, 1980) have stressed the need for cassava screening to locate low cyanide lines (no acyanogenic lines have yet been found) and for extended studies of the effects of processing on residual cyanide contents. This paper reports a study of the residual cyanide levels during the various stages of rural starch extraction.

Materials and methods

Description of the starch factories

The two starch factories studied, La Nacional (referred to as factory A) and

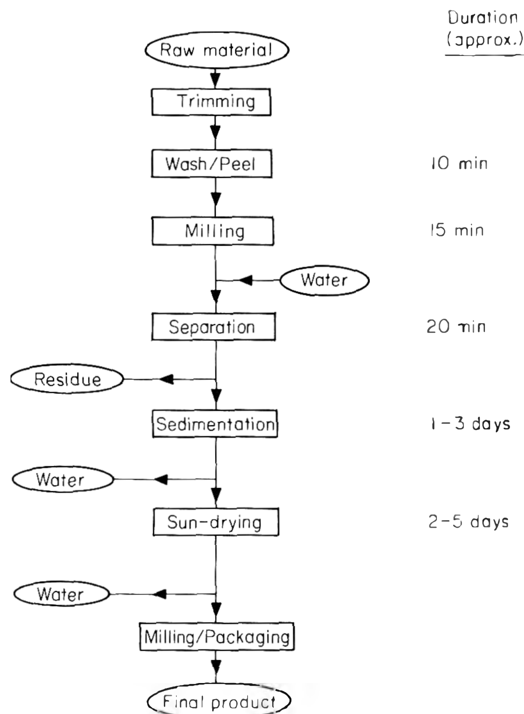

Figure 1. Flow diagram for the starch extraction process.

La Moderna (factory B), are both in Santa Eulalia, Atenas, Costa Rica. The cassava processed is of the two common Costa Rican cultivars, Valencia and Papa, grown in the Alajuela and San Carlos districts of Costa Rica. The different stages of the extraction process are shown in Fig. 1. The equipment used in the two factories is described below:

(1) *Washing/Peeling.* Both factories use a horizontally mounted slatted wooden drum (0.92 × 1.00 m, diameter × length). This is rotated slowly by an electric motor as a water spray washes the roots. Much of the brown, corky layer (referred to as peel) is rubbed off during this stage, leaving the root cortex and parenchymal tissue intact.

(2) *Milling.* Factory A uses a revolving drum grater (0.3 × 0.5 m, diameter × length), against which the roots are pressed with a wooden shovel. Factory B uses a swinging hammer mill of similar processing capacity to the mill in factory A. The hammers are 7 cm long and 0.75 cm wide.

(3) *Starch separation.* Factory A has two vertical cylinders (0.62 × 0.76 m, diameter × depth) with 170 mesh sieve bases. Motor driven paddles mix the pulp with a water spray, and the eluted starch passes through the sieve base. The residue is subsequently removed through a sliding port at the base of the cylinder walls. In factory B the milled pulp flows onto an inclined tray (10.0 × 1.2 m, length × width) which has a sieve base similar to that described above. A series of water sprays irrigate the tray which is supported on six vertical

leaf springs vibrated by means of a motor driven camshaft. The residue progresses along the tray, eventually collecting on the factory floor. The starch-water suspension passes through the sieve and is channelled to the sedimentation tanks.

(4) *Sedimentation.* Factory A has two parallel wooden canals ($21.0 \times 0.61 \times 0.28$ m, length × width × depth) connected laterally and on a slightly different level. Factory B uses a series of concrete tanks ($6.05 \times 0.99 \times 0.49$ m, length × width × depth), which are connected by pipes such that as one is filled it begins to discharge into the second.

(5) *Drying.* Both factories sun-dry the green (sedimented) starch on metal trays (1.75×0.75 m, length × width) supported on wooden frames.

Preparation of samples

Six series of samples were taken: four from factory A and two from factory B. Factory production was interrupted on each occasion and one drum load of roots weighed after the wash/peel operation (average weight 320 ± 30 kg) and followed through the process until all the starch from this batch had entered the sedimentation tanks. This permitted measurement of water used and pulp residue produced. Samples were taken of the milled pulp, residue, freshly sedimenting starch and of the wash water in the sedimentation tanks. Samples for cyanide analysis were weighed into 0.1 M phosphoric acid (160 ml) within 5 min of sampling, mixed and transferred to stoppered bottles. These samples were transported to the CITA laboratories, homogenized, filtered and analysed (later the same day) for total and free cyanide content by the enzymatic assay described earlier (Cooke, 1978; 1979). Linamarin recovery tests (Cooke, 1978) were done on the different samples from two of the test series to ensure that no variation in assay efficiency occurred in the different samples. No appreciable ($< 5\%$) differences were encountered. Samples for starch and moisture content analysis (300–600 g of each sample) were collected in wide-mouthed screw cap bottles, transported to CITA and analysed later the same day. Moisture content determinations were done on 30 g samples dried to constant weight at 90°C in a vacuum oven. Starch analyses were done by the method of Gaines (1973) for both factory A and B samples; and Krochmal and Kilbride (1966) for factory B samples.

A representative sample of the raw material (10–12 roots) was taken on each occasion, transported to the CITA laboratories in perforated polythene bags, and analysed the following day (16–18 hr later, during which period there was negligible change in root weight). These roots were washed in tap water and the brown outer bark removed. Earlier studies (Cooke, 1978) have shown that cassava roots have longitudinal and radial cyanide gradients. A representative sample for cyanide analysis (Cooke & De La Cruz, 1981) was obtained by chopping up the 10–12 roots in a 1 hp Hobart mill model S601 fitted with a 9 in vegetable slicer (Hobart Manufacturing Co., Ohio). The vegetable slicer was fitted with a P 77046 shredder plate. The fragmented tissues were further mixed

manually in a large plastic bucket and four samples (50 g each) taken for cyanide analysis as described earlier (Cooke, 1979). Two subsequent factory visits were made in each analytical series to collect samples of sedimented starch and dried product (Fig. 1).

Stability of cyanide in root homogenates

A sample of disintegrated whole root tissue prepared using a Hobart mill, as described above, was homogenized in a Waring blender (400 g tissue + 250 ml of distilled water) for 2 × 2 min, and spread onto a shallow (1 cm) aluminium tray. Samples (50 g in duplicate) were taken immediately after transfer to the tray and periodically until 4 hr later. The trays were stored at ambient temperature (28°C) during this period. The homogenate was sufficiently viscous to prevent the water from separating out, the layer of material was mixed with a ladle just prior to sampling.

A similar experiment used tissue that was milled in the Hobart using a slicer plate. The average dimensions of such slices were 4.99 (s.d. 1.03) × 1.35 (s.d. 0.17) × 0.11 (s.d. 0.04) cm, length × width × thickness, for ten such slices. These slices were mixed with the same proportion of water (400 g+250 ml of water) and placed in identical trays.

Results

The starch extraction process

The basis of the small scale starch extraction process, common in tropical Latin America, is shown in Fig. 1. This process varies between factories with regard to the efficiency of root washing and peeling, extent of tissue disintegration on milling, manner of washing out the starch and design of the sedimentation tanks. The variants used by the two factories studied are described in the previous section. The raw material comprises mixtures of the two common Costa Rican cassava cultivars grown in different locations, with differing post-harvest storage periods. Six different production runs were analysed at each stage of the process for free and bound cyanide and starch concentrations. This repetition was considered necessary in view of the variable raw material and arbitrary process control, e.g. the decision to cease elution of the milled pulp is based on a visual assessment of the wash water and of the pulp.

The cassava is processed continuously in the factories, but the production was interrupted on these six occasions and the roots corresponding to one drum load weighed (usually about 320 kg) and processed separately; this permitted measurement of the quantity of water used and of the weight of residue produced. The data in Tables 1 and 2 have been standardized to relate to 100 kg dry weight of raw material (which is similar to one washer-drum load). Averages of the data for the four production runs studied in factory A are shown in Table 1. The moisture and starch contents of the four different fractions studied are

Table 1. Starch concentrations at the different process stages: Factory A

Process stage	Moisture content (%)	Dry matter (kg)	Starch conc. (% dry basis)	Quantity of starch (kg)
Milled cassava	72.8	100	78.4	78.4
Residue	85.3	28.4	61.9	17.6
Green starch	44.7	73.9	82.3	60.8*
Dry starch	8.6	71.7	84.8	60.8*

*Quantity of starch present in green (and dry) starch is calculated by subtracting that in the residue from that in the milled cassava, i.e. assuming that there are no further starch losses following separation of the residue (see text). The dry-matter weights of these fractions is calculated from these starch quantities and the measured starch and moisture contents.

Table 2. Starch concentrations at the different process stages: Factory B

Process stage	Moisture content (%)	Dry matter (kg)	Starch conc. (% dry basis)	Quantity of starch (kg)
Milled cassava	88.3	100	73.2	73.2
Residue	89.3	22	41.9	9.2
Green starch	74.4	86.1	74.3	64.0*
Dry starch	6.0	76.5	83.7	64.0*

*As explained in Table 1.

similar for these four sets of analyses (±5%), and are similar to those measured in factory B (Table 2). Factory B used more water than factory A, average data are 600 1/100 kg of roots and 450 1/100 kg, respectively. This, combined with the different milling and elution system, which may be more efficient (though this was not obvious from pulp appearance) could explain the more efficient starch removal from the residue. The residues from factories A and B contain 22.5% and 12.6%, of the cassava starch, respectively.

It is not possible to weigh the sedimenting starch (green starch) from one batch (drum load) of roots, without causing 3 days disruption in the factory (Fig. 1). The quantity of starch in the green starch and dry starch fractions (Tables 1 and 2) is therefore a calculated figure: the starch present in the raw material less that present in the residue. This is an over-estimate, since starch is lost in the water draining from the sedimentation tanks, and during the green starch transfer to the drying trays. The final starch yields in Tables 1 and 2 are 77.5% and 87.4%, respectively. This corresponds to 1 part by weight of starch from about 4.5 parts of raw material, whereas the subjective data of the factory owners is 1 part of starch from 5 or 6 parts of raw material.

The starch concentrations in the factory B samples were determined both by the methods of Gaines (1973) and Krochmal and Kilbride (1966). This comparative study was done because the starch concentration of the green starch by the

Gaines (1973) method is, surprisingly, only slightly higher than that of the raw material. This method depends on the purification of the gelatinized starch by iodine complex formation. Starch-iodine complex formation depends on starch molecular weight (Whistler & Paschall, 1965). The slow sedimentation process (Fig. 1) is accompanied by lactic acid fermentation, which causes starch hydrolysis (Cardenas & de Buckle, 1980). This partial hydrolysis is likely to reduce the starch-iodine affinity and lead to an underestimation of starch contents by the Gaines method.

The Krochmal and Kilbride method (1966) is quicker but less specific: the sample is homogenized in water, sieved and dried to constant weight. Wholey and Booth (1979) recommended this simple method for starch determination in cassava roots. Starch contents are overestimated since all root compounds (e.g. proteins, other polymers, etc.) are estimated as starch. Starch degradation does not affect this method. This may explain the higher starch concentrations in the residue, green and dry starch fractions reported by the latter method: 51%, 91% and 100% respectively as compared with 41.9% 74.3% and 83.7% respectively (Table 2) by the Gaines method. Both methods give similar values for cassava raw material.

Cyanide stability in root homogenates and sampling considerations

Both factories are 2–3 hr from the CITA laboratories and initial experiments were necessary to evaluate the effects of such a delay prior to sample preparation. Homogenization of whole roots in water leads to the rapid conversion of bound cyanide to free cyanide (Fig. 2), which is rapidly lost from the homogenates. The experiment was repeated with a range of different cassava : water ratios between 1 : 0.7 to 1 : 2 (by weight) and similar rates of loss of cyanide to

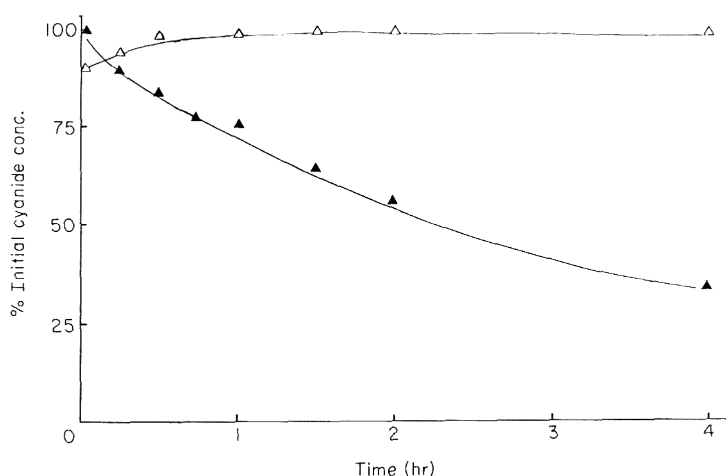

Figure 2. Stability of the total and free cyanide contents of cassava root homogenates. The homogenates were stored at 28°C in shallow trays, as described in the materials and methods section of this paper. The free cyanide content (△) is expressed as a percentage of the total cyanide content (▲) at each time.

those shown in Fig. 2 were observed. This rapid loss of cyanide depends primarily on the initial conversion of bound to free cyanide (Cooke & Maduagwu, 1978). This is emphasized by experiments (p. 246) in which thin slices of cassava water were stored in water under the same conditions as those of Fig. 2. The damaged surface area permits linamarase action on the bound cyanide, but the percentage of free cyanide is much less than that in homogenates. The residual cyanide after 4 hr is about 75%, as compared with 35% in the homogenates.

Figure 2 indicates that samples must be processed in the factory to obtain a representation of cyanide distribution during the process, and to determine the proportion of cyanide that is free. The samples at each process stage were weighed into phosphoric acid within 5 min of sampling, mixed and stored in stoppered bottles. The phosphoric acid inactivates endogenous linamarase and stabilizes the cyanide content of the sample (Cooke, 1978).

Residual cyanide concentrations during starch extraction

The cyanide behaviour in the two factories is shown in Tables 3 and 4. The cyanogenic glucosides are rapidly hydrolyzed during milling; in the milled pulps from both factories 70–80% of the cyanide is present as free cyanide. This percentage remains between 80 and 100% for the cyanide in the wash water and freshly sedimenting starch; but after 2–3 days sedimentation (green starch) and sun-drying (dry starch) this percentage decreases, presumably because the free cyanide is removed faster than the residual bound cyanide is hydrolyzed during these later phases. The endogenous linamarase is wholly or partly inhibited by the acid conditions (Cooke, Blake & Battershill, 1978) and the reduced water activity during these later phases.

The milling processes in both factories reduce the total cyanide content by about 15%. The residue from factory A contains about twice as much of the initial cyanide as that from factory B. This is in accordance with their relative starch contents(Tables 1 and 2) and confirms that the extraction procedure of factory B is more effective than that of factory A.

The greatest proportion of the cassava cyanide appears in the wash water (40–70%). In factory A, the long shallow wooden canals permit a slower sedimentation of the eluted starch than in factory B which has deeper sedimentation tanks. Consequently the wash water sample from factory A has a higher solids and cyanide content than that from factory B; both samples were taken about 1 hr after the start of the milling operation. Samples of sedimenting starch, taken about 2 hr after the start of the milling operation, are variable in solids and cyanide content. This variability arises from the quantity and pressure of wash water used, thickness of starch layer already in the tank, etc. In the six batches tested, the percentage of the initial cyanide present in this sedimenting starch varies between 8 and 14%. This percentage decreases to 2.5–3.9% in the sedimented or green starch (2–3 days later).

Table 3. Cyanide concentrations at the different stages of starch extraction: Factory A

Stage	Moisture content (%)	Dry matter (kg)	Cyanide conc. (mg/100 g, dry basis)	Proportion of cyanide in free form (%)	Quantity of cyanide (g)	
Fresh roots	61.0	100	40.9	14	40.9	(100)*
Milled roots	72.8	100	35.4	81	35.4	(87)
Residue	85.3	28.4	13.2	87	3.74	(9.2)
Wash water	93.8	11.9	229.4	100	27.30	(66.7)
Green starch	44.7	73.9	1.4	96	1.04	(2.5)
Dry starch	8.6	71.7	0.4	59	0.29	(0.7)

*The figures in parentheses are the quantities of cyanide at each stage as a percentage of that in the raw material.

Table 4. Cyanide concentrations at the different stages of starch extraction: Factory B

Stage	Moisture content (%)	Dry matter (kg)	Cyanide conc. (mg/100 g, dry basis)	Proportion of cyanide in free form (%)	Quantity of cyanide (g)	
Fresh roots	66.3	100	68.3	26	68.3	(100)*
Milled roots	88.3	100	58.1	71	58.1	(85)
Residue	89.3	22	14.8	33	3.26	(4.8)
Wash water	98.8	19.6	143.4	83	28.11	(41.2)
Green starch	74.4	86.1	3.1	64	2.66	(3.9)
Dry starch	6.0	76.5	0.12	29	0.09	(0.13)

*As for Table 3.

The green starch is spread onto trays and periodically crumbled by hand to assist drying. Drying is stopped 2–5 days later depending on product appearance; this corresponded to a moisture content range of 5–10%. The residual cyanide concentration of 1–4 p.p.m. corresponds to a quantity of cyanide between 0.1 and 0.7% of the cyanide initially present in the raw material.

Discussion

Minor differences in cyanide and starch distribution during starch extraction are evident in the two factories (Tables 1–4). This probably relates to the differences in process conditions described in the Materials and methods section (p. 252) of this paper. The same basic trends are however evident in all the data. A large percentage of the cyanogenic glucosides are rapidly hydrolyzed to free cyanide following tissue disintegration during milling. The short time involved leaves little opportunity for a microbial role in this hydrolysis. The conversion rate is similar to that shown in a laboratory experiment (Fig. 2) using distilled water and relatively non-contaminating conditions; this indicates that endogenous linamarase is responsible for the hydrolysis.

An earlier study (Cooke & Maduagwu, 1978) showed that free cyanide is removed far more rapidly than bound cyanide by soaking in water, this explains the presence in the wash water of a large proportion of the cassava cyanide. Meuser *et al.* (1978) indicated concern about the possible cyanide content of the wash water used in starch extraction (which they did not analyse), and recommended that this water must be heated to decompose the linamarin present. The relative stability of the β-glucoside would necessitate an extreme heat treatment (Wood, 1966). However the data presented here (Tables 3 and 4) indicate that the problem is simpler since the wash water cyanide is mainly free cyanide.

The freshly sedimenting starch contains about 8–14% of the cyanide present in the raw material, and this is reduced during the 1–3 day sedimentation to less than 4%. This sedimentation is accompanied by a natural fermentation which Cardenas and de Buckle (1980) have shown to be caused by lactic acid bacteria. The pH drops from 6.5 to 3.5 within 2–3 days and then remains stable for the 8–20 day period used in Colombia to produce sour starch. This sour starch undergoes considerable hydrolysis during the 8–20 day fermentation period, which is responsible for the improved functional properties in the traditional baked product, pan de yuca (de Buckle *et al.*, 1978). Ngaba and Lee (1979) have reported that lactic acid fermentation is also responsible for the characteristic flavour and aroma of 'gari', a West African fermented cassava product.

In Costa Rica, the starch is left to sediment a maximum of 3 days. The wash water pH drops sharply after the first 18–24 hr, in agreement with earlier studies (Cooke & Maduagwu, 1978). The reduction in cyanide content during this period may be simply due to the continued elution of the cyanide during sedimentation, or it could reflect microbial activity. Meuser (1978) stated that

fermentation does not remove linamarin, while Dahlberg (1978) stated that fermentation reduces toxicity; neither author presented supporting data.

Cooke and Maduagwu (1978) had shown that slow, low temperature drying processes remove residual cyanide more effectively than rapid high temperature ones. This is in accordance with the considerable reduction in cyanide content caused by sun-drying the green starch. The cyanide concentration of 1–4 p.p.m. corresponds to less than 1% of the cyanide content initially present in the raw cassava. Pieris, Jansz and Kandage (1974) and Meuser (1978) reported a few analyses of cassava starch products using less sensitive assay techniques, the ranges were 4–11 p.p.m. and 0–2.5 p.p.m., respectively. These authors did not measure the cyanide content of the raw material or of the intermediate products. A key step in obtaining these very low residual cyanide concentrations is the conversion of most of the bound cyanide to free cyanide in the initial process stage. Free cyanide is much easier to remove than bound cyanide (Cooke & Maduagwu, 1978), and the extended mixing with water, soaking in water (with associated fermentation) and slow sun-drying constitute an efficient process for removing this residual cyanide.

The starch yields are similar to those reported in Colombia (de Buckle *et al.*, 1978), i.e. about 20% of the fresh weight of the raw material. Improved yields can be obtained by rapid, large-scale processes, depending on centrifugal separation of the starch; this minimizes starch degradation and increases its value for most applications (Dahlberg, 1978). Water requirements are about 20% of those used in the traditional factories studied here, which minimizes pollution problems. However, the rapid sedimentation and drying involved is likely to reduce the efficiency of cyanide removal, for the reasons described above. This may cause problems in view of increasing medical concern (Ermans *et al.*, 1980) about residual cyanide levels in foodstuffs.

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Nutritional, fatty acid and oil characteristics of different agricultural seeds

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Summary

Recently increased attention has been given to the utilization of agricultural waste products to produce food, feed, fertilizer and as a raw material in certain industries. Such utilization could not only help maximize available resources but at the same time minimize waste disposal problems. Nutritional and oil characteristics of different seeds were investigated. On a dry basis protein contents of 43, 34, 16.1 and 36.9% and oil contents of 19, 50.6, 35.9 and 25.7% were found in maple, pumpkin, citrus and apple seeds, respectively. Determined food energy values averaged 626 kcal/100 g (26.2 kJ/g). The seeds were found to have valuable amounts of essential minerals. The major fatty acid composition was 18 : 2 (linoleic) at concentrations of 39.1, 58.9, 31.9 and 45.6% and total saturated fatty acids of 16.8, 19.7, 42.8 and 12.4% and *cis, cis*-PUFA contents were 34.1, 46.8, 19.8 and 45.6%, respectively, for maple, pumpkin, citrus and apple; no measurable amounts of *trans* fatty acids were found.

Introduction

It has been stated (Kramer & Kwee, 1977*a*) that only 20–30% of plant material grown in the U.S. is utilized directly for human consumption. If the remaining portion of this material, or even part of it, could be converted into nutrients for food or feed or into fertilizer, an important contribution to food resources and industrial products could be made. At the same time waste disposal problems would be minimized. Such utilization could be done economically only in locations where such resources are available in large quantities. If different vegetable and fruit seeds were to be utilized they could provide significant

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amounts of calories and proteins and also could provide extra income to farmers.

Different seeds are being utilized for food and feed especially legumes as a result of their high protein content. Guar seed has recently been utilized in India and Pakistan (Arora, 1981). Tomato seed cake was found to contain over 39% protein and its amino acid composition compares favorably with that of soybean protein (Vicchiotti & Piva, 1964). Kramer and Kwee (1977*b*) found that tomato protein concentrates from process wastes have protein quality similar to that of soy protein or cotton seed concentrate. Several seeds of the Cucurbitaceae have been investigated for their oil and protein values. Oil from the seeds of bottle gourd (Luffa) has been used to some extent in Nigeria. The oil content is about 45.7% (Omidiji, 1977), 83.1% of the total fatty acids are composed of oleic and linoleic acid and the protein content is 37.5%. Melon seed oil has also been utilized in many areas of Nigeria. The oil content was 51% and 74% of the fatty acids which were oleic and linoleic (Girgis & Said, 1968).

The problems of industrial and farm waste are becoming more and more difficult to handle and, therefore, every effort has to be made by scientists to investigate the nutritional and industrial potential of available unutilized agricultural products, especially in countries where other resources are limited.

Maple seeds are unutilized and could be collected in large quantities. Citrus and apple seeds are a by-product of food processing plants. Pumpkin seeds are partially utilized by direct consumption but large quantities are wasted.

Recently, much emphasis in research has been directed toward protein shortages and less attention has been directed toward finding new oil and fat resources. The present study was undertaken to determine the nutritional potential, oil content, fatty acid composition and oil characteristics of maple, pumpkin, citrus and apple seeds.

Materials and methods

Materials

Maple seeds (*Acer saccharum*) were obtained from the seed extraction plant, Ministry of Natural Resources, Angus, Ontario. Unroasted pumpkin seed kernels, *Cucurbita mixta*, were obtained from a local health food store and citrus seeds were extracted from fresh tangerines and oranges. McIntosh apple seeds were supplied by the Department of Horticultural Science, University of Guelph.

Methods

The kernels of pumpkin and maple and the whole seeds of apple and citrus (kernel and testa) were used for proximate analysis and mineral composition determination.

Moisture content was determined directly on all seeds using an air oven drying method at 102°C for 6 hr. The rest of the seeds were dried in an air flow oven at 65°C for 6 hr, then ground in a Wiley mill to pass through a U.S. standard 20 mesh sieve. The ground seeds were dried in a vacuum oven at 60°C for 6 hr, stored in glass containers, placed in a desiccator and stored at 4°C until used.

Proximate analysis was performed in duplicate in accordance with the 1975 AOAC Procedures. Ether extraction for crude fat using Soxhlet apparatus, macro-Kjeldahl for crude protein (%N × 6.25), ash by heating at 550°C overnight and crude fiber by digestion with 1.25% H_2SO_4 and 1.25% NaOH using a Fibertec analyzer (Tecator Hoganes, Sweden). Food energy values were determined using an oxygen bomb calorimeter (Parr Instrument, U.S.A.) and calculated energy values were obtained by using the conversion factor 9 for fat and 4 kcal/g for protein and carbohydrate.

The method described by the Health Protection Branch, Canada Department of Health and Welfare (1977) was used for the dry ashing and mineral determinations and an atomic adsorption spectrophotometer was employed to measure Mg, Fe, Zn, Cu, Ca and K. The spectrophotometric molybdovanadate method (AOAC 22.042, 1975) was used for determination of phosphorus.

Fatty Acid Analysis

Oil from the whole seeds of apple, and citrus and the kernels of pumpkin and maple were extracted with a mixture of 2 : 1 v/v chloroform/methanol using a solvent ratio of 20 : 1. The seed-solvent mixture was homogenized using a Sorvall Omni mixer (Ivan Sorvall, U.S.A.) for 3 min and the procedure described by Folch, Lees & Stanley (1957) was followed to obtain the oil. The methyl esters of the fatty acids were prepared using the procedure described by Shehata, deMan & Alexander (1970). Fatty acid methyl esters (FAME) were analyzed with a Varian 3700 gas chromatograph on a 125 cm stainless steel (I.D. 3.2 mm) column packed with 15% DEGS on Chromosorb W-60/80 mesh and operated at 180°C.

The *cis, cis* methylene interrupted polyunsaturated fatty acids (PUFA) were determined by the lipoxidase method described by the Health Protection Branch, Canada Department of Health and Welfare (1967). Isolated *trans* fatty acids were determined according to AOCS procedure (Cd–14–61, 1975) using a Beckman IR 4230 infrared spectrophotometer.

Oil Characteristics

Refractive indices of the different oils were measured at 60°C with a Zeiss refractometer, solidification and melting ranges were determined using differential scanning calorimetry (DuPont 900 thermo-analyzer). Iodine values (AOCS Cd–1–25, 1974), saponification values (AOCS Cd–3–25), unsaponifiable matter (AOCS Ca–60–40, 1974) and acid values (AOCS Cd–3a–63, 1974) were all determined in triplicate and their mean values were reported.

Iodine values were also calculated from the fatty acid composition using the I.V. of the individual fatty acids and free fatty acids expressed as % oleic and palmitic acids were calculated by dividing the acid values by 1.99 and 2.19 for oleic and palmitic acids, respectively.

Results and discussion

The moisture content of pumpkin and maple kernels was 5.1 and 6.4%, respectively, and the whole seeds of apple and citrus had moisture levels of 31.5 and 50.5%, respectively.

The nutrient composition of the seeds is presented in Table 1. The range of protein contents was 16.1 to 43%, and oil contents were 19, 50.6, 35.9 and 25.7% for maple, pumpkin, citrus and apple, respectively. The oil contents of pumpkin and citrus are high and compare well with other oil seeds such as sunflower, soybean and peanut. Pumpkin oil content and characteristics were also comparable to other cucurbitaceae such as melon and luffa (Girgis & Said, 1968; Porterfield, 1955).

Table 1. Proximate composition of the different seeds (% dry basis)

	(%)			
Components	Maple	Pumpkin	Citrus	Apple
Crude protein	43.00	34.04	16.15	36.93
Crude oil	19.03	50.59	35.92	25.68
Ash	7.05	4.13	3.78	3.76
Crude fiber	4.97	3.74	18.31	12.02
Carbohydrate (by difference)	25.95	7.50	25.84	21.61
Determined energy kcal/100 g	569	711	633	593
Calculated energy kcal/100 g	447	621	491	465

The crude fiber content of citrus 18.3% and apple 12.0% were much higher than maple 4.9% and pumpkin 3.7%. This can be explained by the fact that the maple and pumpkin seeds had been dehulled. Determined food energy values of all seeds averaged 626 kcal/100 g which is relatively high compared to other seeds. The determined energy values were higher than the calculated values with an average difference of 19.2%.

Table 2 shows the mineral composition of the different seeds. The Fe content was 8.8, 10.7, 21.2 and 7.6 mg/100 g for maple, pumpkin, citrus and apple, respectively. Ca content of maple and citrus seeds was 740 and 480 mg, respectively. Maple and pumpkin seeds were high in phosphorus containing 910 and 950 mg, respectively. Maple and apple seeds are good sources of Mg with contents of 340 and 320 mg, respectively. The potassium content ranged from 630–1690 mg/100 g. A fair amount of Cu and Zn was also present.

Table 2. Mineral composition of the different seeds (mg/100 g dry basis)

	Minerals mg/100 g						
Seeds	K	Fe	Mg	Ca	P	Cu	Zn
Maple	1690	8.79	340	740	910	3.25	6.14
Pumpkin	630	10.73	90	79	950	1.49	8.01
Citrus	1000	21.15	240	480	250	1.93	2.25
Apple	720	7.56	320	230	600	2.71	3.85

The lipid composition of the seeds is listed in Table 3. The major fatty acid of maple, pumpkin, citrus and apple oil was 18 : 2 (linoleic) as shown in Table 3 with contents of 39.1, 58.9, 31.9 and 45.6%, respectively. Such a composition is a typical characteristic of seed oils. Citrus oil was found to have an exceptionally high content of 16 : 0 (palmitic) at 34%. Maple oil contained 5.8% of 18 : 3. Such compositions are not very common among oils originating from plant products. Total saturated fatty acids were 16.8, 19.7, 42.8 and 12.4% and unsaturated fatty acids were 83.2, 80.3, 57.2 and 86.6% for maple, pumpkin, citrus and apple, respectively.

Table 3. Fatty acid composition of the different seed oils (wt. %)

	Wt. (%)			
Fatty acids	Maple	Pumpkin	Citrus	Apple
16:0	8.5	13.8	34.0	9.0
18:0	6.3	5.9	6.1	2.3
18:1	38.3	21.4	25.3	41.3
18:2	39.1	58.9	31.9	45.6
18:3	5.8	—	—	0.7
20:0	2.0	—	2.7	1.1
Total saturated	16.8	19.7	42.8	12.4
Total unsaturated	83.2	80.3	57.2	86.6
cis, cis-PUFA*	34.1	46.8	19.9	45.6

**cis, cis* methylene interrupted polyunsaturated fatty acid.

The *cis, cis* methylene interrupted polyunsaturated fatty acids (PUFA) determined enzymatically indicated that maple, pumpkin, citrus and apple oil contain 34.1, 46.8, 19.8 and 45.6%, respectively. No measurable amount of *trans* fatty acids were detected in all of the seed oils. *Trans* fatty acids are usually formed during the processing and hydrogenation of fats and oils.

Table 4. Oil characteristics of the different seeds

Characteristics	Maple	Pumpkin	Citrus	Apple
Refractive index $n_D^{60°}$	1.4599	1.4615	1.4592	1.4613
Solidification range C°	—	−4 to −48	−2 to −50	—
Melting range C°	−12.5 to −40	+2 to −46	−2 to −24	−12.5 to −37.5
Iodine value determined	112	119	81	116
Iodine value calculated	116	120	77	114
Saponification value	172	207	189	195
Acid value	4.15	3.55	0.82	4.35
Free fatty acid as:				
% Oleic	2.09	1.78	0.41	2.18
% Palmitic	1.89	1.62	0.37	1.99
Unsaponifiable matter	1.6	0.9	2.3	1.8

Different oil characteristics are presented in Table 4. The solvent extracted oils stayed liquid at room temperature as indicated by their melting and solidification ranges. Visual colors of the oils were dark green, pale green, yellow and brown for pumpkin, maple, citrus and apple, respectively. Small variations between calculated and determined iodine values were observed. The determined iodine values were 112, 119, 81 and 116 compared to the calculated values of 116, 120, 77 and 114 for maple, pumpkin, citrus and apple, respectively. The saponification values were 172, 207, 189 and 195, respectively. This is within the range of other plant oils. Acid values of maple, pumpkin and apple seed oil were high, with values of 4.15, 3.55 and 4.35, respectively. The acid value for citrus seed oil was 0.82. The unsaponifiable matter was in the range of 0.9–2.3 which falls within the range of that of other plant oils.

The results of this investigation show that maple, pumpkin, citrus and apple seeds constitute useful products of high nutritional value. The seeds could be utilized successfully in oil extraction and as a source of protein concentrates for human consumption, and as a high energy feed source for animal and poultry. The seeds may also be used as a fertilizer due to their high mineral and nitrogen content. Research on possible toxicity and economical feasibility should be conducted. For full utilization of the seeds, more work is also needed to establish different possible uses.

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Bioconversion of sugar cane bagasse for cellulase enzyme and microbial protein production

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Summary

A pilot scale (10 l) fermenter trial was conducted for the production of cellulase enzyme and single cell protein by growing a cellulolytic mould *Aspergillus terreus* GN_1 on 1.0% alkali-treated sugar cane bagasse substrate. A maximum crude protein content of 29.8% in the biomass was obtained during 4 days fermentation from the initial alkali-treated bagasse having 3.0% crude protein content. The carboxymethyl cellulase (CMCase) activity was almost 0 u/ml during the first 24 hr, which increased during 96 hr incubation to 0.52 u/ml, and corresponded with the highest filter paper enzyme (FPase) activity of 0.032 u/ml. There was an alternate decrease and further increase in water soluble carbohydrate during 96 hr incubation, which increased finally to 7.9%. There was no cellulose utilization during the first 24 hr of incubation. However, the cellulose of sugar cane bagasse substrate was utilized subsequently and a maximum of 72.5% of the initial cellulose was utilized up to 96 hr fermentation time.

Introduction

Nearly 90% of the cellulolytic waste is derived from agricultural sources, and the rest from urban and industrial wastes (Han, 1978). Although the annual production of these wastes is sufficient to satisfy the yearly cellulosic demands, little is utilized because of poor technology to develop feasible systems for waste management and its utilization. The cellulosic wastes have a great potential for their utilization as substrates for single cell protein (SCP), cellulase enzyme, energy and fuel production (Garg, Batish & Neelakantan, 1980). For these purposes, these wastes have to be first hydrolyzed by cellulolytic enzymes. Bacteria and fungi have been of particular interest in fermentation of cellulosic

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agricultural wastes because of their relatively short fermentation times, which enable them to be used economically for converting cellulosic wastes to glucose, which in turn could serve as the substrate, through secondary fermentation, for the production of microbial protein (Rogers, 1973), glucose syrups, fuels, chemicals, food and alcohol (Appell *et al.,* 1971; Allen, 1976; Andren, 1977; Spano, Medeiros & Mandels, 1976; Davis, 1975; Wilke *et al.,* 1976; Savarese & Young, 1978; Cysewski & Wilke, 1976). *Trichoderma viride* and *T. koningii* are two main moulds being utilized for cellulase production. However, Chahal and Hawksworth (1976) and Garg and Neelakantan (1981*a*) have recently reported that the mould species of *Geotrichum cellulolyticum* and *A. terreus* GN_1 were good cellulase, as well as protein producers. Single cell protein production through bioconversion of substrates by algae, bacteria, moulds and yeasts has become increasingly important because of their shorter generation time, rapid biomass production, lack of land requirement and the wide range of substrates on which they can be grown. The use of single cell protein has already become familiar in livestock and poultry feed components and can possibly be a potential source for human consumption as well in under nourished countries.

The microbial utilization of native cellulose in substrates is slower than the pretreated or denatured cellulose. Although various forms of pretreatment of cellulosic materials have been proposed, their effectiveness varied depending upon the nature of the substrate (Han, Dunlap & Callihan, 1971). Han and Callihan (1974) reported that treatment of rice straw and sugar cane bagasse with 4.0% NaOH for 15 min at 100°C increased the digestibility of cellulose from 29.4 to 73%. Garg and Neelakantan (1982*a*) reported that eventhough the alkali-treatment of sugar cane bagasse resulted in 45% loss in dry matter, the delignified and decrystallized cellulose was made more biodegradable through microbial enzyme action. Garg and Neelakantan (1981*b*, 1982*a*) standardized the optimum cultural and nutritional conditions for the growth of *A. terreus* GN_1 on 1.0% alkali-treated sugar cane bagasse substrate as sole carbon source in a modified Czapek's broth *viz.*, pH 4.0, 5.0% (v/v) inoculum of week-old culture, 28 ± 1°C incubation and continuous aeration. The present investigation deals with the fermenter trial for single cell protein and cellulase enzyme production by growing *A. terreus* GN_1 on 1.0% alkali-treated sugar cane bagasse substrate under optimum cultural and nutritional conditions.

Materials and methods

Micro-organisms

A. terreus GN_1, an efficient cellulolytic mould isolated from a mixture of sugar cane bagasse and cellulosic agricultural waste undergoing biodegradation and characterized in our laboratory, was used for the present investigation. The standard pure culture was maintained on Czapek's agar slants impregnated with filter paper strips as a sole carbon source (Garg & Neelakantan, 1981*a*).

Preparation of substrate

One part of sugar cane bagasse powder was pretreated with twenty parts of 4.0% sodium hydroxide solution by autoclaving for 30 min at 121°C. The recovered material was washed repeatedly with distilled water 'til neutrality. The pretreated bagasse was dried in an oven at 60°C and ground to 40 mesh size.

Cultivation conditions

To investigate the optimum fermentation time in a pilot scale fermenter of 10 litres capacity with optimum cultural conditions *viz.*, pH, aeration, shaking, temperature, dose of inoculum, nutritional factors, different nitrogen sources and charging rate of bagasse substrate on microbial protein and cellulase enzyme production, a modified Czapek's broth (Haynes, Wickerham & Hesseltine, 1955) was used containing (per litre): 600 mgN of cornsteep liquor; 1.0 g KH_2PO_4; 0.5 g KCl; 0.5 g $MgSO_4.7H_2O$ and 10.0 g alkali-treated sugar cane bagasse substrate. It was adjusted to pH 4.0, autoclaved and after cooling poured aseptically into a sterilized fermenter and then inoculated with 5.0% (v/v) inoculum of a week-old *A. terreus* GN_1. Also added was 0.2% (v/v) sterilized anti-foaming agent (10% v/v Aerocil in liquid paraffin). The fermenter was operated at 28±1°C with a continuous aeration of 2.0 l/min. However, the agitator was driven at 120 rev/min at alternate 6 hr intervals, during 96 hr fermentation for occasional mixing of the fermenting slurry.

Harvesting

The samples of fermenting slurry were drawn periodically, every 12 hr for 4 days. About 200 ml of each sample was centrifuged at 10 000 rev/min for 15 min and the residual solid biomass was mixed with distilled water and filtered through Whatman No. 1 filter paper to harvest fungal biomass (SCP) along with unfermented bagasse substrate. The contents were washed repeatedly with distilled water to remove any soluble nitrogen (absorbed from medium) adhering to the biomass. The filter paper along with biomass was oven dried at 60°C to a constant weight, and was analyzed for its nitrogen content, water soluble carbohydrate and residual cellulose.

The supernatant liquid after centrifugation was used for the assaying of cellulase enzyme activity.

Analytical determination

Crude protein. The total nitrogen content of the dried biomass was estimated by the conventional Kjeldahl's method (AOAC, 1975).

Assay of cellulase enzyme activity

(1) *Filter paper enzyme activity (FPase).* A quantity, 0.4 ml of the supernatant liquid was added to 0.6 ml of 0.05M citrate buffer at pH 4.8 and 50 mg Whatman No. 1 filter paper strip (1×6 cm) and incubated for 120 min at 50°C.

(2) *Carboxymethyl cellulase activity (CMCase or CX).* A quantity, 0.2 ml of supernatant liquid was added to 4.5 ml of 1.0% carboxymethyl cellulose (CMC) (1.0% CMC was prepared in 0.05M citrate buffer, pH 4.8) and 0.3 ml of 0.05M citrate buffer, and incubated for 120 min at 50°C.

The quantity of reducing sugars released in each FPase and CMCase assay after incubation was measured by the dinitrosalicylic (DNS) method (Miller, 1959). The enzyme activity is expressed as μmol glucose released/min/ml of supernatant liquid as enzyme solution.

(3) *Water soluble carbohydrates (WSC).* One gram dried and milled sample of microbial biomass was treated with 100 ml of 0.25N sulphuric acid. One millilitre of this extract was diluted to 25 ml with distilled water and 1.0 ml of the diluent was mixed with 0.15 ml of 80% aqueous phenol and 5.0 ml of concentrated sulphuric acid. The intensity of yellow-orange colour was measured with an Elico-Spectrocol at 490 nm and compared with the sugar standard curve. Water soluble carbohydrate was expressed in terms of percentage on dry weight basis (Dubois *et al.*, 1956).

(4) *Cellulose estimation.* Two hundred mg of sample in 10.0 ml distilled water was homogenized and centrifuged at 2000–3000 rev/min for 5 min and the supernatant liquid was discarded. The residue was mixed thoroughly in 3.0 ml acetic/nitric reagent (acetic/nitric reagent was prepared by mixing 150 ml of 80% acetic acid and 15.0 ml concentrated nitric acid). The tubes were placed in boiling water bath for 30 min. Tubes were again centrifuged and the supernatant liquid was discarded. The residue was washed thoroughly with 10.0 ml distilled water and after discarding the supernatant liquid, 10.0 ml of 67% sulphuric acid was added and kept for 1 hr at room temperature. One millilitre of this was diluted to 100 ml with distilled water. One millilitre of the above diluent was mixed with 4.0 ml distilled water and kept in an ice bath for cooling. Ten millilitres chilled anthrone reagent (prepared by adding 0.2 g anthrone to 100 ml concentrated sulphuric acid) was added to each tube. The contents were mixed gently on a vortex-mixer and returned to the ice bath. These tubes, with glass marbles on top (for condensing the evaporating steam), were placed in a boiling water bath for 16 min. The intensity of green colour was read on an Elico-Spectrocol at a wave-length of 620 nm against a reagent blank and cellulose content was extrapolated against cellulose standard curve (Updegraff, 1969).

Results and discussion

The effect of incubation time on cellulase enzyme production by *A. terreus* GN_1 on alkali-treated sugar cane bagasse substrate is presented in Fig. 1. The

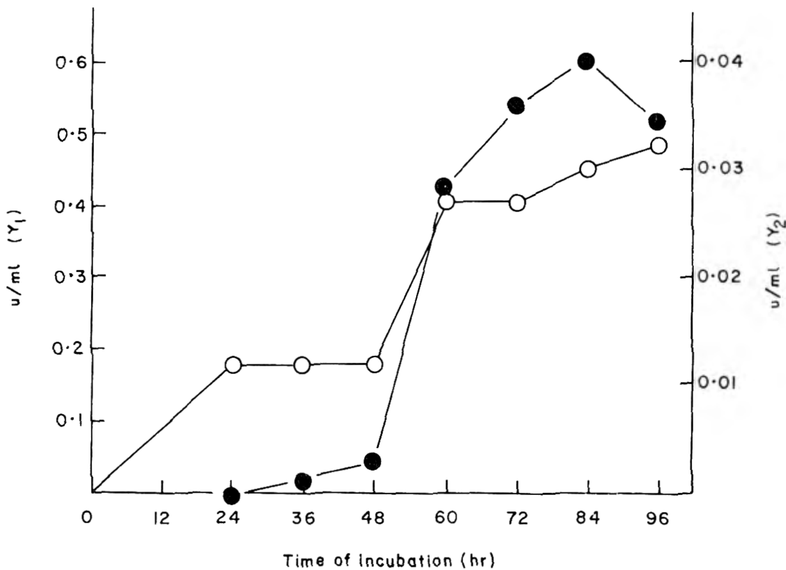

Figure 1. Effect of growth of *A. terreus* GN_1 with alkali-treated bagasse on cellulase enzyme activity in 10-l fermenter. ●, carboxymethyl cellulase (CMCase) activity (Y_1); O, filter paper enzyme (FPase) activity (Y_2).

carboxymethyl cellulase (CMCase) activity was zero during the initial 24 hr of fermentation and then slightly increased up to 48 hr. Then there was a significant increase in CMCase activity up to 84 hr. The CMCase activity was in the range of 0.02–0.6 u/ml after 84 hr fermentation and then decreased subsequently, when fermentation was prolonged up to 96 hr. The filter paper enzyme (FPase) activity ranged between 0.012–0.032 u/ml during 96 hr fermentation and corresponded positively with CMCase activity up to 84 hr incubation. Even though the CMCase activity was 0 u/ml during the first 24 hr fermentation, the culture filtrae showed a little FPase activity suggesting thereby that CMCase and FPase activities are associated with two different polypeptide components of a multicomponent cellulase enzyme complex system, which are induced independently. The maximum FPase activity of 0.032 u/ml was achieved at 96 hr incubation as compared to CMCase activity, which was maximum at 84 hr fermentation. However, Garg and Neelakantan (1982*a*) reported that the maximum CMCase activity of 0.9 u/ml and FPase activity of 0.076 u/ml could be obtained with alkali-treated bagasse substrate in a continuously shaken flask trial for 4 days under the same set of conditions. The reduction in the activity of cellulase enzyme in the fermenter trial can be attributed to its intermittent shaking. The similar results on CMCase and FPase activities of 0.4 and 0.04 u/ml were obtained in intermittently shaken flasks with $NaNO_3$ as nitrogen source with alkali-treated bagasse substrate (Garg & Neelakantan, 1981*b*). When untreated bagasse substrate at 1.0% concentration (w/v) with cornsteep liquor as nitrogen source was used in the continuously shaken flask trial with *A. terreus* GN_1 for 4 days, the CMCase and FPase activities were 0.45 and 0.019 u/ml (Garg & Neelakantan, 1982*b*).

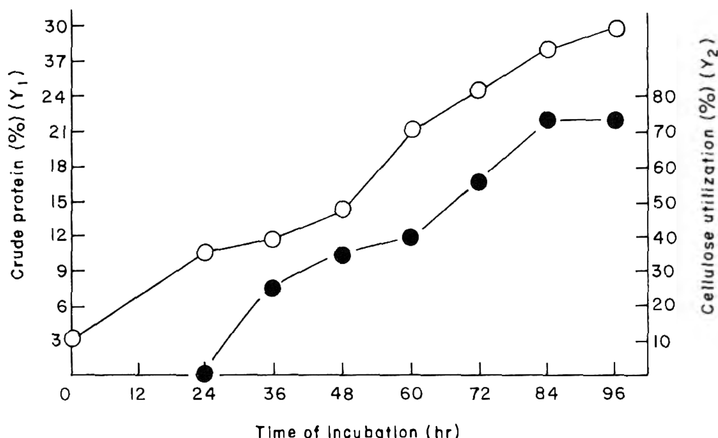

Figure 2. Effect of growth of *A. terreus* GN_1 with alkali-treated bagasse on crude protein and cellulose utilization in 10-l fermenter. O, crude protein (Y_1); ●, cellulose utilization (Y_2).

The efficiency of sugar cane bagasse cellulose utilization and its correlation with the crude protein content of the biomass produced by growing *A. terreus* GN_1 is presented in Fig. 2. The mould utilized bagasse cellulose in the range of 25.6–72.5%, when it was allowed to grow for 96 hr in the fermenter. There was no initial utilization of cellulose substrate in alkali-treated sugar cane bagasse during the first 24 hr. This might be due to the preferential utilization of the initial water soluble carbohydrate present in the substrate and the repression of cellulase enzyme synthesis due to the presence of the threshold concentration (approximately 8.0%) of water soluble carbohydrate. When the sugar level went below the critical repression level after 24 hr, the bagasse cellulose induced the synthesis of cellulase enzyme and concomitantly increased the cellulose utilization from 0 to 25.6% at 36 hr fermentation. No further cellulose was utilized as there was no more increase in cellulase enzyme activity. Hence, there was a positive correlation between cellulase enzyme induction and efficiency of sugar cane bagasse cellulose utilization. The crude protein content of the fungal biomass ranged between 10.5–29.8% as compared to 3.0% crude protein in control treated bagasse sample. The crude protein content of 28.0% was obtained with 72.5% utilization of bagasse cellulose at 84 hr incubation period. However, 29.8% crude protein was present in the biomass harvested after 96 hr fermentation but the bagasse cellulose utilization did not increase from 72.5%. It indicates that after 84 hr, the fungus grew at the expense of available water soluble carbohydrate, which decreased from 9.70% to 7.89% during 84 to 96 hr fermentation. Hence, crude protein content of biomass and bagasse cellulose utilization had a direct correlation with each other 'til 84 hr incubation time. However, *A. terreus* GN_1 produced 25.7% crude protein and utilized a maximum of 70.1% cellulose from alkali-treated sugar cane bagasse, when grown in continuously shaken submerged culture flasks for 4 days under similar nutritional and cultural conditions (Garg & Neelakantan, 1982*a*). When untreated bagasse substrate at 1.0% concentration (w/v) with cornsteep liquor as nitrogen

source was used in continuously shaken flask trials with *A. terreus* GN_1 for 4 days, the biomass crude protein was 14.0% and the bagasse cellulose utilization was only 8.5% (Garg & Neelakantan, 1982*b*). Araujo & D'Souza (1978) reported that *A. terreus* Thom Pale strain brought about 68.0–85.0% utilization of treated rice straw cellulose with 44.4% of protein in residual biomass during 1 week fermentation.

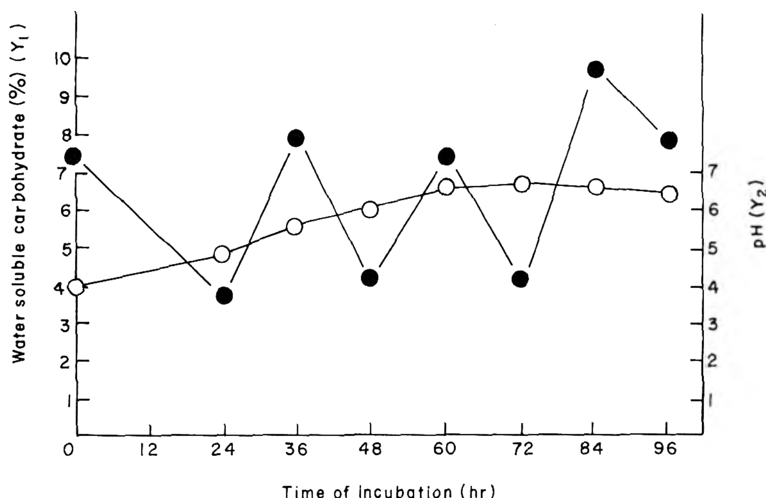

Figure 3. Effect of growth of *A. terreus* GN_1 with alkali-treated bagasse on water soluble carbohydrate and pH in 10-l fermenter. ●, water soluble carbohydrate (Y_1); O, pH (Y_2).

Figure 3 depicts the levels of water soluble carbohydrate and change in hydrogen ion concentration (pH) during fermentation of alkali-treated sugar cane bagasse by *A. terreus* GN_1 for 96 hr in the fermenter. The results indicate an initial decrease in water soluble carbohydrate from 7.36%, which was present originally in alkali-treated sugar cane bagasse substrate to 3.68% during the first 24 hr of fermentation. The alternate decrease and increase in water soluble carbohydrate in a regular fashion may be explained on the grounds of induction and repression of cellulase enzyme at a particular concentration of water soluble carbohydrate. This behaviour of water soluble carbohydrate variation can also be explained on the basis of end product (feed back) inhibition of cellulase enzyme system (Sternberg, 1976; Ryu & Mandels, 1980). The oscillations in water soluble carbohydrate may also be due to the changes in fermentation conditions of the slurry, which was agitated at 6 hr intervals for the occasional mixing of the fermenting slurry along with continuous aeration of 2.0 l/min. The variation in free sugars in the biomass might also be due to simultaneous preferential utilization of initially available sugars and subsequent hydrolysis of bagasse cellulose by cellulase enzyme complex. The initial pH of

4.0 in the fermentation broth increased gradually to 6.7 during 72 hr fermentation followed by a slight decrease up to 6.42 at 96 hr fermentation time. The extent of sugar fermentation remains directly related to acid production, but the decrease in hydrogen ion concentration i.e., increase in pH, in the present study, could possibly be due to simultaneous utilization of acids by the mould (Mandels, Sternberg & Andreotti, 1975).

The beneficial effect of cornsteep liquor with alkali-treated bagasse substrate was significantly higher over sodium nitrate nitrogen source (Garg & Neelakantan, 1981*b*). The enhanced growth and activity of *A. terreus* GN_1 with cornsteep liquor may be attributed to the fact that besides nitrogen, it also supplies a balanced source of carbon, sulphur, mineral salts, amino acids and growth factors, which might have contributed to enhanced cellulose utilization and protein production (Rhodes & Fletcher, 1977).

Conclusion

From the above results, it was observed that by using alkali-treated sugar cane bagasse (1.0%) as substrate with *A. terreus* GN_1 during 4 days fermentation, a maximum biomass crude protein content of 29.8% with 72.5% bagasse cellulose utilization could be obtained. In the case of 4 days fermentation in a continuously shaken flask trial using untreated bagasse substrate, the crude protein content in biomass was only 14.0% and in comparison, the fermenter trial using alkali-treated bagasse yielded 112.9% more crude protein content in biomass (Garg & Neelakantan, 1982*b*). The untreated sugar cane bagasse has 30.8% cellulose content and much of it is ligno-cellulose form and during 4 days fermentation with *A. terreus* GN_1, only 8.0% of the cellulose was utilized to yield biomass crude protein content of 14.0%. In the case of alkali-treated bagasse substrate with a dry matter recovery of 55.0% containing 51.7% cellulose, much of it in available form, the present fermenter trial has yielded a biomass crude protein content of 29.8% with 72.5% cellulose utilization. However, the present fermenter trial has yielded 16.0% more crude protein content in the biomass than did the continuous shaken flask trial for 4 days.

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Cholesterol content of poultry meat and cheese determined by enzymic and gas-liquid chromatography methods

J. KARKALAS, A. E. DONALD AND K. M. CLEGG

Introduction

An upsurge of interest in the cholesterol content of foods has inspired the application of new techniques to replace the older methods of analysis. For example, some of the data included in the recent edition of 'The Composition of Foods' (Paul & Southgate, 1978) are based on analysis by gas–liquid chromatography (GLC) and a laboratory kit is available from Boehringer Corporation Ltd. (BCL) for the enzymic determination of cholesterol. The principle of the BCL method is based on oxidation by cholesterol oxidase with the liberation of hydrogen peroxide which then oxidizes methanol to formaldehyde in the presence of catalase; the formaldehyde reacts with acetylacetone and NH_4^+ to yield lutidine which is spectrophotometrically measured at 450 nm.

The present study was undertaken to compare the merits and reproducibility of the above two methods. Most of the test materials were poultry meat but two varieties of cheese were also analysed for cholesterol content.

Materials and methods

To provide samples for investigating the two methods of analysis, a whole chicken was cut in half longitudinally; one half was cooked in a 'Roastabag' at 190°C for 1 hr. The raw and cooked chicken were divided into skin, leg, wing, light meat (breast) and dark meat from the spinal and pelvic regions of the bird. Samples of roasted light and dark turkey meat were also obtained. The cheese varieties were Double Gloucester (hard) and Edam (semi-hard). All samples were mechanically homogenized in a Moulinex chopper® before taking portions for analysis.

Moisture content was determined by drying after mixing with sand, as described by Pearson (1976) for meat and cheese. Fat content was determined by

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the Werner-Schmid method (Pearson, 1976).

The GLC procedure of Kovac *et al.* (1979) has been slightly modified. A Pye 104 chromatograph fitted with a flame ionization detector and a glass column (1.5 m×2 mm i.d.) packed with 3% OV-17 on Gas Chrom Q (100/120 mesh) was used; the injector, column and detector temperatures were 250°C and the nitrogen, hydrogen and air flow rates were 30, 30 and 500 ml/min respectively. Samples of test material (0.5 g) containing approximately 0.4–0.6 mg cholesterol were refluxed with ethanolic KOH in 25 ml pear-shaped Quickfit flasks for 1 hr and the unsaponifiable components were extracted with four 5 ml portions of *n*-hexane. The combined extracts were dried by distillation and, finally, in an air oven. On cooling, 0.5 ml *n*-hexane containing 0.3 mg 5α-cholestane as internal standard was added. A sample (3–4 μl) was injected on to the column. Derivatization was unnecessary.

BCL provide an instruction sheet (Cat. No. 139050) for the enzymic determination of cholesterol. Samples (1.5 g) were refluxed with aqueous methanolic KOH, the supernatants were transferred to 25 ml volumetric flasks and the residues were twice extracted by further refluxing with isopropanol; the combined supernatants were made up to volume with isopropanol and 0.4 ml samples were taken for analysis. Separation from the saponified material was unnecessary.

All the results are the average of a minimum of duplicate analyses.

Results and discussion

Both the GLC and the enzymic methods gave a linear response with increasing concentration of cholesterol; recovery values for cholesterol subjected to the extraction procedure were 97.7 and 99.2%, respectively. Table 1 shows the cholesterol contents of 14 samples determined by GLC and enzymic analysis. The agreement between the two methods is very good. Moisture and fat values are included so that the cholesterol content may be related to the dry weight or fat content.

The commercially available oxidase derived from *Nocardia erythropolis* reacts with any sterol having the hydroxyl group at carbon atom 3 in the β-position. Thus, phytosterols such as stigmasterol and sitosterol, present in vegetable oils, will interfere in the enzymic analysis of cholesterol. Rietsch and Entressangles (1980) have found a similar situation with the sterol oxidase of *Mycobacterium,* except that the rate of reaction is faster for cholesterol relative to the phytosterols. For the purpose of this comparative study, the investigation was therefore restricted to animal products. However, it was established that interference from other sterols would not occur in GLC analysis under the conditions employed as 5α-cholestane, cholesterol, stigmasterol and β-sitosterol were eluted after approximately 8, 19, 27 and 31 min respectively.

The GLC procedure (Kovac *et al.*, 1979) no longer requires the preparation of derivatives; the possibility that removal of the unsaponifiable components might

Table 1. Moisture, fat and cholesterol contents of poultry meat and cheese

Sample	Moisture (g/100 g)	Fat (g/100 g)	Cholesterol (mg/100 g fresh wt.)		
			GLC	Enzymic	Published*
Chicken (raw)					
skin	41.3	48.6	128	130	—
leg	75.4	5.9	90	92	—
wing	76.4	5.9	98	97	—
light meat	74.7	5.0	67	70	69
dark meat	69.1	11.7	107	109	110
Chicken (cooked)					
skin	46.8	44.2	73	78	—
leg	71.8	8.0	120	122	—
wing	71.0	8.4	136	140	—
light meat	67.7	5.3	80	80	74
dark meat	60.2	15.4	92	93	120
Turkey (cooked)					
light meat	70.3	4.1	82	79	62
dark meat	62.2	5.3	89	84	100
Cheese					
Edam	44.3	23.2	59	61	72
Double Gloucester	39.7	32.3	83	83	—

*(Paul & Southgate, 1978)

also be unnecessary was investigated by injecting the extract prepared for enzymic analysis on to the GLC column. However, the saponified fatty acids and other components interfered and since there was danger of damage to the column packing this short-cut is not recommended.

It is suggested that both the enzymic and the GLC methods are equally reliable and reproducible for the determination of cholesterol in animal products but GLC analysis is recommended for foods known to contain a mixture of animal fats and vegetable oils.

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Technical note: Drying fruit purées on an improved pilot plant drum-drier

J. A. KITSON AND D. R. MACGREGOR

Introduction

Fruit purées are consumed directly as desserts or baby food and used as ingredients in such varied products as jams, fruit flavour yoghurt and chutney sauces. A major portion of fruit purées are preserved by heat sterilization in consumer-size metal or glass containers or by aseptic processes involving sterile filling into bulk containers. Since purées contain 85–90% water there are distinct shipping/cost advantages to reducing water content by concentration or dehydration. Dehydrated fruit purées containing 2–4% water have other advantages attributable to product form. The dry material as flake, granule or powder may be packed in inexpensive single serving vapour proof pouches. Pouch contents may be reconstituted with water and used as a dessert or as infant food. Formed into small tablets, dry purées may be used as ingredients in baked goods and products such as cake or muffin mixes. Fruit purée flakes, usually combined with spices, are used as an ingredient in cooked breakfast cereals. Flakes may also be mixed with fruit juice concentrates and honey or other sweeteners to produce reformed dry fruits (Kitson & Britton, 1978).

Various methods of producing dry fruit purées that have been used commercially include:

(1) drying fruit pieces to low moisture, generally using vacuum driers, then crushing or grinding the dry product to form powder or granules;

(2) foam mat-drying by mixing purée with a foaming agent, spreading the foam on a perforated belt or trays, blowing craters in the foam to improve the drying rate then drying in a flow of warm air and finally grinding or crushing the foam to granules or powder;

(3) spray or tower-drying using either warm air or low humidity cool air;

(4) drum-drying using either a double drum-drier or a single drum with applicator rolls.

Of these methods, drum-drying is mechanically the simplest, most rapid and probably least expensive means of producing low-moisture dry purées. The main drawbacks are heat damage to some sensitive products and the physical problems of forming and handling dry product sheets.

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Double drum-driers or single drums with applicator rolls to provide an even thickness of product feed have been used for drying a number of food products including dry cereal foods, mashed potatoes, mashed potato with meat or fish purée, apple sauce, milk and tomato purée to name a few (Hohenschuh, 1970). Products with low sugar and high insoluble solids content such as mashed potatoes or pea purée form friable dry sheets at the doctor-blade that can simply slide down a chute to a product receiver.

Products, such as fruit purées, with relatively high sugar contents form cohesive sheets when they are still hot. These tend to roll into an amorphous mass at the doctor-blades and will not slide down the product chutes.

In order to produce an acceptable flaked product from fruit purées it is necessary to remove the sheet at the doctor blade and cool it to room temperature before it reaches the product receiver. To achieve this the product sheet may be taken from the doctor blades and passed over take-off rolls rotating at a suitable speed to either stretch or compress the sheet. Simultaneously with product removal via take-off rolls it is desirable, when drying thermoplastic products such as most fruit purées, to cool the hot plastic product as it is being removed from the drums at the doctor blades. To meet these requirements it is necessary to add independently controlled variable speed take-off rolls and a cool-air supply to a basic double drum-drier.

Methods

This note describes how a commercially built pilot plant size double drum-drier was modified to incorporate variable speed take-off rolls, cool-air flow directed at the doctor blade area and ventilation system to remove saturated air from the area beneath the drums. Removing moist air from beneath the drums significantly increases the moisture removal rate during the drying process.

The basic machine described here is a Model 2 GF drier flaker built by Mathis Machine Corp., Indiana, U.S.A. The drums are 12.7 cm diam. by 23.4 cm long, chrome-plated steel driven by a sprocket chain from a 7–70 rev/min variable speed motor. Steam is fed in and condensate removed via co-axially mounted lines led through slip joints on one end of the drums. The complete unit is installed on a 147×81×2 cm steel plate. The plate sits on 90 cm legs to provide a convenient working height. The factory supplied unit has adjustable drum clearance, adjustable doctor blades, and spring-mounted end plates to hold the product pool in the nip of drums.

In order to make possible drum-drying of fruit purées the following modifications were made to the basic unit:

(1) Drum rotation speed was changed to a lower range by installing a jackshaft to give a 4 : 1 speed reduction in the sprocket drive system. This allows a drum rotation speed range of 0.5–6.6 rev/min and product residence time on the drum of 6.4–85 sec.

(2) A mounting base for both the take-off rolls and the cool air supply tubes was installed to fit inside and underneath the drum-drier support assembly. Approximate locations of the take-off rolls and cool-air supply are indicated in Fig. 1. Working drawings of this base, take-off roll and cool-air supply assembly are also available in a technical report prepared by the authors and associates (Kitson *et al.*, 1981).

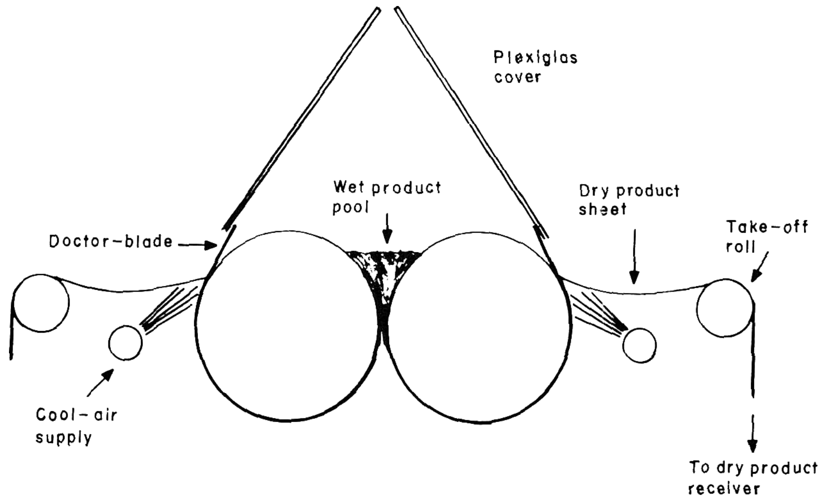

Figure 1. Modified drum-drier.

(3) Take-off rolls 42 mm diam. were mounted on the base and provided with a separate variable speed drive and jackshaft to give a roll rotation speed range of 0.4–6.6 rev/min. Light-weight, round, rubber drive-belts were used throughout this system. The lightness and elasticity of these belts eliminates any need for safety guards.

(4) The two 30 mm i.d. cool-air supply tubes each with seven 6 mm holes spaced 30 mm apart were mounted as shown in Fig. 1. Air flow from these outlets was directed at the doctor-blade area. Cool air was supplied to this system by connecting a 15×33×3 cm core size heat exchanger via a plenum and two 40 mm diam. flexible hoses. A 10 cm diam. squirrel cage blower was installed to provide air flow through the system. In the authors' pilot plant, line water at 15°–20°C is passed through the radiator to provide air cooling. In warmer locations a supply of refrigerated water might be needed.

(5) A 12 cm diam. hole was cut in the base plate directly beneath the nip of the drums and connected via tubing of the same diameter to a 15 cm diameter squirrel cage blower. This system was designed to remove moist air from below the drums thus improving the drier's capacity.

(6) A plexiglass cover was built for the feed portion of the drums. This prevents product splatter from the feed pool and promotes a natural draft to aid in removal of water vapour. Product feed into the pool is via a Masterflex WX 1R057 peristaltic pump with a No. 7018 head. This pump feeds through an 8 mm i.d. flexible tygon tube. Feed rate can be varied from 114–2280 ml/min using this pump system.

Conclusion

With the described modifications the drier has been used to produce low-moisture flakes from a wide range of fruit purées. Products with relatively high fibre contents such as apple, banana, apricot, guava, papaya, and cranberry can be dried successfully without additives, provided the fruit is not over-ripe. The degree of maturity that can be handled varies with fruit species and variety. Purées with lower fibre contents such as raspberry, strawberry, and blueberry require addition of fibre to aid in sheet formation at the doctor-blade. Fibre additions in the form of low methoxyl pectin at up to 1% have been found satisfactory for drying berry purées. As an alternative, berry purées mixed 1+1 with unsweetened apple purée have been drum-dried satisfactorily.

To retard oxidation during storage of drum-dried fruit sauce flakes it is desirable to add an antioxidant such as sulphur dioxide to the purée before it is dried. The addition of 400 p.p.m. SO_2 to apple purée before drying, for example, will retard off-flavour formation in the dry product stored for two years at 20°C in polyethylene bags. Oxidative off-flavours in dry purées may be further controlled by storing the product in gas tight containers in inert atmospheres such as nitrogen or carbon dioxide.

By using suitably modified equipment, a minimum of additives and adequate packaging it should be possible to develop a large selection of dry purées from tropical and temperatate zone fruits. These products can be packaged inexpensively, are lightweight for shipping, easily reconstituted and have a number of potential uses for remanufacture.

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Book reviews

Food Packaging Materials: Aspects of analysis and migration of contaminants. By N. T. Crosby.
London: Applied Science Publishers Ltd, 1981. Pp xiii+190. ISBN 0–85334–926–6. £14.00

Glass, paper and tinplate have been used as food packaging materials for many years. Plastics are a relatively recent innovation, and the amount and type used continues to grow. It is thus reasonable that plastics should be the major topic in this book. The author has sensibly included in his subtitle the words 'Aspects of'. This has enabled him to concentrate particularly on the areas of the subject with which he is knowledgeable and the areas which he knows are important. This is preferable to misleadingly comprehensive titles seen on other books, that subsequently disappoint both readers and reviewers with their uneven coverage.

In the first chapter food packaging requirements are reviewed. The author considers the history of food packaging and food preservation, the role of micro-organisms in food spoilage and the control of spoilage by heat processing, freezing etc. Simple tables illustrate the proportions of different packaging materials used since 1971. A number of selected food groups are listed and there is a paragraph or two on the particular packaging requirements of each group. This chapter is a short and excellent introduction to the book and, indeed, to the subject.

The second chapter is devoted to a description of the different types of polymers used for food packaging. The reviewer does not believe that all vinyl polymers may be referred to as polyolefins as is implied on page 22. Chapter 3 covers the determination of monomers and, as might be expected, most space is devoted to the problems associated with vinyl chloride.

Chapters 4 and 5 discuss toxicological aspects and international legislation respectively. Under the toxicological heading are included wide ranging items on toxicity, risk benefit analysis, carcinogenesis, epidemiology and the extrapolation of animal data to man. It is against this background that any risks from those polymer monomers that may be carcinogens, are assessed. International legislation cannot be covered comprehensively in seventeen pages but nevertheless much information is given, including an introduction to the Materials & Articles in Contact with Food Regulations SI 1978 No. 1927.

The theory of migration is discussed in some depth in Chapter 6. Much of the value of this chapter is lost by some unfortunate lapses, possibly at the proof-reading stage. Thus Ficks' first law is misquoted on page 109, as is the Freundlich

adsorption isotherm on page 116. The error function term is omitted from the solution of a diffusion equation quoted on page 112 making nonsense of the resultant expression.

The experimental determination of migration is well-described with particular emphasis on the concept of global migration with olive oil as the extractant. Many analytical chemists have struggled with the EEC draft method of this procedure. The final chapter contains short items on non-polymeric packaging materials.

Each chapter has a generous list of references and there are some suggestions for further reading. One or two of the references, known to the reviewer, have been misquoted or inadequately described in the text.

This is a very interesting book and should prove useful both to students who need to study food packaging and to persons in the packaging industry who are not normally concerned with potential migration problems. Among its many virtues is the author's ability to see and assess the subject of migration of contaminants into foodstuffs against a technical, social and historical backcloth. Its weak points are the occasional lapses referred to above.

J. T. Davies

Protein Functionality in Foods. Ed. by J. P. Cherry, (A.C.S. Symposium Series 147)
Washington, D.C.: American Chemical Society, 1981. Pp xi+332. ISBN 0–8412–0605–8. $36.75.

Students and research workers, and industrial product development and quality control specialists, who take up this very topical subject, will quickly appreciate that it is in a very active state of evolution. Even the definition of 'functionality' is a subject for debate. The Editor of this compendium accepts it as 'any property of a substance, beside the nutritional ones, that affects its utilization'. He therefore includes appearance and flavour, though to many workers in this field functionality means physico-chemical properties.

The subject is beset with problems of methodology. Very few experimental methods are standardized and it is virtually impossible to compare results obtained in two different laboratories. Variability in raw material and its processing history, as well as the presence of non-protein components, add to the problems, but also to the opportunities. Protein of biological origin is never 'pure', and even after the most elaborate processing one is still dealing with a complex mixture of chemical entities.

Despite these pitfalls, this volume is a valuable compilation. It comprises a general introduction by A. Pour-El (whose contribution to the same series—No. 92 on 'Functionality and Protein Structure'—must already be well known), followed by chapters by recognized authorities on colour, flavour volatiles,

texturization, solubility and viscosity, adhesion and cohesion, gelation and coagulation, whippability and aeration, water and fat absorption, emulsifiers and emulsification, nutrient bioavailability, enzyme modification and multiple regression modelling of functionality.

The stimulus for functionality studies comes from the developing availability and utilization in the food and feeds industries of raw materials such as milk and soyabean proteins. These are likely to be forerunner of many others, and the time may not be far off when such products are accepted as normal ingredients of most formulated foods. This book can be recommended as a 'must' for food specialists concerned with the relevant technology. It is up-to-date, well referenced and informative, particularly in relation to methods and applications.

B. J. F. Hudson

Microbiological Applications of Gas Chromatography. By D.B. Drucker. Cambridge: Cambridge University Press, 1981. Pp. viii+478. ISBN 0-521-22365-2. £45.00.

This book is intended to be a practical guide for users of the technique of gas chromatography in microbiology. It consists of seven chapters and reviews the literature up to the end of 1978. The first chapter is concerned with the principles and practices of gas chromatography and is quite thorough, although injection systems for capillary columns and techniques for switching gas flows between columns could have been covered in more detail. The next four chapters deal directly with applications and cover the analysis of fermentation products, detection of micro-organisms, analysis of structural components (lipids, proteins, nucleic acids, polysaccharides, teichoic acids and peptidoglycans) and the analysis of metabolites. As the author freely admits the term 'microbiological' in the title has been interpreted rather liberally and this is especially apparent in Chapter 5 which includes analysis for components of alcoholic beverages without considering which of them are directly attributable to microbial action. The last two chapters are more specialized and deal with pyrolysis gas chromatography and the rapidly developing subject of computation of data.

As a practical users guide the book succeeds, and the technique of condensing information on the evolution of particular methods into tables is particularly effective. The chapter on detection of micro-organisms is stimulating and food technologists may well wonder why gas chromatography is not more widely used for studies with spoiled foods. Unfortunately the 'cookery book' style that the author has chosen to adopt has promoted an uncritical acceptance of gas chromatographic methods for applications, such as fatty acid profiling, where

other methods of analysis, especially high performance liquid chromatography, may be more appropriate.

The more specialist chapters on pyrolysis gas chromatography and data handling are the least effective in the book. The pyrolysis chapter describes equipment which is obsolete and well behind the 'state of the art' and also confuses pyrolysis mass spectrometry with pyrolysis gas chromatography without proper description or explanation. In fact the interface between gas chromatography and mass spectrometry is not well described in this book.

The data handling chapter provides an adequate description of calculations for measures of association but fails to emphasize the growing importance of multivariate analysis. The author reveals his unease with this subject by describing a principal components plot as an example of discriminant analysis, which it is not! He is also obsessed with the transfer of digital information to a computer by punched paper tape when most modern data processing devices utilize magnetic storage media (e.g. floppy disc).

Despite these criticisms the book will undoubtedly prove useful for those microbiologists who are considering the use of gas chromatography for the first time or want to extend the usefulness of an existing instrument. It will certainly produce an appreciation of the full range of applications and the variety of available methods.

C. S. Gutteridge.

Microbial Ecology of Foods (Micro-organisms in Foods 3): Volume I: Factors affecting life and death of micro-organisms, Volume II: Food commodities. By The International Commission on Microbiological Specifications for Foods.

New York: Academic Press, 1980. Vol. I: Pp xvi+332. ISBN 0-12-363501-2. £16.60. Vol. II: Pp xxii+665. ISBN 0-12-363502-0. £33.60. (Set Price: £43.20.)

The long awaited sequel to *Micro-organisms in Foods 1: Their Significance and Methods of Enumeration* and *Micro-organisms in Foods 2: Sampling for Microbiological Analysis* has at last arrived. Each one of these books represents the culmination of many years of work by a large international body of authors supported by a large number of consultants. In the case of *Microbial Ecology of Foods,* twenty-five authors, helped by a further thirty-nine named consultants, and seventeen other contributors were involved over a 4 year period. Although each chapter was the responsibility of one or two authors, it is stressed in the Preface that the work is 'not a collection of separately authored chapters, but each chapter fits into an overall scheme to provide complete coverage of all important aspects of food microbiology'. Since the editorship of the volumes resided in an editorial committee of eight, it might be feared that this would result in something analogous to the camel—the animal purportedly designed

by a committee! In fact *Microbial Ecology of Foods* proves to be a cohesive and well integrated work. Also it is much more *readable* than its predecessors (in which the précis-style of writing tended to discourage leisurely reading of the whole books), since not only does it have an attractive literary style, but also it is presented in a pleasant typeface and is well laid out.

Volume I contains 12 chapters covering the environmental factors that affect micro-organisms in foods, namely: Temperature; U.V. Irradiation; Ionizing Irradiation; Reduced Water Activity; pH and Acidity; Oxidation-Reduction Potential; Organic Acids; Curing Salts; Antibiotics; Gases; Packaging; and Cleaning, Disinfection and Hygiene. A further two chapters deal with Injury and Its Effect on Survival and Recovery and Interacting Factors Affecting Mixed Populations. The ICMSF suggest that Volume I can stand alone as a basic text in food microbiology, for example for undergraduates or postgraduates who have already had a course in general microbiology. In this context, it would be useful if the publishers were to issue a cheaper, soft-back version of this Volume. The Volume is also intended to give the theoretical background required to appreciate fully the material presented in Vollume II.

Volume II contains 15 chapters, all but one of these dealing with specific commodities: Meats and Meat Products; Poultry and Poultry Meat Products; Feeds of Animal Origin and Pet Foods; Milk and Milk Products; Eggs and Egg Products; Fish and Shellfish and Their Products; Vegetables, Fruits, Nuts and Their Products; Soft Drinks, Fruit Juices, Concentrates and Fruit Preserves; Cereals and Cereal Products; Spices; Fats and Oils; Sugar; Cocoa, Chocolate and Confectioneries; Miscellaneous Foods; and Natural Mineral Waters. The remaining chapter is on Preventing Abuse of Foods after Processing and includes much information relevant to catering establishments.

Two points need to be made about *Micro-organisms in Foods 3,* in the context of its relationship to the previous two works. Firstly, *Micro-organisms in Foods 1* and *2* are handbooks suitable for constant reference in laboratories etc., and requiring, for day-to-day use, minimal cross-reference to the publications referred to in the bibliographies. In the case of this latest work, however, the emphasis is slightly different. Intended as 'a source of information for those who must interpret the results of microbiological analyses conducted on foods', it nevertheless cannot give totally comprehensive and self-contained coverage of the whole subject in 1000 pages. The reader must therefore use the books to orientate thinking and to give the background information which must then be built on by reference to specific works given in the list of references. For example, in the one page allotted to Precooked Frozen Foods—a type of commodity of increasing importance in countries with highly industrialized food sectors—it is stated that 'the production of safe products depends upon careful hazard analyses', which 'begins with the development of a process flow diagram . . . specific for the food, process, equipment and plant involved', and 'requires the establishment of critical control points'. However, 'because of the wide variety of products involved, no attempt will be made either to generalize or to deal with specific products'. The success of these books must therefore depend

in part on the comprehensiveness of the literature references that can be followed up, and in part on the delineation and illustration of the general principles and provision of essential data of a generalized nature. To give just one example—the table and graph to show relationship between pH and proportion of undissociated organic acid (such as acetic, benzoic, citric etc.) can be used in conjunction with the table on anti-microbial spectra of these organic acids to make a prediction of the likely effect of adding a specific organic acid to a specific food product of known pH. Mostly, the information in Volume I provides just this sort of background information. However, in Chapter 1 it would have been helpful in the discussion on the effect of the freezing process to indicate whether this was dependent on the freezing rates encountered in practice. Also the multiple-hit target theory of death caused by high temperatures was not mentioned, although it has important implications in the application of experimentally determined D-values and *z*-values.

Secondly, the nature of the international character of the books needs to be understood. The ICMSF, since its inception, has been concerned with international co-operation amongst food microbiologists in an endeavour to encourage both the introduction of standard methodologies and agreement on microbiological specifications for foods moving in international trade. These latest volumes therefore continue this emphasis, and the discussions on food commodities in Volume II tend to concentrate on such foods with only passing mention of the microbial ecology and problems of food produced and consumed entirely within a country or region of a country. Of course only by making such a decision could the ICMSF have produced a work on the microbial ecology of foods which was of an economically feasible length. However, in consequence, the food microbiologist, epidemiologist or public health worker cannot expect to find detailed discussion of problems specific to their country or region. Nevertheless it would be wrong to give the impression that the scientist or technologist concerned with regional foods would not find relevant information in these books. Volume I, by its very nature, should assist food microbiologists in identifying the likely microbiological problems of a given food or predicting those of a proposed new product, provided that relevant production methods for the constituent materials and food, and the physical and chemical parameters are known. In addition useful information may be obtained in Volume II by reference to analogous foods.

The production of *Microbial Ecology of Foods* represents a significant achievement on the part of all involved, and its publication a landmark in the science of food microbiology. We must hope that the bookshelves of every microbiological laboratory in the food industry and related government establishments will have these books added to them, alongside the previous two publications.

W. F. Harrigan

Fats and Oils: Chemistry and technology. Ed. by R. J. Hamilton and A. Bhati.
London: Applied Science Publishers Ltd., 1980. Pp. xii+255. ISBN 0-85334-915-0. £24.00.

In the Preface the editors make the point that, while there are many good books on the chemistry and biochemistry of lipids, there is a conspicuous lack of modern texts on the chemistry and technology of oils and fats. This book was intended to fill the gap, and comes near to doing so in some chapters but falls woefully short in others. The book is a collection of papers originally presented at a symposium, and its scope was thus restricted by the availability of speakers. Some authors have written good chapters, but others appear to have made little effort to convert their original lecture into a form suitable for publication. The literature cited at the end of each chapter ranges from 6–108 correctly presented references to a 'Bibliography' consisting of two issues of *J. Am. Oil Chem. Soc.* (with no authors or page numbers!). There is even one chapter with a single reference to the obsolete 3rd edition of Bailey's *Industrial Oils and Fats* where 50–100 recent references could easily have been given!

The first five chapters deal with aspects of the chemistry of lipids. In Chapter 1, W.W. Christie updates his book *Lipid Analysis* (1973) without much overlap of material. He describes most of the modern methods for qualitative and semi-quantitative analysis of complex lipids and discusses their limitations, but avoids the ultimate problem of how to obtain reference materials for accurate calibration of instrumental systems.

D. Waddington's chapter on applications of continuous wave and pulsed nuclear magnetic resonance (NMR) in the oils and fats industry gives a useful summary of NMR theory, and a critical appraisal of the applications of NMR compared with the longer and tedious classical procedures such as dilatometry.

F.D. Gunstone's review of natural oxygenated acids (Chapter 3) is interesting but regrettably brief (12 pages). It is surprising that there is almost no mention here (or in Chapter 10) of the numerous oxygenated acids initiated by lipoxygenases and subsequently modified by enzymic or non-enzymic mechanisms to produce, for example, bitter tasting trihydroxy acids and precursors of numerous desirable and undesirable flavour volatiles.

Chapter 4 is an extensive review by A. Bhati, R. J. Hamilton and D.A. Steven of methods for the synthesis of simple glycerides, ending with details of some recommended procedures. In their opening remarks they mention biochemical methods for synthesis of glycerides (which are probably superior where labile fatty acids are to be incorporated), but do not mention reversed lipolysis using lipases and phospholipases. They erroneously state (page 59) that phospholipase-A (-A_2) hydrolyses the secondary ester group of L-triglyceride, failing to point out that it is first necessary to partially hydrolyse to *sn*-1, 2 and *sn*-2, 3-diacylglycerols which are then converted to *sn*-1, 2-diacyl-3-glycero-

phosphate and *sn*-1-glycerophosphate-2, 3-diacyl derivatives, the former being the specific substrate for the enzyme.

The first half of the book ends (Chapter 5) with a concise description by S.M. Roberts and R.F. Newton of prostaglandins, which are potent hormone-like substances synthesized in various mammalian tissues from arachidonic and other fatty acids. The chapter is relevant as a continuation of Chapter 3, but seems a little out of place in the context of the rest of the book.

The second half of the book is concerned with the technology of oils and fats. V. Young (Chapter 7) gives a good, though far from exhaustive, description of the processing of oils and fats, with many clear diagrams. The preceding chapter on extraction of vegetable oils and fats by J. Davie and L. Vincent compares very unfavourably in all respects.

The next two chapters are concerned with specialized fats. H. Nickless and A. F. Sidaway (Chapter 8) describe confectionery fats, with emphasis on cocoa butter and equivalent vegetable fats which have similar glyceride structures or polymorphic forms (these topics are not mentioned in the index). J. Hanford (Chapter 9) then explains how polyunsaturated fatty acids in ruminant feed can be protected from biohydrogenation in the rumen, so as to be available for subsequent absorption and incorporation into milk and body fats.

Problems of fats in the food industry are discussed by M.L. Meara (Chapter 10) in a rambling review. This is an immense subject, and it is understandable that the author should be selective in his choice of topics, but there can be no excuse for failing to give references so that the reader can obtain further information on particular points.

In the final chapter M. Pike follows the growth in importance of palm oil in the 1970's with the main emphasis on the Malaysian palm oil industry.

From these remarks it will be evident that *Fats and Oils: Chemistry and Technology* is of very mixed content and quality and that some subjects worthy of separate chapters (e.g. hydrogenation, margarine, surfactants) are largely neglected. Nevertheless, the book contains a lot of useful information and is worth having until a truly comprehensive treatise is written.

W. R. Morrison

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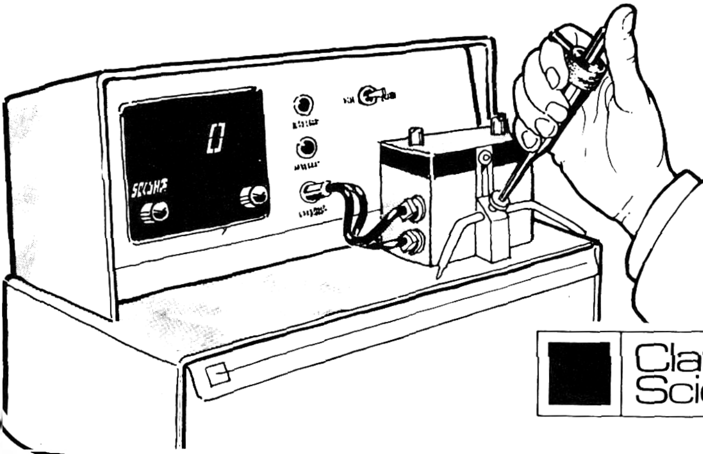

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SI UNITS

gram	g	Joule	J
kilogram	kg $= 10^3$ g	Newton	N
milligram	mg $= 10^{-3}$ g	Watt	W
metre	m	Centigrade	°C
millimetre	mm $= 10^{-3}$ m	hour	hr
micrometre	$\mu = 10^{-6}$ m	minute	min
nanometre	nm $= 10^{-9}$ m	second	sec
litre	l $= 10^{-3}$ m^3		

NON SI UNITS

inch	in	$= 25{\cdot}4$ mm
foot	ft	$= 0{\cdot}3048$ m
square inch	in^2	$= 645{\cdot}16$ mm^2
square foot	ft^2	$= 0{\cdot}092903$ m^2
cubic inch	in^3	$= 1{\cdot}63871 \times 10^4$ mm^2
cubic foot	ft^3	$= 0{\cdot}028317$ m^3
gallon	gal	$= 4{\cdot}54611$
pound	lb	$= 0{\cdot}453592$ kg
pound/cubic inch	lb in^{-3}	$= 2{\cdot}76799 \times 10^4$ kg m^{-3}
dyne		$= 10^{-5}$ N
calorie (15°C)	cal	$= 4{\cdot}1855$ J
British Thermal Unit	BTU	$= 1055{\cdot}06$ J
Horsepower	HP	$= 745{\cdot}700$ W
Fahrenheit	°F	$= 9/5\ T°C + 32$

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