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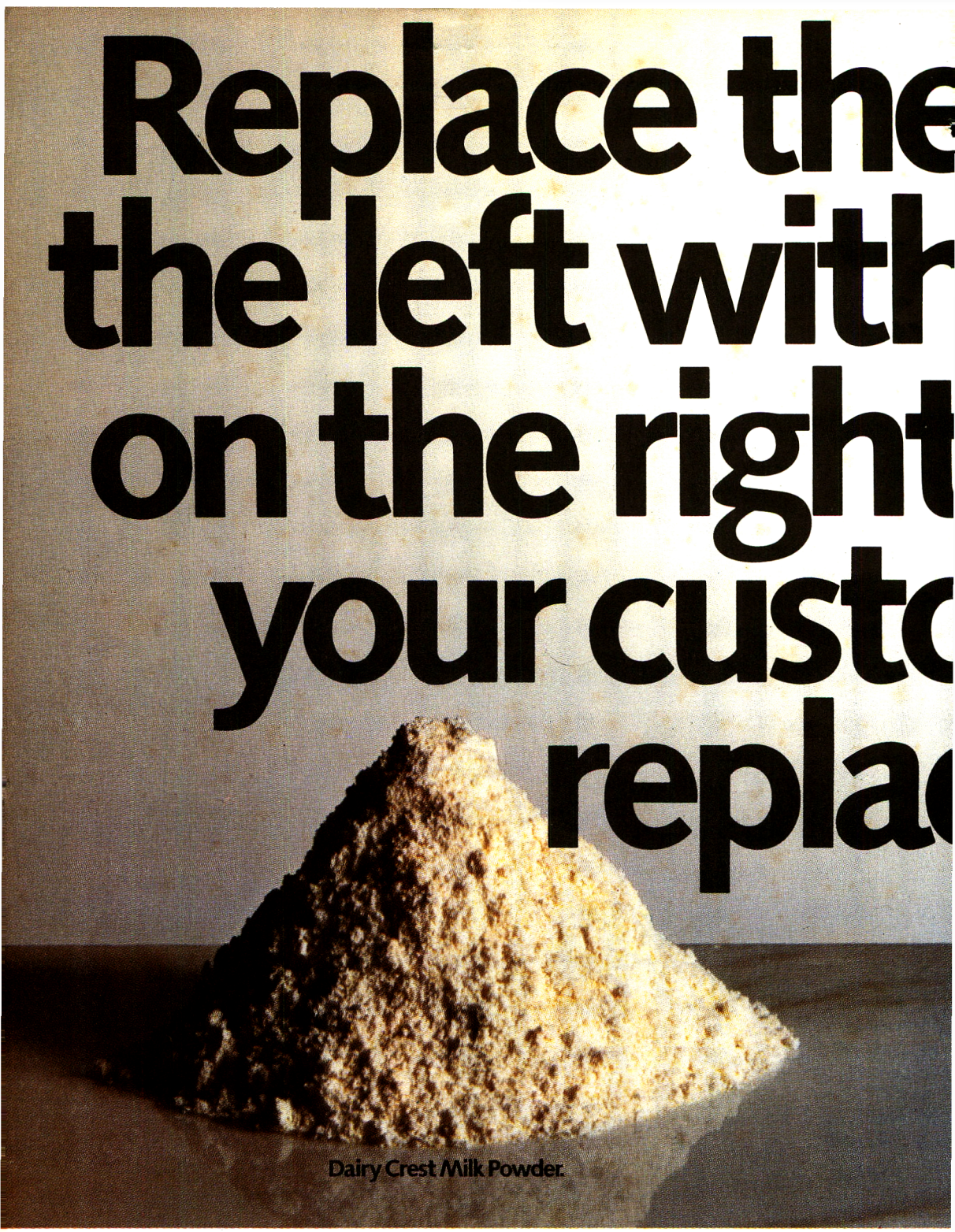

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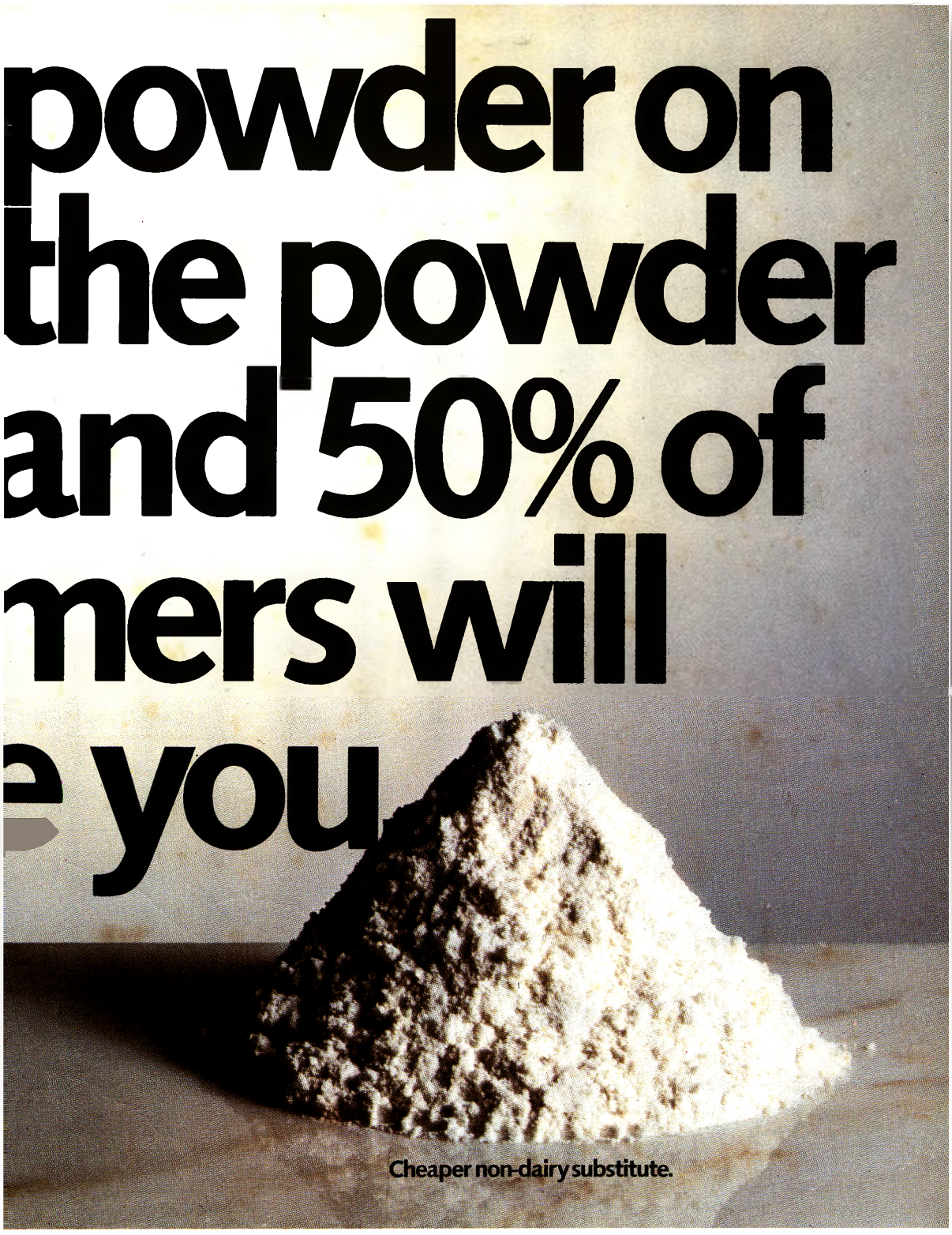

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J. Fd Technol. (1983) **18**, 401–410

The use of low resolution nuclear magnetic resonance for determining avocado maturity by oil content

G. A. BARRY, B. I. BROWN* and L. R. BARKER*

Summary

A method for determining oil content of dried avocado flesh using a low resolution nuclear magnetic resonance (NMR) spectrometer is described. Forty samples were analysed for oil content by NMR, Soxhlet extraction and refractive index (RI) methods. Percentage of oil by NMR was more closely correlated with Soxhlet extracted oil (r=0.978) than was percentage of oil determined by a RI method (r=0.853).

Data from approximately 700 avocado samples collected from the 1975, 1976 and 1978 Queensland growing seasons showed that percentage of oil determined by NMR was better correlated with percentage of dry matter than was percentage of oil determined by RI. Furthermore, the relationships between dry matter and oil were far more consistent between seasons for the NMR method than for the RI one.

Advantages of using the NMR technique in avocados are simplicity, speed, low operator errors and the elimination of the use of dangerous or inflammable solvents. A reproducibility relative standard deviation (s.d.) of 0.6% was obtained.

Introduction

Considerable research has been reported on the chemical properties of avocado fruit (Stahl, 1933; Haas, 1937; Davenport & Ellis, 1959; Hatton, Harding & Reeder, 1964), and their use in assessing avocado maturity. The most generally accepted index of maturity was oil content, and this led to development of methods such as Soxhlet extraction and refractive index measurement for determining oil content (Shannon, 1949). The refractive

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index method of Shannon (1949) became the standard method internationally for oil content in avocados, and was used to obtain data for market standards in Queensland (Hope, 1963).

However, for years, workers have questioned the precision and accuracy of refractometric methods (Stahl, 1933) while Soxhlet extractions are time consuming. A technique for oil content determination developed in South Africa (Hughes, 1971) was based on the linear relationship between oil and water and this allowed the determination of dry matter content to be used as an index of maturity. Maturation studies carried out on the major varieties of avocados grown in Queensland and northern New South Wales led to the avocado maturity standards in Queensland being changed to a regulatory determination of percent dry matter rather than oil content (The Fruit and Vegetable Grading and Packing Regulations, 1979). In spite of this improvement, which was designed to simplify the determination of maturity as well as the market standard, there is still considerable scientific discussion concerning the method by which avocado maturation should be measured. Many regulatory bodies use oil content as the index while others prefer to use dry matter content.

During the course of the above maturation studies, we also compared three methods for determining oil content in avocados: Soxhlet extraction with petroleum ether, refractometric and a new technique based on the principle of low resolution nuclear magnetic resonance (NMR). We also examined the relationship between oil and water by comparing linear regressions of percentage of dry matter against percentage of oil determined by the three methods.

The NMR technique measures total hydrogen nuclei in the liquid phase (oil and water) independent of hydrogen associated with the solid phase. Provided the moisture content of the sample is low enough not to contribute significantly to the NMR readout, response of the instrument is directly proportional to the quantity of oil in the sample (Wolff, Karleskind & Vallmalle, 1970). Conway (1960) first applied the method to whole corn seeds while other workers (Alexander *et al.*, 1967; Collins *et al.*, 1967; Madsen, 1976) have since found the technique suitable for a variety of oilseeds. The method is now under investigation as the official method for the domestic trading of sunflower seed in the U.S.

The objectives of this study were to investigate the feasibility of using the low resolution NMR spectrometer to determine oil content in dried avocado flesh and to examine the relationship between oil and dry matter content in Queensland avocados sampled over several growing seasons.

Materials and methods

Sample preparation

All avocado fruits were hard and green when sampled. They were ripened at 20°C to an 'eating ripe' condition and then halved longitudinally, peeled,

and the seed removed. Ripe flesh was sectioned into longitudinal wedges which were diced into small pieces. A sub-sample of diced flesh was taken for the refractive index (RI) method of Shannon (1949). The remainder was dehydrated to constant weight by freeze drier (60°C platen temperature at 1 mm total pressure and −20°C product temperature for 12 hr), dry matter content calculated, and the dehydrated residue sub-sampled for analysis by NMR and Soxhlet extraction.

Conversion of percentage of oil to a 'fresh weight' basis for NMR and Soxhlet determinations was done using the appropriate moisture factor.

NMR spectrometer calibration and reproducibility testing

The nuclear magnetic resonance (NMR) instrument used in this study was the Newport Quantity Analyser, Mk I, equipped with a temperature control unit Type WR2 Mk II and Supplementary Modulation Unit Type A. All measurements were made at 25°C using a 50 ml sample coil assembly with the following optimum operating conditions being previously determined: integration times of 35 sec with external voltmeter readout, RF level 400 μA and AF gain 400.

The NMR analyser was calibrated for avocado oil analysis in the following manner. Dehydrated samples of avocado were read on the NMR to select eight samples with as wide a range in readout values as possible. These samples were divided into eight matching pairs by repeated scanning with NMR. One sample of each matched pair was flame sealed under vacuum in a glass NMR tube to make eight permanent standards. The other eight samples were again read in duplicate by NMR on five separate occasions then divided and analysed in duplicate for oil content by extracting the oil into petroleum ether for 16 hr in a Soxhlet extractor using a fast boiling rate. A calibration curve between percentage of oil in dried avocado flesh and NMR readout was then constructed. All NMR readouts were corrected to 10 g dried sample weights.

Reproducibility of the NMR spectrometer method was tested by measuring six samples with varying oil contents on six different occasions over the course of 1 month.

Comparison of NMR, RI and Soxhlet methods

Forty samples from the 1975 season were selected at random and analysed for oil content by NMR, RI and Soxhlet methods. For Soxhlet extraction, petroleum ether was used with a fast boiling rate for 16 hr. Dry matter contents were also calculated.

Relationship between oil and dry matter

A total of 736 avocado fruit, representing six varieties (Table 5) were collected in 1975 (281 samples), 1976 (170 samples), and 1978 (285 samples). All had dry matter contents determined and were analysed for oil content by NMR and RI methods.

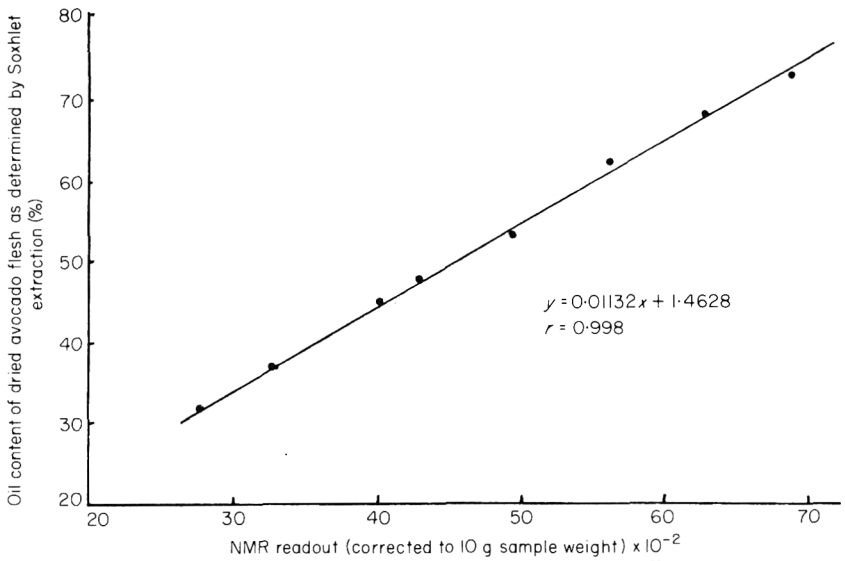

Figure 1. Relationship between oil content determined by Soxhlet extraction and NMR response calculated to a 10 g sample basis.

Results

NMR spectrometer calibration and reproducibility testing

The standard curve was linear over the range tested (Fig. 1). The response of the NMR instrument was highly correlated (r=0.998) with oil content in dehydrated avocado determined by Soxhlet extraction. All subsequent oil determinations by NMR were calculated from this equation (Fig. 1). The reproducibility of the NMR spectrometer is seen in Table 1 which shows results obtained for single measurements as well as means for the six occasions. Using the method of Youden & Steiner (1975) a reproducibility relative standard deviation (r.s.d.) of 0.6% was obtained.

Table 1. Reproducibility of determining oil content in avocados by the NMR spectrometer

Sample no.	Occasion number						Mean
	1	2	3	4	5	6	
1	43.6	43.1	42.9	44.0	44.0	43.7	43.6
2	55.6	55.0	54.9	55.4	55.4	54.9	55.2
3	64.3	64.3	64.0	64.8	65.0	64.0	64.4
4	66.2	65.4	65.6	65.8	66.1	65.5	65.8
5	71.2	71.3	70.7	71.1	71.3	70.6	71.0
6	72.4	71.9	72.3	72.5	72.4	72.0	72.3
Mean	62.2	61.8	61.7	62.3	62.4	61.8	

Reproducibility r.s.d. = 0.6%.

Table 2. Oil contents determined by Soxhlet extraction, refractive index (RI) and low resolution NMR and percentage dry matter (% DM) in avocados collected from the 1975 growing season

Sample no.	Percentage of oil determined by			% DM
	Soxhlet*	RI†	NMR*	
1	10.5	8.5	11.7	20.1
2	17.2	21.8	17.8	27.4
3	10.1	7.6	10.7	19.3
4	13.4	8.6	14.2	22.5
5	17.5	14.9	17.9	26.2
6	20.9	25.9	21.8	30.7
7	20.8	17.2	21.5	31.4
8	22.2	27.6	22.4	31.6
9	14.4	21.6	15.0	24.1
10	18.7	25.0	19.0	28.5
11	33.2	34.6	25.3	44.4
12	24.6	35.8	20.7	38.6
13	35.3	36.0	41.5	45.3
14	29.2	28.4	29.6	39.5
15	33.6	33.8	33.9	43.2
16	22.1	22.8	20.4	29.5
17	5.9	6.5	6.6	15.8
18	8.5	9.5	9.1	18.1
19	12.6	15.0	13.2	21.5
20	7.6	8.1	8.2	18.8
21	5.4	5.9	6.1	15.7
22	24.5	24.1	24.9	33.6
23	30.0	29.4	29.8	38.9
24	34.0	33.2	34.2	43.5
25	37.1	36.0	36.7	47.0
26	6.8	6.7	7.0	15.0
27	15.7	16.8	16.2	25.3
28	17.0	17.7	17.3	27.3
29	19.9	20.0	20.3	30.3
30	32.0	32.5	30.4	39.4
31	17.2	21.8	17.7	27.1
32	18.2	24.1	18.3	25.1
33	12.4	12.4	12.8	19.4
34	24.6	15.9	24.4	35.1
35	22.7	28.1	23.5	33.0
36	20.9	11.7	21.1	29.2
37	28.5	14.9	28.5	39.4
38	14.4	25.1	14.6	21.3
39	13.4	20.7	14.3	22.4
40	22.3	27.0	22.7	30.0

* Analyses carried out on dried samples and corrected to a 'fresh weight' basis.

† Analyses carried out on undried samples.

Table 3. Linear regression equations,* and correlation coefficients (r) for the regression of oil content (y) determined by NMR and RI methods against oil content (x) determined by Soxhlet extraction. Data are for forty avocado samples from the 1975 season.

Method	$b_1 \pm$ s.d.	$b_0 \pm$ s.d.	r
NMR	0.9987 ± 0.0349	−0.1239 ± 0.7585	0.978
RI	0.9176 ± 0.0909	2.585 ± 1.969	0.853

* Linear equation is in the form $y = b_1x + b_0$.

Comparison between NMR, RI and Soxhlet methods

Oil values determined by three methods for the forty samples selected from the 1975 growing season are shown in Table 2. Values ranged from less than 10% oil early in the growing season, to 30–40% oil in the later part.

We compared NMR and RI methods with Soxhlet values by performing linear regressions of percent oil determined by Soxhlet against percentage of oil determined by NMR and RI. Regression equations and correlation coefficients are shown in Table 3. The two lines did not differ significantly ($P<0.05$) in their slopes and intercepts, which were not significantly different from 1 and 0 respectively. However, a much higher correlation coefficient was found for the NMR regression ($r=0.978$) than for the RI one ($r=0.853$).

Relationship between oil and dry matter

Percentages of dry matter for the forty samples are also shown in Table 2. Linear regressions between dry matter data and oil values from three methods once again indicated their slopes and intercepts were not significantly ($P<0.05$) different (Table 4). However, a much greater variation existed about these parameters for the RI regression than for the other two lines, and an indication of the uncertainty in making predictions from these equations is

Table 4. Linear regression equations,* population size (n), correlation coefficients (r) and confidence intervals CI (y) about a predicted y for a future x for the regression of percentage of dry matter (y) against percentage of oil (x) determined by Soxhlet, NMR, and RI methods.

Method	n	$b_1 \pm$ s.d.	$b_0 \pm$ s.d.	$y \pm$ CI (y)† when $x = 13$	r
Soxhlet	40	1.0224 ± 0.0251	9.0349 ± 0.5436	22.33 ± 1.98	0.99
NMR	40	1.0149 ± 0.0479	9.0323 ± 1.0336	22.23 ± 3.67	0.96
RI	40	0.8114 ± 0.0838	12.4621 ± 1.908	23.01 ± 7.09	0.84

* Linear equation is in the form $y = b_1x + b_0$.
†95% confidence limits.

shown by the values of the confidence intervals when oil content is 13%. (This was the level found in maturation studies to be generally satisfactory for marketing avocados.)

Equations given in Table 4 were based on combined data from six varieties for 1 year. We subsequently analysed over 700 samples of the six major varieties of avocados from three growing seasons to see how the relationship between oil and dry matter changed with time and varieties. Linear regressions of percentage of dry matter against percentage of oil determined by the NMR and RI methods for each variety for each year produced the correlation coefficients shown in Table 5. In every case percentage of oil

Table 5. Correlation coefficients (r) and population size (n) for the regression of percentage of dry matter (y) against percentage of oil content (x) determined by NMR and RI methods for each variety for each year

		1975		1976		1978	
Variety	Method	n	r	n	r	n	r
Zutano	NMR	9	0.972	12	0.984	20	0.945
	RI	9	0.899	11	0.792	20	0.567
Fuerte	NMR	52	0.993	39	0.991	91	0.979
	RI	52	0.847	26	0.908	91	0.774
Rincon	NMR	44	0.995	38	0.986	30	0.969
	RI	44	0.934	26	0.783	30	0.834
Sharwill	NMR	60	0.989	21	0.979	74	0.972
	RI	60	0.888	14	0.092	74	0.759
Hass	NMR	62	0.983	15	0.951	55	0.933
	RI	62	0.762	11	0.827	55	0.601
Hazzard	NMR	54	0.993	45	0.973	15	0.994
	RI	54	0.917	29	0.662	15	0.769

determined by NMR was more highly correlated with percentage of dry matter than was percentage of oil by RI. The low correlation for Sharwill in 1976 for the RI method was due to the fourteen samples all having approximately the same oil content.

Poolability of linear regression lines was assessed by combining data from all varieties for each year. Regressions for the NMR method showed equal slopes, normality in errors and equal variances, with only the intercepts differing significantly ($P<0.01$), i.e. the lines constructed for each year were parallel (Table 6). However, the regressions for the RI method failed to show equal slopes, equal intercepts and normality in errors and therefore each year had its own linear relationship.

Table 6. Linear regression equations, correlation coefficients (r) and population size (n) for the regression of percentage of dry matter (y) against percentage of oil (x) determined by NMR and RI methods for each year, combining varieties

	NMR			Refractive index (RI)		
Year	n	Equation	r	n	Equation	r
1975	281	$y = 1.03349x + 8.318$	0.990	281	$y = 0.88915x + 11.563$	0.893
1976	170	$y = 1.04959x + 7.034$	0.979	117	$y = 0.65756x + 12.528$	0.707
1978	285	$y = 1.02937x + 8.485$	0.961	285	$y = 0.66492x + 15.468$	0.739
Pooling allowed	Slopes equal, variances equal intercepts differ**			Not poolable		

** 1% Level of significance.

Discussion

The very high correlation (r=0.998) between oil content determined by Soxhlet extraction and NMR response, coupled with an instrument reproducibility r.s.d. of 0.6% make the NMR spectrometer an excellent instrument for measuring oil content in dried avocados. Provided the avocado flesh is dried, response of the NMR spectrometer gives a direct measurement of the quantity of oil in the sample. In earlier work (unpublished data) we compared freeze drying and vacuum oven techniques for dehydrating samples and found the latter one tended to slightly burn samples with high oil content. In addition, freeze dried samples were easier to insert into a Nessler tube prior to reading on the instrument. Tests carried out on diced material with different Soxhlet extraction times confirmed 16 hr was necessary to ensure total extraction of oil from the dried avocado flesh. Grinding the sample more finely would probably reduce the extraction time since Lewis, Morris & O'Brien (1978) found that 4 hr was sufficient to obtain total extraction of oil from finely ground avocado flesh.

The main advantages of using low resolution NMR to determine oil content are simplicity, speed and non-reliance on the use of dangerous or flammable solvents. Once the initial operating conditions have been found and the instrument calibrated, no further tests are necessary apart from daily checks on the permanently sealed standards. Operator errors are reduced to a minimum. To weigh, read twice and calculate each sample takes approximately 3 min so that a single analyst may complete twenty analyses per hour. Samples can be dried overnight and read the next day so that a minimum of operator time per sample is achieved.

The relationship between oil and water contents of avocados has been studied by many workers (Stahl, 1933; Hughes, 1971; Pearson, 1975; Swarts, 1976) who found that the sum of these variables remains fairly constant (approximately 91) over a growing season. Since the composition of edible

flesh or mesocarp of the avocado is oil, water and other fibrous solids, then by calculating the amount of fibrous solids, the quantity of oil plus water can be deduced. In the regression equation of percentage of dry weight against percentage of oil the value of b_0 (the intercept on y axis) gives a rapid estimate of the amount of fibrous solids in the avocado sample, allowing a value for oil plus water to be determined. The results in Tables 4 and 6 indicate that for the Soxhlet and NMR regressions this constant value for oil plus water holds, but that much wider values are obtained in the RI regressions. This is due to a much higher variability in oil measurements by the RI method, a fact which is further supported by the lower correlation coefficients and the changing relationships between oil and dry matter occurring for each year (unequal slopes). One reason for the higher variability in the RI method could be the inherent operator error associated with the series of manual steps incorporated in it as opposed to the nearly automatic NMR method.

Oil content in avocados determined by low resolution NMR compares very favourably (r=0.978) with Soxhlet extraction values and appears to give more reliable results than the RI method. Because of the simple and rapid nature of the method, the NMR spectrometer has great value from a regulatory point of view in not requiring specialized skills to operate it. The results reported here suggest that low resolution NMR is a suitable technique for measuring oil in dried avocado flesh and should help regulatory bodies to simplify the determination of maturity and improve the avocado market standard. With more recent low resolution NMR models it may be possible to determine oil content directly on a fresh weight basis and so eliminate the need to dehydrate the sample. This would further enhance the NMR spectrometer as a means for determining avocado fruit maturity.

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The effect of irregular cooling phenomena on the lethality of thermal processes

A. C. CLELAND and M. F. GESTERKAMP

Summary

Irregular temperature/time curves in the cooling phase of thermal processes for cans containing semi-fluid foods have been previously attributed to internal boiling and mixing of the can contents. A physical model is proposed for this behaviour. This model can in part be simulated by numerical (finite difference) heat transfer calculations, but the actual physical behaviour is too complex for complete simulation. Experimental investigation for a baby food showed that substantial variations in total process lethality (±18% around the mean value) occurred as a result of changing process variables that affected the extent of internal boiling. Of these retort pressure during cooling was most important, but there were also complex interactions between this variable and three others: headspace, steam temperature and can pressure during seaming. Because the extent of internal boiling has a substantial effect on the total process lethality, determination of heating phase target F values for foods in which irregular cooling phenomena occur must consider these effects. The canner should consider the sensitivity of F values to variations that could occur in practice of parameters such as can seaming pressure, headspace and retort pressure during cooling, as well as steam temperature.

Introduction

In the canning process it is usually accepted that the successful control of the preservation of food by canning is dependent on the slowest heating point in the container receiving a heat treatment sufficient to reduce the concentration of bacterial spores (typically *Clostridium botulinum*) to some pre-determined level.

Calculation of the required heat treatment and the consequent spore reduction has been the subject of a large number of publications during the 60

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years since Bigelow (1921) introduced the thermal death time (TDT) concept. The literature can be conveniently grouped into three categories: (1) methods for determining total lethality (commonly referred to as the F value) from experimental temperature/time data. These are known as graphical methods; (2) methods for determining total lethality based on theoretical predictions of temperature/time profiles, or heat transfer theory to extrapolate experimental temperature/time data. These are commonly referred to as formula methods; (3) examination of the sensitivity of lethality calculations to data uncertainties.

There are recent reviews on the thermal processing literature (Loncin & Merson, 1979; Hayakawa, 1977). Briefly, formula methods are based on either a pure conduction or a pure convection heat transfer model. In the latter, the can contents are assumed to be both homogeneous and perfectly mixed so that the temperature is uniform throughout the can at any time. This model may be used for completely liquid foods. The more practical and common pure conduction model assumes that the can contents are totally immobilized and thus heated internally by conduction only. The model further assumes that the surface heat transfer coefficient is infinitely large, so that at the onset of heating or cooling the surface temperature, all over the can, changes instantaneously to that of the heating or cooling medium. Implicit assumptions in the use of the conduction model in 'formula' method lethality calculations are that: (1) the can contents are homogeneous and have thermal properties that are independent of temperature; (2) the can contents totally fill the can and are at uniform initial temperature; (3) at time zero the can surface temperature changes instantaneously to the heating medium temperature (θ_{ah}), and this latter parameter does not vary with time; (4) at the onset of cooling the can surface temperature changes instantaneously to the cooling medium temperature (θ_{ac}), and this latter parameter does not vary with time.

Many foods heat totally, or almost totally by conduction, including some high viscosity fluid products such as purées (Loncin & Merson, 1979). Provided the can dimensions and product thermal diffusivity are known any accurate method for modelling heat conduction in a short cylinder can be used to predict temperature/time profiles. Using these profiles the lethality can be calculated at any point in the can either graphically or by superimposing a lethality calculation on the heat conduction calculation, for example, the method of Hicks (1951). It is normal to estimate lethality only at the thermal centre of a can. It may not be the point at which the chance of survival of a spore is greatest, but it is easiest to use (Loncin & Merson, 1979). For any semi-fluid, or heterogeneous canned products, formula methods cannot be used to predict the temperature/time history. Experimental measurements must be made, and the lethality determined graphically (or by numerical integration) using the measured temperature history. Calculation aids such as special graph paper, or the tabulated 'L' values of Board (1977) are available. Studies on the effects of data uncertainties on lethality calculations have examined possible inaccuracies in thermal properties and kinetic parameters (e.g. Lenz & Lund, 1977), but reasons for differences in physical behaviour

when processing apparently similar cans under supposedly identical conditions have not been systematically investigated.

Ultimately the level of safety to the consumer of a canned product is determined by the total lethality of the thermal process used. Both the heating phase and cooling phase contribute to the total lethality, but the decision to terminate the thermal process must be made before the cooling phase contribution is known. The canner must therefore choose a target lethality for the heating phase based on his expectation of the cooling phase contribution. A conservative approach is to achieve the required total lethality in the heating phase, and treat the cooling phase contribution as a safety margin. Weaknesses in this approach are that the process is longer, and therefore more expensive than it need be, and that the destruction of heat sensitive nutrients may be substantially greater than would occur if the minimum safe process was used.

The alternative approach is to conduct experiments to establish the likely cooling phase lethality. The heating phase target lethality is then set lower

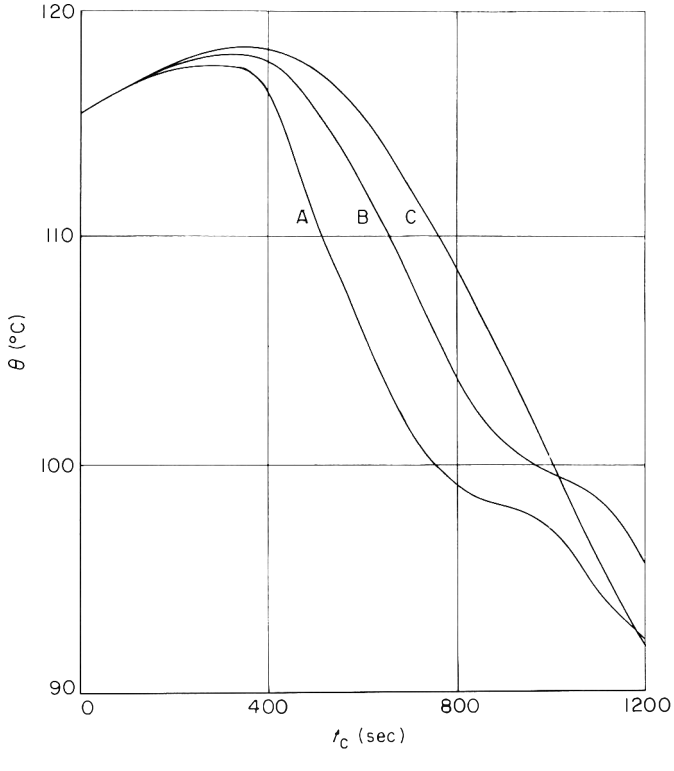

Figure 1. Plot of temperature (°C) at the geometric centre of a can of baby food during cooling following heating in steam at 129.0°C for 2950 sec. Can dimensions: 74 mm diameter×108 mm height; net head space: 2 mm; retort pressure during cooling: 101 kPa.a; cooling water temperature: 20.8°C. (a) Experimental; (b) predicted using the calculation flow sheet of Fig. 2, (c) predicted using the pure heat conduction model.

than the required total lethality in the expectation of achieving a certain cooling phase contribution. This approach is widely used in Australia and New Zealand, and concern over canning costs, and nutrient destruction will probably lead to its adoption more widely elsewhere. It does pre-suppose that the cooling phase contribution can be estimated in advance. This paper examines how the cooling phase contribution can change with processing conditions and is intended to warn canners of some hidden difficulties that can be enountered.

One physical phenomenon that has been observed, but not studied in detail is the presence of irregular temperature/time profiles during cooling. Occurrences have been reported by Jackson & Olsen (1940), Townsend *et al.* (1949), Hemler *et al.* (1952), Powers, Pratt & Norris (1952), Board, Cowell & Hicks (1960) and Taimmanenate (1980), but no systematic appraisal of their cause has been made. If these irregular effects occur consistently the graphical method can be used to determine the lethality of a cooling process. However, if the occurrence or size of the irregular profile is not consistent when processing conditions remain constant, there is no guarantee that an experimentally determined F value is representative of all cans undergoing the process. An investigation into the causes and effects of observed irregular temperature/time profiles in cooling was therefore warranted to show whether under-processing of some cans could occur. This could be in one of two situations. The first of these is where some cans show the irregular cooling effect and others do not, and the second is where the extent of the irregular cooling phenomena varies between cans. The system chosen for study was that of Taimmanenate (1980) who processed cans of an homogeneous, high viscosity, puréed baby food which heated by conduction, but exhibited effects such as those shown in Fig. 1 during the cooling phase.

A feasible physical model

Irregular cooling phenomena are characterized by rapid rates of temperature change over short times, and by apparent 'plateau' or inflections in the cooling curve. These effects have been blamed on a combination of conduction and convection (i.e. partial mixing) by Jackson & Olson (1940), and on internal boiling by Townsend *et al.* (1949), Powers *et al.* (1952) and Board *et al.* (1960). The rapid temperature drops were when boiling was occurring, transferring sensible heat to latent heat of evaporation (without removing the heat from the can), and the plateau or inflection regions corresponded to condensation where heat was removed by the can with little or no temperature change. The boiling and condensation effects may promote mixing of the can contents.

In order to be able to understand these effects it is useful to postulate the sequential nature of the physical behaviour of the can. This discussion is related to materials which heat by conduction, but are fluid in nature, such as the bentonite suspensions used by Powers *et al.* (1952), the baked beans

processed by Board *et al.* (1960), and the baby food used by Taimmanenate (1980).

In the heating phase the external (steam) pressure is always greater than or equal to the internal pressure in the can and either no boiling (or condensation) occur, or the gradual evaporation of water to form steam in any headspace in the can does not substantially affect conduction elsewhere in the can. Therefore measured temperature/time histories at various points fit a pure conduction model.

At the onset of cooling any steam in the headspace will rapidly condense, thus lowering the pressure in the can. Boiling (formation of steam bubbles) will occur until the internal pressure of the can reaches the vapour pressure of the hottest material in the can. The total volume (and hence mass) of steam formed will depend on the volume not occupied by the food material. This will be the head space, plus any increase in can volume due to bulging which will occur if the external pressure during cooling is less than the internal pressure. If non-condensable gases are present in the headspace (depending on the can seaming pressure) the mass of steam formed will be less than if non-condensable gases are not present. Initially the steam bubbles will form in the outer regions of the can where the temperature is highest. The transfer of sensible heat to latent heat will lead to accelerated temperature change in these regions. As cooling proceeds the hottest region in the can will move towards the centre so new steam bubbles will occur and old ones condense as the region of 'phase change' moves. Eventually the phase change region will be at the thermal centre of the can, and at, or close to the geometric centre. The arrival of the phase change region at the centre will be characterized by rapid temperature drop at this point. During the cooling process the hottest temperature in the can will drop, thus lowering the internal pressure. If bulging had occurred this will gradually disappear as the pressure drops, thus reducing the volume in which steam could form. Finally, condensation at the can centre will occur when the temperature drops sufficiently. The formation and disappearance of steam bubbles may lead to mixing and convection heat transfer.

This proposed mechanism suggests four parameters, all externally controllable which may be of importance: (1) the size of the can headspace; (2) the retort pressure during cooling; (3) the pressure during can seaming; and (4) the maximum temperature in the can at the end of heating. This is a function of the steam temperature and the length of the heating process.

Variation of any of these parameters would be expected to lead to variation in the extent of the boiling/condensation phenomena, and hence to variation in the overall process lethality (F).

Materials and methods

The investigation was carried out in two parts. Firstly, the validity of the postulated physical model was tested using experimental data from Taim-

Table 1. Levels of the four process variables used in the 2^4 factorial experiment

Variable	Coded level		
	+1	0	−1
Can headspace (mm)	16	8	0
Retort pressure during cooling (kPa.a)	101	151	201
Can seaming pressure (kPa.a)	41	71	101
Steam temperature (°C)	124	118.5	113

manenate (1980). Second, the effect of the four variables discussed above was investigated by varying them in a 2^4 factorial experiment. The levels for each variable are shown in Table 1. These were chosen to encompass commonly used ranges yet be practical for the laboratory equipment available. The method of can preparation and heat processing was identical to that of Tahmmanenate (1980) so only a summary is presented here. The baby food formulation is shown in Table 2. The raw materials were disintegrated and homogenized, then frozen until required. Preheating to 70°C over a period of 7 min was carried out in a steam jacketed pan with continuous agitation. This procedure maximized the baby food viscosity (Taimmanenate, 1980). Anti-sulphur lacquered cans (75 mm height, 106 mm diameter) were then filled and seamed according to the plan in Table 1. These cans each had a central 25 gauge copper/constantan thermocouple junction mounted by the tension method (Board, 1977). For this the copper and constantan wires had been separated and threaded through holes on the opposite sides of the can, joined and soldered. The junction must be as small as possible. The holes in the can walls were sealed with epoxy resin and the resin hardened. After filling and seaming the cans were immersed in a water bath held at a constant temperature between 60 and 65°C to equilibrate prior to processing.

The retort used was a laboratory-scale, horizontal autoclave, equipped with two venting valves, steam inlet, cooling water inlet, cooling water outlet and compressed air inlet controls. Steam pressure was controlled manually, but the compressed air pressure was automatically controlled to a preset value.

Two cans were processed in each experimental run. The thermocouples in each of these were connected to a 12 point recording potentiometer operating on a 30 sec print cycle.

Table 2. Composition of the baby food used in experimental work

Beef	5.0%	Cornflour	2.5%
Carrots	10.0%	Barley flour	0.8%
Peas	5.0%	Yeast extract	0.8%
Potatoes	30.0%	Salt	0.4%
Flour	1.2%	Water	44.7%

Parallel wiring to four junctions was used so that the temperature at the centre of each can was printed every 7.5 sec. Two thermocouples were placed in the retort to measure steam temperature, and these also connected to the recording potentiometer. The measurement junctions were placed in the retort at the same height as the cans to be processed so that at the onset of cooling these would become immersed at the time that the water level was halfway up the cans.

At the commencement of each run all thermocouples were connected to the recording potentiometer while the cans were still in the waterbath. The retort was pre-heated to 100°C by steam addition with the vents open for several minutes. The cans were then inserted into the retort (in a wire basket), the retort door closed, and the steam supply turned on. After 2 min venting the drain and vent valves were closed and the steam supply modulated by hand to control the steam temperature. Heating was continued until the F value (determined using the tabular data of Board (1977) was approximately 6.0. Board's data are based on $Z \times 10$°C and a reference temperature of 121.1°C. At the onset of cooling the steam supply was turned off and the compressed air (pre-set to the required pressure) turned on. Cooling water was admitted as rapidly as possible while still maintaining the required pressure during the cooling phase. The retort was filled to approximately 75% full of water, and the drain valve opened sufficiently to maintain this level. Cooling was continued until the can centre temperatures had dropped to 60°C.

Results and discussion

The first aspect considered was whether the physical model proposed was consistent with measured temperature/time data. Experimental data from Taimmanenate (1980) were used. Figure 1 shows experimental centre temperature data. Predictions based on the assumption that heat transfer was purely by conduction are also shown. These were calculated by finite differences in preference to the analytical heat conduction equations (Newman, 1936), or finite elements. This choice was made because finite differences were more suitable for the second stage of the modelling, and because physical data uncertainties are sufficient that the differences in temperature predictions are negligibly small provided space and time steps ($\triangle r$, $\triangle x$ and $\triangle t$) are chosen wisely. As thermal properties are assumed to be constant, a simple explicit finite difference scheme is almost as accurate as more complex difference schemes. This is

$$h_{jm}^{i+1} = h_{jm}^{i} + \frac{C\alpha\Delta t}{\Delta x^2}(\theta_{j+1m}^{i} - 2\theta_{jm}^{i} + \theta_{j-1m}^{i}) + \frac{C\alpha\Delta t}{\Delta r^2}(\theta_{jm+1}^{i} - 2\theta_{jm}^{i} + \theta_{jm-1}^{i})$$

$$+ \frac{C\alpha\Delta t}{\Delta r^2}(\theta_{jm-1}^{i} - \theta_{jm+1}^{i}) \cdot \frac{1}{2(M+1-m)} \qquad (1)$$

where $h=C\theta$ if C is constant, and nodes are numbered from $j=1$ and $m=1$ at the can centre to $j=J+1$ on the end of the can, and $m=M+1$ on the curved outer surface. Special cases are surface nodes:

$$\theta_{jM+1} = \theta_{J+1\,m} = \theta_a \tag{2}$$

(i.e. all nodes on the can surface are assumed to be at the temperature of the heating or cooling medium), nodes on the central axis of the can ($m=1$), where the method of Albasiny (1960) is employed to give:

$$h_{j1}^{i+1} = h_{j1}^{i} + \frac{C\alpha\Delta t}{\Delta x^2}(\theta_{j+11}^{i} - 2\theta_{j1}^{i} + \theta_{j-11}^{i}) + \frac{4C\alpha\Delta t}{\Delta r^2}(\theta_{j2}^{i} - \theta_{j1}^{i}) \tag{3}$$

and nodes at which $m=1$ where a symmetry boundary condition can be applied:

$$h_{1m}^{i+1} = h_{1m}^{i} + \frac{2C\alpha\Delta t}{\Delta x^2}(\theta_{2m}^{i} - \theta_{1m}^{i}) + \frac{C\alpha\Delta t}{\Delta r^2}(\theta_{1m+1}^{i} - 2\theta_{1m}^{i} + \theta_{1m-1}^{i}) + \frac{C\alpha\Delta t}{\Delta r^2}(\theta_{1m-1}^{i} - \theta_{1m+1}^{i}) \cdot \frac{1}{2(M+1-m)} \tag{4}$$

This scheme predicted temperatures that were within 0.1°C of the analytical solution (Newman, 1936) when eleven nodes across the radius, eleven nodes across half the can height, and a time step of 12 sec were used. Therefore the line shown in Fig. 1 for the pure conduction model would have been derived almost identically from any of the other temperature prediction methods. The thermal diffusivity had been measured by Taimmanenate as 1.535×10^{-7} m^2/sec.

The finite difference calculations were then modified to take into account the boiling and condensation effects. Figure 2 shows the calculations involved, and results are shown on Fig. 1. Data for can expansion in response to pressure difference were taken from Charm (1978). This modelling approach takes account of only the thermodynamic effects, and ignores any mixing caused by the formation and collapse of steam bubbles. In fact, it assumes that in spite of the presence of steam thermal properties do not change and the can contents always fill the can; assumptions which are probably untrue. Thus the inability of the results to match the experimental curve on Fig. 1 is hardly surprising. However the similarity in shape indicates that the basic concept of the physical model is probably sound.

Although this fundamental modelling approach did not lead to useful predictions of cooling phase lethality some conclusions could be drawn from it. Firstly, the proposed physical model (boiling/condensation effects which create mixing, and a moving phase change region) is probably valid, thus answering previous conjecture on the reasons for irregular cooling curves. Second, more detailed mathematical modelling is unlikely to be useful as the

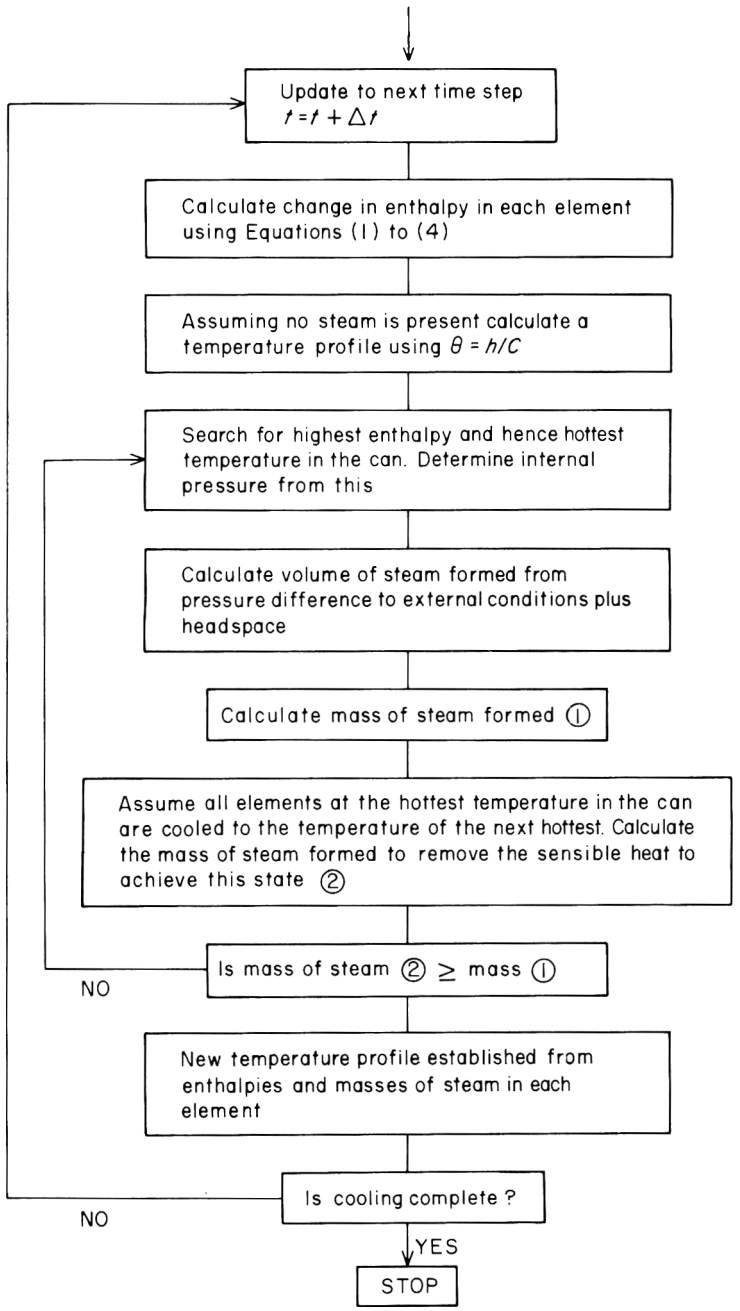

Figure 2. Calculation flowsheet to take account of some thermodynamic effects of boiling and condensation in cooling of cans.

mixing effects are probably very complex, depending on both the heat transfer and the food physical properties.

An experimental investigation of how the four processing variables discussed earlier affect lethality was therefore the most useful approach.

Table 3. Experimental conditions for the 2^4 factorial experiment. Codings are shown in Table 1

Run	Pressure during sealing	Head-space	Pressure during cooling	Steam tempera-ture	θ_{in} (°C)	θ_{ah} (°C)	t_h (°C)	θ_{ac} (°C)	t_c (s)
1	−1	−1	−1	−1	62.8	113.9	6270	40.2	2400
2	1	−1	−1	−1	63.5	113.8	6730	33.7	2400
3	−1	1	−1	−1	62.9	113.8	6730	33.7	2400
4	1	1	−1	−1	62.5	113.9	6270	40.2	2400
5	−1	−1	1	−1	62.8	113.8	6760	38.2	2400
6	1	−1	1	−1	63.8	113.8	6700	38.2	2400
7	−1	1	1	−1	63.0	113.8	6760	38.0	2400
8	1	1	1	−1	62.8	113.8	6700	38.2	2400
9	−1	−1	−1	1	62.0	123.0	3960	28.0	2400
10	1	−1	−1	1	61.6	124.3	3950	34.1	2400
11	−1	1	−1	1	60.4	124.3	3950	34.1	2400
12	1	1	−1	1	62.1	123.0	3960	28.0	2400
13	−1	−1	1	1	64.2	124.0	3940	38.5	2400
14	1	−1	1	1	63.9	124.0	3940	38.5	2400
15	−1	1	1	1	63.9	123.9	3790	40.9	2400
16	1	1	1	1	64.4	123.9	3790	40.9	2400
17	0	0	0	0	64.0	118.6	4860	37.2	2400
18	0	0	0	0	64.0	118.6	4860	37.2	2400

Table 4. F_0 values for cans receiving various heat treatments. (A) experimental; (B) predicted from the pure heat conduction model.

	F_{0h}		F_{0c}		F_{0t}		$F_{0h\,(exp)}$	$F_{0c\,(exp)}$	$F_{0t\,(exp)}$
Run	A	B	A	B	A	B	$F_{0h\,(pred)}$	$F_{0c\,(pred)}$	$F_{0t\,(pred)}$
1	5.67	5.69	1.57	1.63	7.24	7.32	1.00	0.96	0.99
2	6.88	6.89	1.49	1.62	8.37	8.51	1.00	0.92	0.98
3	6.30	6.85	1.29	1.62	7.59	8.47	0.92	0.80	0.90
4	5.94	5.67	0.75	1.63	6.69	7.30	1.05	0.46	0.92
5	6.88	6.91	0.82	1.65	7.70	8.56	1.00	0.50	0.90
6	6.77	6.82	0.87	1.64	7.64	8.46	0.99	0.53	0.90
7	6.54	6.93	0.58	1.64	7.12	8.57	0.94	0.35	0.83
8	7.26	6.75	0.46	1.63	7.72	8.38	1.08	0.28	0.92
9	5.38	5.36	5.38	5.47	10.76	10.83	1.00	0.98	0.99
10	6.35	6.47	7.12	6.97	13.47	13.44	0.98	1.02	1.00
11	7.73	6.24	3.42	6.84	11.15	13.08	1.23	0.50	0.85
12	7.02	5.38	4.28	5.48	11.30	10.86	1.30	0.78	1.04
13	7.07	6.76	2.26	7.16	9.33	13.92	1.05	0.32	0.67
14	6.83	6.70	2.04	7.12	8.87	13.82	1.02	0.29	0.64
15	7.20	5.10	2.23	6.21	9.43	11.31	1.41	0.36	0.83
16	6.71	5.24	1.19	6.30	7.90	11.54	1.28	0.19	0.68
17	6.71	6.41	1.73	3.52	8.34	9.93	1.05	0.49	0.84
18	7.05	6.41	1.73	3.52	8.78	9.93	1.10	0.49	0.88

Tables 3 and 4 show the conditions and results for the eighteen experimental cans. Experimental F_0 values were calculated using the tabular 'L' values of Board (1977). Predicted F_0 values were based on the pure conduction model (finite difference calculations). The baby food used in the second set of experiments was a new batch so its thermal diffusivity was re-determined using the slope of a plot of ln Y *versus* t for cans in which there was no headspace. This method has been used by Olson & Jackson (1942) and Teixeira (1969). The new average value was 1.60×10^{-7} m^2/sec. At each time step the increase in process lethality was calculated using

$$F_0^{i+1} = F_0^i + \frac{k_\theta}{k_{121.1}} \cdot \frac{\Delta t}{60} \tag{5}$$

where k_θ was calculated at temperature θ_k:

$$\theta_k = {}^1/_2(\theta_{11}^i + \theta_{11}^{i+1}) \tag{6}$$

To match Board's tables Z=10°C was used in evaluation of values of $k_\theta / k_{121.1}$.

All runs with no headspace would be expected to match the pure conduction model during the heating phase. This was the case. Further, those runs with no headspace, and with high pressures during cooling would be expected to match the pure conduction model during cooling. There were four such runs (1, 2, 9 and 10), and their F_0 values were close to those predicted.

All other runs showed irregular temperature/time profiles during cooling. Some of these showed plateaus or inflections, these occurring at different temperatures for different cans, while others showed sharp changes in slope as if violent mixing of the can contents had occurred. These effects could be qualitatively related to the four process parameters: (1) Plateau/inflection regions occurred in all cases where head space was present, as well as in other runs where the internal can pressure exceeded the retort pressure during cooling, thus indicating can expansion was significant; (2) the can pressure during seaming (index for presence of non-condensable gases) affected the temperature at which the plateau/inflection regions occurred. At seaming pressures of 41 kPa.a. these were most often between 85–95°C, whereas the inflection occurred at 100–110°C for most cans with a seaming pressure of 101 kPa.a; (3) at low cooling pressures the irregularities in the shape of the temperature/time profiles were more pronounced than at high cooling pressures; (4) steam temperature was used as an indicator of maximum internal temperature. At higher temperatures the irregular cooling phenomena were more pronounced.

The ratio of $F_{0(\mathrm{exp})}/F_{0(\mathrm{pred})}$ was used as an index of the extent to which the phenomena affected the process lethality. The data in Table 4 show that the total process lethality does alter significantly with the four process variables. The cooling phase F_0 varied between 19% and 100% of that predicted assuming that no boiling occurred, and total lethality was as low as 64% of the

prediction. This indicates that underprocessing is a real possibility if the effects of internal phase change are not taken into account in choosing the heating phase target lethality.

Attempts were made to fit a quantitative mathematical model to the data set in Table 4. The data set forms an orthogonal experimental design from which the main effects of each variable, and their interactions with each other can be calculated. The significant terms (at the 95% level of confidence) were retained, these giving the following equations:

$$\frac{F_{0h\,(\exp)}}{F_{0h\,(\mathrm{pred})}} = 1.078 + 0.074\,X_2 + 0.082\,X_4 + 0.074\,X_2X_4 \tag{7}$$

$$\frac{F_{0c\,(\exp)}}{F_{0c\,(\mathrm{pred})}} = 0.568 - 0.112\,X_2 - 0.226\,X_3 \tag{8}$$

$$\frac{F_{0t\,(\exp)}}{F_{0t\,(\mathrm{pred})}} = 0.877 - 0.081\,X_3 - 0.052\,X_3X_4 - 0.039\,X_4 - 0.028\,X_1X_3X_4 + 0.026\,X_2X_3 \tag{9}$$

where X_1=coded value of pressure during sealing, X_2=coded value for head space, X_3=coded value of pressure during cooling, X_4=coded steam temperature.

Fitting of equations in this manner assumes that a linear model will adequately describe the data set. Suitability of a model can be tested by comparing the constants in Equations (7), (8) and (9) with results for Runs 17 and 18 (at the 0, 0, 0, 0 level). There is significant lack of fit in the case of Equation (8) indicating that a linear model is probably not suitable. Therefore in interpretation of Equations (7) to (9) it must be remembered that other effects may be of importance, and could be found to be statistically significant if another form of model was used.

Considering first Equation (8). Variation in can headspace and retort cooling pressure led to substantial differences in F_0. Enlarged head space and lower cooling pressure compared to the test can will lead to lower cooling phase lethality. The effects of seaming pressure and steam temperature on F_{0c} were not statistically significant.

Equation (7) is more difficult to interpret. The effect of increasing head space is to increase heating phase lethality due to an increasing rate of temperature rise as headspace increases (and mass of baby food decreases). Surprisingly, steam temperature is apparently important, both on its own, and as an interaction with headspace. The important interaction with headspace suggests that the effect of headspace is accentuated as the steam temperature is increased. There is no apparent physical reason for steam temperature on its own leading to deviation from the heat conduction model. It may be that the

way steam temperature and headspace interact is complex, and is inadequately explained by the simple $X_2 X_4$ term.

Equation (9) looks at the cumulative effect of heating and cooling. For the baby food the two effects of headspace largely cancel so X_2 does not appear in the equation by itself. Overall cooling pressure (X_3) is the most important parameter. Lethality decreases as cooling pressure drops, and this effect is accentuated by increasing retort temperature ($X_3 X_4$). The effect of steam temperature has been carried through from Equation (7), and this term can only be assumed to arise from the inadequate shape of a linear model in this situation. The $X_2 X_3$ term suggests that the cooling pressure effect is accentuated by increasing headspace, and a slight effect due to can seaming pressure is seen in the $X_1 X_3 X_4$ term. The linear model represented by Equations (7) to (9) is not a mechanistic one so it is difficult to relate the terms in it to the physical model proposed earlier. The numerical values of the coefficients in Equations (7) to (9) are probably specific to the baby food and should therefore not be used for other foods.

To develop a more accurate equation relating process lethality to the four process variables would require further experimentation to collect data suitable for a non-linear model. Such an equation would not necessarily be useful in practice because it is the variation in the extent of boiling rather than accurate prediction of the absolute magnitude under a particular set of conditions that is important. Further, any model would only apply to a particular food. Therefore the prime aim of the work was not to obtain an accurate quantitative equation, but to describe how internal phase change affects process lethality, and to show canners the effects that would result from poor process control. Use of heating phase target lethalities which are less than the required total lethalities (for example to reduce nutrient losses) can only be successful if the various process variables are controlled sufficiently well to consistently obtain the expected cooling phase contribution.

Conclusions

Differences in a thermal process that alter the extent of internal boiling can significantly alter total process lethality. This has practical implications in that poor control of the four process variables could lead to underprocessing of some cans. The way in which the four factors interact is too complex to be accurately represented by a linear model. Further, the relative size of the effects of the variables will probably vary for different foods. Therefore further investigations of the type discussed in this paper are probably not justified. However, if irregular cooling phenomena are observed in establishing processing conditions and heating phase target lethality by experiment, the canner should investigate the sensitivity of the cooling phase lethality to process variables that affect the extent of internal boiling in the can. The

heating phase target lethality should then be chosen to ensure that underprocessing does not result.

Appendix

C	Volumetric specific heat capacity ($J/m^3{}^\circ C$)
F_0	Equivalent processing time at 121.1°C (min) based on a Z value of 10°C
F_{0c}	F_0 value for cooling phase (min)
F_{0h}	F_0 value for heating phase (min)
F_{0t}	F_0 value for overall thermal process (min)
h	Enthalpy (J/m^3)
i	Number of elapsed time steps in finite difference calculation
j	Finite difference node numbering in height of can
J	Number of Δxs in half-height of can
k_θ	First order rate constant for spore destruction (per sec)
$k_{121.1}$	k value at 121.1°C (per sec)
m	Finite difference node numbering in radius of can
M	Number of Δrs in can radius
Δr	Space increment in r direction (m)
t	Time (sec)
t_c	Cooling time (sec)
t_h	Heating time (sec)
Δt	Time step in finite difference calculations (sec)
Δx	Space increment in x direction (m)
X_1–X_4	Coded values of process variables (Table 1)
Z	Temperature interval for a ten-fold change in rate constant (°C)
α	Thermal diffusivity (m^2/sec)
∇	Extent of first order process = $\int k\,dt$
θ	Temperature (°C)
θ_a	External medium temperature (°C)
θ_{ac}	Cooling medium temperature (°C)
θ_{ah}	Heating medium temperature (°C)
θ_{in}	(Uniform) initial temperature in can (°C)
θ_k	Temperature at which rate constant is evaluated (°C)

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A study of the apparent diffusion coefficients for solute losses from carrot tissue during blanching in water

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Summary

The apparent diffusion coefficients (D_a) for total solute loss from cylinders of Chantenay carrot cortex tissue were calculated after blanching under various conditions. Cell sap concentration was measured before and after blanching for times up to 1800 sec, in the temperature range 333–363°K. Values of D_a were found to be in the range 3×10^{-10}–8×10^{-10} m^2/sec, and could be related to temperature by an Arrhenius type equation, having an activation energy of 28.2 kJ/mol. D_a was independent of cylinder diameter between 0.005 and 0.007 m, but appeared to increase in blanch media concentrations that were higher than the initial carrot cell sap concentration. The formal numerical solutions for unsteady state diffusion mass transfer were recalculated in the relevant cell sap concentration range (7.8–10.4% as sucrose), and are given in tabular form for the shapes of slab of infinite extent, sphere, and cylinder of infinite length. In design, these results will allow the prediction of mean cell sap concentration, and hence the overall losses incurred, after a given blanch or wash treatment in this time and temperature range.

Introduction

Water blanching is one of many processes in which the mass transfer properties of the food constituents influence both the final product quality and related operations, such as effluent disposal. Much attention has been given to the losses of nutrients during vegetable blanching and the work has been well reviewed elsewhere (Lee, 1958; Cain, 1967; Selman, 1977). Relatively few studies have involved carrots (Lee, 1945; Gooding, 1956; Sistrunk, 1969; Dan & Jain, 1971; Mirza & Morton, 1974; Baloch, Buckle & Edwards, 1977). In

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most cases little consideration has been given to the mechanisms of loss involved (Selman & Rolfe, 1979).

Several researchers have studied the loss of solid or liquid during the processing of a variety of foodstuffs, under conditions where the surface resistance has been removed by agitation and is just diffusion controlled. For example Becker & Sallans (1955) studied the drying of wheat kernel which was considered as spherical in shape. They estimated apparent diffusion coefficients (D_a, m^2/sec) for water at several temperatures, and found that D_a was related to temperature by an Arrhenius type equation:

$$D_a = D_0 \exp(-E_a/RT)$$

where D_0=constant (m^2/sec), E_a=activation energy, (kJ/mol), R=universal gas constant (8.314 J/K per mol), T=absolute temperature (°K).

At 334°K the D_a for water was 1.58×10^{-10} m^2/sec. Saravacos & Charm (1962) reported D_a values of $10^{-9}-10^{-10}$ m^2/sec for water in the air drying of potato and other fruits and vegetables, again exhibiting strong temperature dependence. Urie & Shahbenderian (1968) found the desalting of pickled gherkins to be controlled by simple diffusion, with a D_a of 1.35×10^{-10} m^2/sec. Suarez, Viollaz & Chirife (1980) dried grain sorghum, treated the grain as spherical, and also showed that D_a obeyed an Arrhenius-type equation with temperature; at 333°K, $D_a = 4.0\times10^{-11}$ m^2/sec. Lathrop & Leung (1981) examined the water blanching of peas. These were treated as spherical in shape and the D_a for ascorbic acid at 358°K was determined as 1.4×10^{-8} m^2/sec, a significantly higher value compared to related work. Similar experiments have been conducted on other foods, for example Bressan *et al.* (1981, 1982) measured the D_a for solids loss from cheese curds during washing. They related D_a to temperature using a linear relationship; at 331°K the value of D_a was 5.54×10^{-10} m^2/sec.

The objective of this work was to examine the mechanisms of solute loss from carrot tissue during water blanching, and to estimate values of D_a for solute loss by diffusion under various conditions of tissue size, blanch time, temperature and concentration of the blanch medium.

Materials and methods

Carrot samples

Chantenay carrots (supplied by the National Vegetable Research Station, Wellesbourne) were sorted to select those with a length of 0.12–0.15 m and a diameter of 0.025–0.035 m. Cylinders of carrot tissue were cut longitudinally using a sharp No. 3 size cork borer, to give cylinders of either cortex or core tissue 0.06 m in length and 0.006 m in diameter. This effectively 'infinite' cylinder was chosen to be the studied shape due to the availability of a formal solution for unsteady state diffusion, and the similarity to the shape of a whole

Table 1. Cell sap concentration (percentage refractometric solids, as sucrose) of Chantenay carrot cortex cylinders cut from eight different carrots

Cylinders cut from the given carrot, at the same radius	Cell sap concentration (as % sucrose w/w) in cylinders of carrot cortex cut from the given carrot							
	A	B	C	D	E	F	G	H
1	7.8	9.6	10.1	10.1	8.5	9.0	9.2	8.3
2	7.9	10.1	10.4	10.1	8.4	9.0	9.5	8.0
3	8.3	10.2	—	10.2	8.3	9.1	—	8.0
4	7.8	10.1	—	—	—	9.6	—	8.0
5	—	10.1	—	—	—	—	—	—
Means	8.0	10.0	10.2	10.1	8.4	9.2	9.4	8.1

carrot. Where indicated, other work involved carrots of unknown variety (nameless) but which had similar dimensions and initial cell sap concentration to the Chantenay carrots.

Several cylinders were cut out from a number of carrots and placed in a covered Petri dish to minimize evaporation during the short delay prior to use. Each test sample consisted of two cylinders (approx. 0.005 kg) which were carefully blotted with absorbent paper to remove surface cell sap before being weighed. As characteristics of the Chantenay carrots used, the dry solids and alcohol insoluble solids contents of the carrots were measured on a number of samples. Mean dry solids content of cortex samples was 11.64±0.98% as measured by drying to constant weight at 343°K and 13.33 kN/m^2 pressure. Mean alcohol insoluble solids content of whole carrot samples was 3.75±0.10% as determined by the method of Moyer & Holgate (1948).

Cell sap was expressed from the carrot tissue using a pestle and mortar and the concentration of solutes was measured as percentage sucrose using an Abbé refractometer calibrated with sucrose solutions (w/w) at 293°K. Leveille *et al.* (1974) reported that the solutes in carrots (soluble solids content=8.4±0.4%) consisted typically of about 50% sucrose, 20% reducing sugars and 30% vitamins, minerals and soluble protein. The concentration of solutes in the cell sap of Chantenay carrots was only slightly less in core samples compared to cortex samples. Cylinders of cortex taken from different positions within the cortex had the same cell sap concentration. The number of cylinders that could be cut from one carrot without defect varied from two to five. The typical variation of cell sap concentration between cylinders cut from eight carrots is shown in Table 1. Solute content of the cell sap ranged from 7.8–10.4%, with the mean for all twenty-seven cylinders being 9.1±0.2% (as sucrose).

Blanching

The blanching technique was developed from that described by Selman & Rolfe (1979). In this case it was desired to maintain a constant solute content

in the blanch water, and so a 29 litre tank (0.45×0.29 m diameter) was filled with 27 litre of either distilled water or sucrose solution, to effect an infinite volume. The blanch medium was thermostatically controlled to ±0.5°K, and was agitated by an impeller to promote a uniform and constant concentration in the medium at the surface of the carrot samples. Evaporation from the surface of the blanch medium was minimised by a layer of plastic spheres. Cotton thread was passed diametrically through the centre of each carrot cylinder with a fine sewing needle, so that two carrot cylinders were about 0.10 m apart. The lower end of the thread was weighted and several such threads could be simultaneously suspended in the blanch medium from cross bars. Blanch time was recorded from the moment of immersion. After the required blanch time the samples were removed, lightly blotted, separated from the cotton thread and placed in a closed Petri dish. One cylinder in each sample was weighed and the dry solids content determined, and the second cylinder was weighed and the cell sap concentration determined. A post blanch cooling procedure was not incorporated.

Calculation of apparent diffusion coefficient (D_a)

The two contributions to the total resistance to mass transfer in the blanching of vegetables are the surface resistance due to convection, and the 'internal' resistance due to mass diffusion. These two can be represented by Fick's first and second laws together with a mass balance at the interface (surface):

$$\frac{\partial C}{\partial t} = D_a \cdot \frac{\partial^2 C}{\partial x^2}$$

and

$$-D_a \cdot \frac{\partial C}{\partial x} = k(C - C_0) = \frac{1}{A} \cdot \frac{\partial N}{\partial t}$$

at $x=a$ (surface), where A=total surface area for mass transfer (m^2), C=solute concentration at any point in the sample (%), C_0=concentration of the (blanch) medium (%), k=surface mass transfer coefficient (kg/m^2 per sec), N=mass diffusing (kg), t=(blanch) time (sec) and x=any position in the sample where the concentration is C(m).

The solution to these equations is given by Newman (1931a) for the three geometric shapes of slab of infinite extent, cylinder of infinite length, and a sphere. The average concentration is obtained after integration with respect to position, as a function of time for given values of surface mass transfer coefficient, all in non-dimensionalized form. Newman also shows how the results for a slab of infinite extent can be used to obtain the solution for a strip

of infinite length, a rhomboid (in particular a cube) and a cylinder of finite length.

If there is sufficient agitation of the blanching liquid, then the surface resistance becomes small and it can be assumed that the total resistance is due to only the 'internal' resistance. We then require only the solution to Fick's second law. This is given by Newman (1931b) for the geometrical shapes slab of infinite extent, cylinder of infinite length, and sphere, with the average concentration obtained after integration with respect to position as a function of time, again in non-dimensionalized form as follows:

$$\text{Slab: } E = \frac{\bar{C} - C_0}{C_1 - C_0} \quad \text{(non-dimensionalized concentration)}$$

$$= \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left[-(2n+1)^2 \left(\frac{D_a t}{a^2}\right)\left(\frac{\pi}{2}\right)^2\right]$$

$$\text{Sphere: } E = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left[-n^2 \left(\frac{D_a t}{a^2}\right)\pi^2\right]$$

$$\text{Cylinder: } E = 4 \sum_{n=1}^{\infty} \frac{1}{R_n^2} \exp\left[-\left(\frac{D_a t}{a^2}\right) R_n^2\right]$$

where R_n is the root of $J_0(x) = 1 + \sum_{n=1}^{\infty} (-1)^n \frac{x^{2n}}{(2n)^2!} = 0$

where the first ten values are:

n	1	2	3	4	5	6	7	8	9	10
R_n	2.4048	5.5201	8.654	11.792	14.931	18.071	21.212	24.352	27.493	30.635

and here, a=characteristic linear dimension; half the diameter for a cylinder and sphere, half the thickness for a slab (m), $\bar{C}$=average solute concentration in the (blanched) sample at time t (%), C_1=uniform initial (cell sap) solute concentration in the fresh (unblanched) sample (%), J_0=the Bessel function of order zero, n=number of roots and $D_a t/a^2 = \tilde{t}$, a non-dimensional time.

Newman (1931b) developed these solutions for drying applications over a wide range of concentrations and presented the results as a set of tables. In the case of blanching vegetables, the concentrations are much smaller and near to zero, and Table 2 shows the solutions recalculated for smaller increments in this range of concentrations, using the first ten terms for the three series. This table has been applied in this blanching work. Values of D_a were calculated by first estimating $E=(\bar{C}-C_0/C_1-C_0)$ from the experimenting data, and then

Table 2. Values of $\bar{t}(D_a t/a^2)$ for various values of $E(\bar{C} - C_0/C_1 - C_0)$ for the geometrical shapes: slab of infinite extent, sphere, and cylinder of infinite length.

$\bar{t}$	Slab (E)	Sphere (E)	Cylinder (E)
0.0002	0.9773	0.9305	0.9527
0.0004	0.9734	0.9198	0.9455
0.0006	0.9697	0.9097	0.9387
0.0008	0.9663	0.9003	0.9323
0.0020	0.9493	0.8536	0.9003
0.0040	0.9286	0.7978	0.8612
0.0060	0.9125	0.7557	0.8312
0.0080	0.8990	0.7212	0.8063
0.0100	0.8871	0.6914	0.7845
0.0200	0.8404	0.5812	0.7014
0.0300	0.8045	0.5036	0.6402
0.0400	0.7743	0.4429	0.5904
0.0500	0.7476	0.3930	0.5478
0.0600	0.7236	0.3508	0.5105
0.0700	0.7014	0.3143	0.4772
0.0800	0.6808	0.2825	0.4470
0.0900	0.6614	0.2544	0.4195
0.1000	0.6431	0.2295	0.3941
0.1100	0.6257	0.2072	0.3707
0.1200	0.6091	0.1873	0.3489
0.1300	0.5931	0.1694	0.3286
0.1400	0.5778	0.1532	0.3096
0.1500	0.5630	0.1387	0.2918
0.1600	0.5487	0.1256	0.2751
0.1700	0.5349	0.1137	0.2595
0.1800	0.5215	0.1030	0.2447
0.1900	0.5085	0.0932	0.2309
0.2000	0.4959	0.0845	0.2178
0.2100	0.4836	0.0765	0.2055
0.2200	0.4717	0.0693	0.1939
0.2300	0.4600	0.0628	0.1830
0.2400	0.4487	0.0569	0.1727
0.2500	0.4377	0.0515	0.1629
0.2600	0.4270	0.0467	0.1538
0.2700	0.4165	0.0423	0.1451
0.2800	0.4063	0.0383	0.1370
0.2900	0.3964	0.0347	0.1293
0.3000	0.3867	0.0314	0.1220
0.3100	0.3773	0.0285	0.1151
0.3200	0.3681	0.0258	0.1087
0.3300	0.3591	0.0234	0.1025
0.3400	0.3503	0.0212	0.0968
0.3500	0.3418	0.0192	0.0913
0.3600	0.3334	0.0174	0.0862
0.3700	0.3253	0.0157	0.0814
0.3800	0.3174	0.0142	0.0768

Table 2—*continued*

$\bar{t}$	Slab (E)	Sphere (E)	Cylinder (E)
0.3900	0.3096	0.0129	0.0725
0.4000	0.3021	0.0117	0.0684
0.4100	0.2947	0.0106	0.0645
0.4200	0.2875	0.0096	0.0609
0.4300	0.2805	0.0087	0.0575
0.4400	0.2737	0.0079	0.0543
0.4500	0.2670	0.0071	0.0512
0.4600	0.2605	0.0064	0.0483
0.4700	0.2541	0.0058	0.0456
0.4800	0.2479	0.0053	0.0430
0.4900	0.2419	0.0048	0.0406
0.5000	0.2360	0.0043	0.0383
0.5100	0.2302	0.0039	0.0362
0.5200	0.2246	0.0035	0.0341
0.5300	0.2192	0.0032	0.0322
0.5400	0.2138	0.0029	0.0304
0.5500	0.2086	0.0026	0.0287
0.5600	0.2035	0.0024	0.0271
0.5700	0.1986	0.0021	0.0256
0.5800	0.1937	0.0019	0.0241
0.5900	0.1890	0.0017	0.0228
0.6000	0.1844	0.0016	0.0215

finding the corresponding value for $\bar{t}$ from the graph of $\bar{t}$ *versus* E for a cylinder, as constructed from the values in Table 2. D_a was finally determined by solving $\bar{t} = D_a t/a^2$.

Results and discussion

Preliminary work involved nameless carrots from which standard cylinders of cortex and core tissue were cut and then blanched at 343°K for several times up to 1800 sec. Solute concentration in the cell sap was measured after each blanch time and the results are shown in Fig. 1. The trend for both cortex and core is similar, both exhibiting the most rapid decrease in cell sap solute concentration during the first 300 sec. Similar studies on carrot cortex by Selman & Rolfe (1979) suggested that losses during these first 300 sec at 343°K were due not simply to diffusion, but also to the expulsion of cell sap as turgor was lost on cell death. Values for D_a as calculated from the curves are shown in Table 3, and this shows the mean values for D_a during the period from 600–1800 sec when solute losses appear to arise solely by diffusion. At 343°K the mean D_a for movement of solutes out of the core tissue was 5.30×10^{-10}, being slightly less than the mean D_a of 6.40×10^{-10} m^2/sec for the cortex tissue.

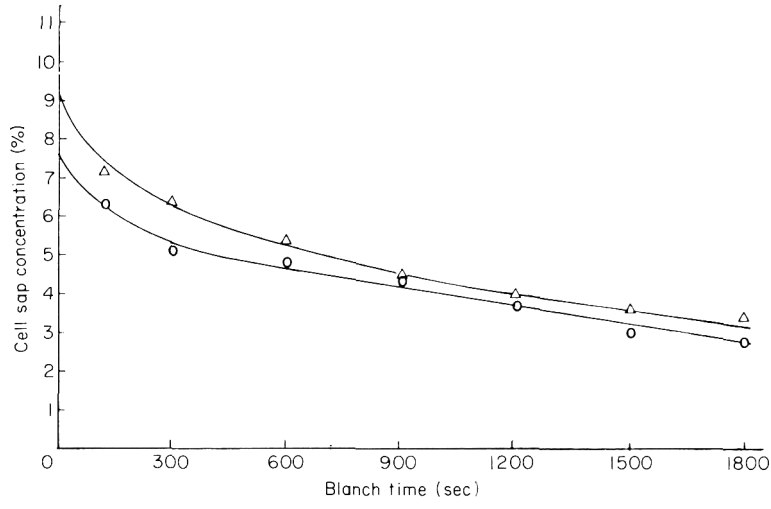

Figure 1. Percentage cell sap concentration of nameless carrot cortex and core cylinders after the given blanch time at 343°K (means of two duplicated replicates). △, Cortex; ○, core.

This difference may be due in part to initial concentration differences between the tissues, and to small structural differences between the tissues. The expulsive losses during the first 300 sec are reflected in some of the D_a values given in Table 3, which tend to be lower as might be expected if whole solution is being lost during loss of turgor.

Cylinders of Chantenay cortex tissue were blanched for several times up to 1800 sec at 333, 343, 353 and 363°K and Fig. 2 shows the resulting cell sap concentrations. A similar pattern of decreasing cell sap concentration is observed at each temperature, but with increasing temperature the trend is for

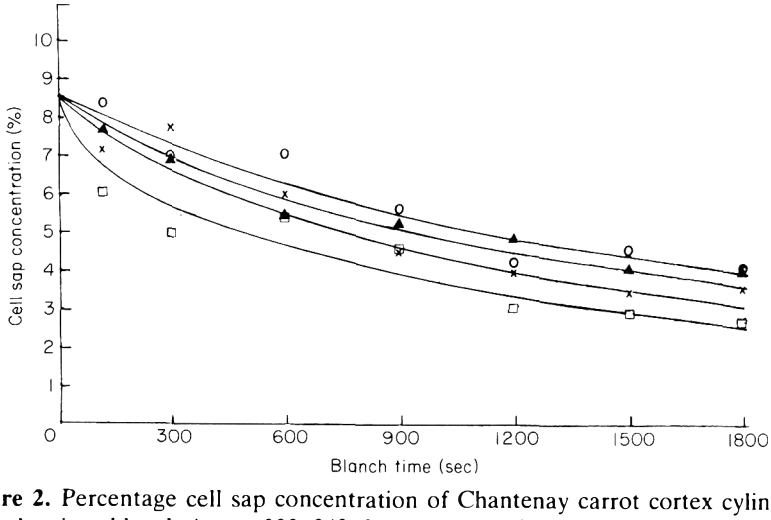

Figure 2. Percentage cell sap concentration of Chantenay carrot cortex cylinders after the given blanch time at 333, 343, 353 and 363°K (means of two replicates). ○, 333°K; ▲, 343°K; ×, 353°K; □, 363°K.

Table 3. Apparent diffusion coefficients (D_a) of cell solutes of carrot cylinders when blanched in water under the given conditions (data from curves in Figs 1, 2, 4 and 5)

Data from figure number	Sample type	Conditions varied from standard*	$D_a \times 10^{10}$ (m²/sec) values at the given blanch time (sec)							Mean 600–1800
			120	300	600	900	1200	1500	1800	
1	Nameless									
	Core	Standard	4.50	6.12	5.52	4.84	5.10	5.35	5.70	5.30
	Cortex	Standard	6.15	6.60	6.62	6.70	6.56	6.06	6.05	6.40
2	Cortex	333°K	3.75	1.20	2.34	2.10	3.41	3.68	3.82	3.07
		Standard	1.00	2.04	3.42	4.00	4.35	4.35	4.55	4.13
		353°K	1.50	3.30	4.58	5.12	5.72	5.58	5.80	5.36
		363°K	6.98	7.35	7.68	7.62	7.69	7.80	7.43	7.64
4	Cortex	Diam. = 0.005 m	3.07	4.83	5.31	5.14	5.05	4.92	4.80	5.04
		Standard	2.34	4.20	5.25	5.10	5.10	4.86	5.08	5.08
		Diam. = 0.007 m	0.96	2.86	4.04	4.76	4.98	5.15	5.21	4.83
5	Chantenay Cortex	3% Sucrose	5.93	7.14	6.33	5.30	4.73	4.62	5.15	5.23
		15% Sucrose	19.90	24.30	24.50	22.40	19.50	15.60	13.40	19.10
	Nameless Cortex	15% Sucrose	4.65	7.50	7.80	6.95	6.30	5.99	6.00	6.61
		20% Sucrose	13.50	13.00	11.60	9.50	8.29	7.76	8.30	9.09

* Standard conditions: blanch temperature = 343°K; blanch medium = distilled water; carrot cylinder diameter = 0.006 m.

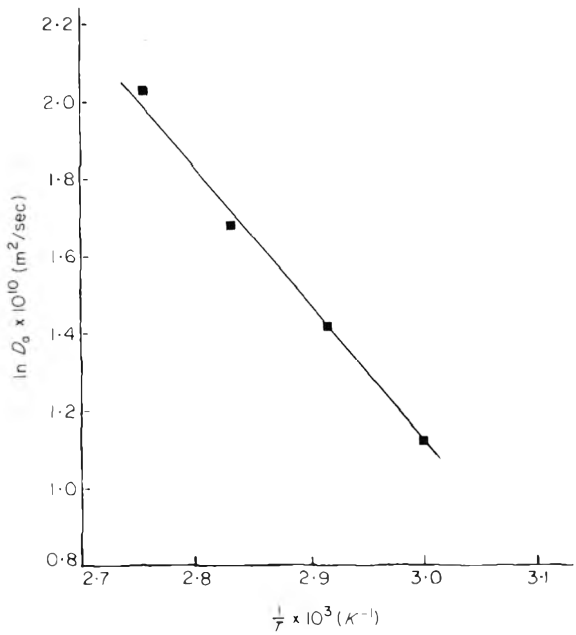

Figure 3. Graph of ln D_a (mean apparent diffusion coefficients at 333, 343, 353 and 363°K from Table 3) *versus* the reciprocal of absolute temperature.

a more rapid decrease in concentration during the first 300 sec, resulting in a greater overall loss at each blanch time. The results for both the Chantenay and nameless carrots at 343°K are very similar, and indicate that different types of carrots having similar characteristics such as initial cell sap concentration, may exhibit a similar pattern of diffusive solute loss during blanching. Mean D_a values (Table 3) show that D_a increases with increasing temperature, having values of 3.07×10^{-10}, 4.13×10^{-10}, 5.36×10^{-10}, and 7.64×10^{-10} m^2/sec at 333, 343, 353 and 363°K respectively. A graph of ln D_a (mean values) versus $1/T$ (Fig. 3) shows that D_a can be related to temperature by an Arrhenius type equation: $D_a = D_0 \exp(-E_a/RT)$. From this graph the value of E_a was calculated as 28.2 kJ/mol. This compares well with reported activation energies for other temperature dependent changes occurring in plant foods during processing. Vaccarezza, Lombardi & Chirife (1974) reported an E_a of 28.9 kJ/mol for water diffusion during drying of sugar beet, and Suarez, Viollaz & Chirife (1980) found an E_a of 31.4 kJ/mol for water diffusion in sorghum grain drying. Paulus & Saguy (1980) found E_a values of 113.0, 92.1 and 117.2 kJ/mol for texture change in Rothild, Kundulus and Rubika carrots respectively during cooking.

Three different cork borers were used to prepare Chantenay cortex cylinders having diameters of 0.005, 0.006 and 0.007 m. These were blanched for several times up to 1800 sec at 343°K and the changes in cell sap concentration are shown in Fig. 4. The rate of decrease of cell sap concentration appears to increase as cylinder diameter decreases. From Table

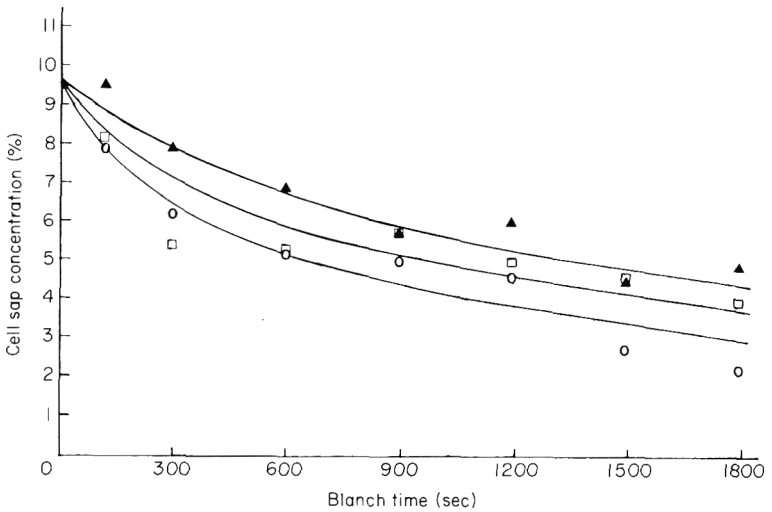

Figure 4. Percentage cell sap concentration of Chantenay carrot cortex cylinders of 0.005, 0.006 and 0.007 m diameter after the given blanch time at 343°K (means of two replicates). ▲, 0.007 m; □, 0.006 m; ○, 0.005 m.

3 it is seen that the D_a values are also influenced by diameter, but only during the first 300 sec blanching, thereafter the D_a values in all three cases were similar with mean D_a values of 5.04×10^{-10}, 5.08×10^{-10}, and 4.83×10^{-10} m^2/sec for the 0.005, 0.006 and 0.007 m diameter cylinders respectively. This indicates that D_a is independent of diameter during the time when solute loss occurs by diffusion, and would be expected. Actual losses of solutes would however be expected to increase with surface area and this was also shown to be true.

In the final set of experiments, cylinders of Chantenay cortex tissue were blanched in three different strengths of sucrose solution, the concentrations being 3, 9 and 15% (w/w) to give blanch medium concentrations that were 6% smaller than, the same as, and 6% greater than the initial mean cell sap concentration of 8.7%. The changes in the cell sap concentration after blanching are shown in Fig. 5. Essentially the results are what might be expected, with sucrose diffusing into the carrot from the 15% solution, little change in cell sap concentration in the 9% solution, and diffusive loss of solutes from the carrot into the 3% solution. This is in agreement with similar observations reported by Dan & Jain (1971) who blanched asiatic carrots (4.5% initial cell sap concentration) for 300 sec at 373°K in solutions containing up to 9% sucrose. The mean D_a values for solute movement in the 3 and 15% solutions are shown in Table 3, D_a for the 9% solution being indeterminate due to the negligible concentration difference. Mean D_a values for diffusion of solutes into the 15% sucrose solution were about four times greater (19.1×10^{-10} m^2/sec) than those for the 3% sucrose solution (5.23×10^{-10} m^2/sec). In both cases D_a decreased with blanch time particularly

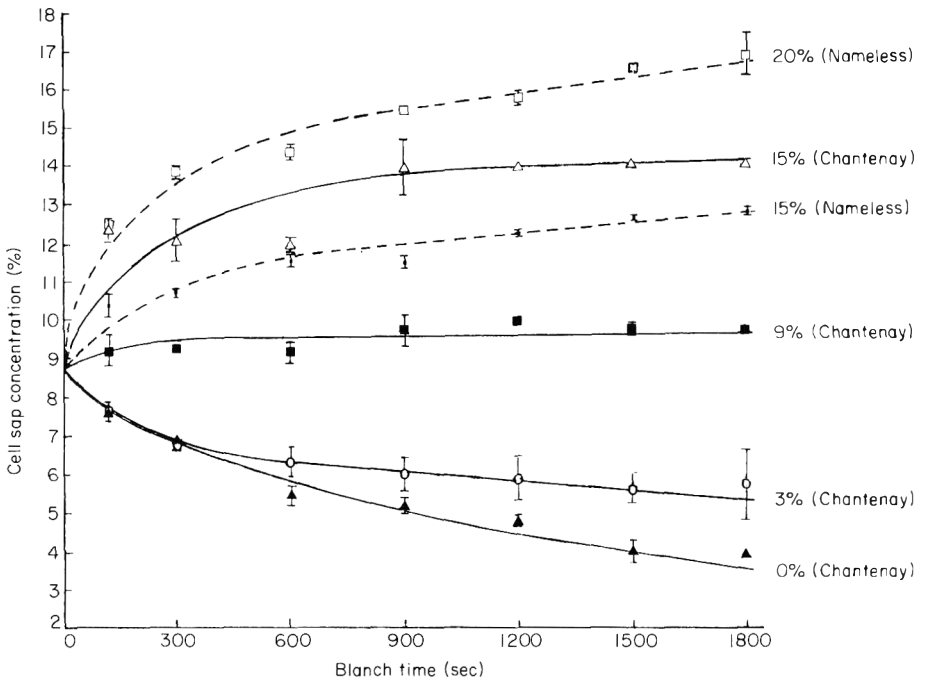

Figure 5. Percentage cell sap concentration of Chantenay and nameless carrot cortex cylinders after the given blanch time at 343°K in 3, 9, 15 and 20% (w/w) sucrose solutions, and water (from Fig. 2) (means of two duplicated replicates).

in the case of the 15% sucrose solution where very small concentration differences existed after 900 sec.

It might be expected that cell sap concentration and blanch water concentration would reach equilibrium at about the same time for both the 15 and 3% conditions. However cell sap concentration rose more rapidly towards an equilibrium in 15% sucrose than did the fall in cell sap concentration towards equilibrium in the 3% sucrose. The higher D_a values observed blanching in 15% sucrose support this, although it is not clear whether D_ε is influenced by the rise of cell sap concentration or vice versa. After 1800 sec cell sap concentration had reached 14% in 15% sucrose, and 5.5% in 3% sucrose when in both cases the initial concentration difference was 6%. Compared to the pattern in water, 9 and 3% sucrose, the pattern at 15% appeared to be unexpected. The difference in the 15% curve seems to arise during the first 300 sec blanching, when the changes occurring are not entirely due to diffusion and yet will influence the calculated value of D_a; for if the initial cell sap concentration (C_1) is taken as that recorded after 300 sec blanching (=12.2%) then D_a at say 1200 sec is 8.7×10^{-10} m^2/sec. Similarly if C_1 is taken as that recorded after 600 sec blanching (=13.2%), then D_a at 1200 sec becomes 3.8×10^{-10} m^2/sec, i.e. more similar to the values of D_a obtained for the other conditions.

However, considering the inherent variability of the carrot material, it was suggested that the 15% result might simply be a reflection of this. And so, bearing in mind the similarity between the results previously obtained for the Chantenay and the nameless carrot cortex cylinders, blanching in 15% sucrose was repeated and also in 20% sucrose using nameless carrots of similar characteristics. These results are shown in Fig. 5. The curves and D_a values (Table 3) are more nearly what might be expected in relation to the results for water, 9 and 3% sucrose, with a mean D_a of 6.61×10^{-10} in 15% sucrose, and 9.09×10^{-10} m^2/sec in 20% sucrose. It is concluded that the results for Chantenay carrots at 15% may well have reflected variability in the carrot tissue, but that D_a is influenced by the blanch medium concentration where it is higher than the initial cell sap concentration. Typically a commercial blanch water concentration for carrots might be about 3–5% during continuous blanching (Gooding, 1956).

Conclusions

The mean D_a values for solutes loss from 0.006 m diameter Chantenay carrot cortex cylinders blanched in water at 343°K were 4.13×10^{-10} and 5.08×10^{-10} m^2/sec for the two experiments. The difference between these results would be expected from the variation in initial cell sap concentration shown in Table 1. For the nameless carrot cortex D_a was found to be 6.40×10^{-10} m^2/sec. Nameless carrot core losses had a D_a of 5.30×10^{-10} m^2/sec, being slightly less, possibly due to an initially lower cell sap concentration and the small structural differences between the tissues. The mean D_a for losses in the 3% sucrose solution was 5.23×10^{-10} m^2/sec suggesting that solute contents in the blanch water up to 3% do not significantly affect diffusive solids loss under these conditions. These D_a values compare with a D_a for 0.38% sucrose in water at 298°K of 5.21×10^{-10} m^2/sec (Weast, 1977). D_a was found to depend on temperature as expected, with an E_a of 28.2 kJ/mol, but was independent of cylinder diameter between 0.005 and 0.007 m. For Chantenay carrots, D_a appeared to increase with increasing blanch medium concentration, being four times larger in 15% sucrose than in 3% sucrose solution. However it is suggested that the result was atypical as repeated tests with nameless carrots in 15 and 20% sucrose gave D_a values of 6.61×10^{-10} and 9.09×10^{-10} m^2/sec respectively. In general D_a values were of the same order of magnitude as those reported for diffusive solids loss from other foodstuffs under various conditions, the value being in the typical range 3×10^{-10} to 8×10^{-10} m^2/sec.

If D_a is calculated for a given temperature, assuming an E_a of 28.2 kJ/mol and a constant D_0 of 8.1×10^{-6} m^2/sec, then $D_a\, t/a^2$ can be evaluated and E may be found from the graph constructed from Table 2. Hence the cell sap concentration ($\bar{C}$) can be calculated for the required blanch time (t). Due to the inherent variability of plant material this will give only an approximate

value of $\bar{C}$. However it should be possible to predict the resulting mean cell sap concentration in the carrot tissue, and hence the overall losses incurred, after a given blanch treatment in the temperature range 333 to 363°K. Further work is now being carried out on potatoes, paying particular attention to the prediction of losses of reducing sugars during blanching and washing operations.

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A comparison of the binding properties of fish flesh

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Summary

Binding properties of loaves prepared from the flesh of ten fish species were evaluated on the basis of visual scores for binding and instrumental measurements of tensile strength. The volume of liquor released on cooking and its content of solids and proteins were measured. The only parameters that corresponded with the binding properties were the moisture and fat content of the raw materials. Fish flesh with high fat content (and low moisture content) did not bind well. Bluefish exhibited a big variability with regard to its binding properties, and this corresponded with its variability in fat and moisture content.

Introduction

Poultry carcasses and fish are small in size and therefore it is difficult to produce large formed products as from red meats. Reforming of various trimmings into larger loaves or sticks might have economical advantage. For red meats binding of small odds and ends might also be worthwhile. This could be accomplished through the mechanism of heat induced binding.

Studies on the mechanism of heat induced binding followed the publication of patented procedures that used salts for binding (Mass, 1963; Torr, 1965; Hansen, Hills & Schwall, 1966). Maesso *et al.* (1970a) studied various effects on the binding quality of poultry loaves. The best binding was obtained by mixing the particles with 2% NaCl and 0.5% polyphosphates (a mixture of sodium tripolyphosphates and sodium hexametaphosphates). Vadehra & Baker (1970) suggested that the binding involved the breakage of cellular structure, the myofibrillar and connective tissue proteins and water holding

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capacity. MacFarlane, Schmidt & Turner (1977) studied the binding ability of beef muscle proteins in a model system. Myosin and actin had good binding ability, whereas sarcoplasmic proteins resulted in poor binding. Siegel, Theno & Schmidt (1978) showed by electrophoresis that myosin and actin were the most important protein constituents of the tacky exudate that developed good binding. In fish, Lee & Toledo (1976) studied the textural properties of products prepared from frozen comminuted Spanish mackerel. They found that salt and phosphates enhanced the binding properties of the fish.

The source of raw material also apparently affects the binding quality. Maesso *et al.* (1970b) showed that broiler leg meat resulted in weaker binding as compared with breast meat. Sato & Nakayama (1970) compared the binding properties of various parts of cockerel meat and one fish species *(Trachurus japanicus)*. They suggested that the binding quality might be related to the amount of myofibrillar proteins in the raw material, and that high fat levels adversely affected binding.

The purpose of this study was to compare the binding properties of the flesh of ten commercially available fish species, and to establish if they could be related to compositional measurements of the fish flesh.

Materials and methods

Raw materials

Fresh fillets of various species were purchased from a local fish market. The species were: bluefish, catfish, cod, haddock, halibut, mackerel, monkfish, sole, white sucker and turbot.

Preparation of loaves

Fillets were cut into 1 cm cubes. The skin, if present, was removed before cutting the flesh into cubes. Fish cubes (200 g) were mixed for 3 min with or without 1.5% NaCl in a small Hobart mixer (Model N50, speed no. 2). After mixing, the samples were stuffed into standard 250 ml pyrex beakers by hand and care was taken to avoid the formation of air pockets during packing. The beakers were covered with aluminium foil, which was punctured with several small holes before placing in an oven at 176°C. The cooking was discontinued when the internal temperature of the fish reached 70–75°C. The beakers were cooled at room temperature to 31–33°C. Each treatment was performed on duplicate samples, and then repeated.

Liquor loss on cooking

The cooked loaves were removed from the beakers and placed in the conical portion of plastic funnels. The liquor released from the loaves under the force of gravity, was collected in graduated cylinders, and the volumes measured after 5 min.

Visual binding scores

Slices of 10 mm width were cut from the centre of the fish loaves and tested for binding by three judges who had experience with this type of product. Binding scores were assigned on an ordinal scale of equal intervals from 1 (very poor binding) to 9 (very strong binding).

Objective measurements of binding

Dumbell shaped samples, approximately 10 mm thick were cut from the centre of the loaves. The samples were secured tightly at both ends of home made jaws of a floor model Instron (Instron Co., U.S.A.). The tensile force required to tear the sample apart when the upper jaw was raised at a constant speed of 2 cm/min was recorded. The peak heights, representing the force required to tear the sample apart, were calculated (in Newtons). The areas under the peaks representing the work performed, were determined by cutting the paper under the peak profile and weighing it.

Chemical analysis of moisture and fat

Moisture and fat analyses were done in duplicate on two different batches of fish according to Official Methods (AOAC, 1975).

Total solids

Samples of the liquor released on cooking from loaves of each fish species were weighed in aluminium dishes and dried overnight at 105°C. After cooling in a desiccator, the samples were re-weighed and the percent total solids calculated.

pH measurements of the fillet

The pH of whole fillets was determined by directly inserting a glass electrode (Metrohm EA147) of a Metrohm/Brinkman pH-103 meter, into the fresh fillets in several spots.

Protein content of the cookout

Protein content of the drip was determined by the method of Lowry *et al.* (1951).

Statistical analysis

Analysis of variance and Duncan's multiple range test were performed on the data with an IBM 1370 using the Statistical Analysis System (SAS). Linear correlations were obtained with the MINITAB computer program.

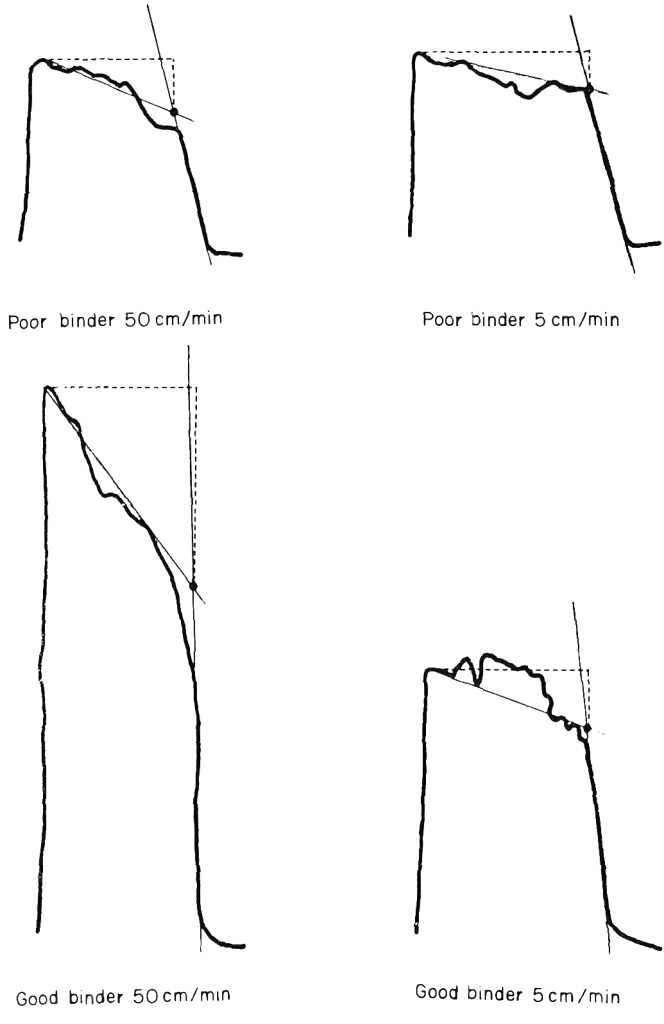

Figure 1. Rheological properties of different bluefish batters.

Objective evaluation of bluefish batters

Bluefish cubes (300 g) were mixed for 3 min in a Hobart mixer (model N50, speed No. 2) with and without 1.5% NaCl. The batters were then placed in a back extrusion cell (7 cm diameter) of an Instron. The full scale load of the cell was 5000 N. Maximal shear-compression forces were recorded at various cross head speeds ranging from 5–50 cm/min. Maximal peak heights, areas under the peaks and slopes (Fig. 1) were measured.

Results and discussion

Table 1 shows the results of the pH of the raw fillets and the moisture and lipid content of the ten fish species. Most of the samples varied in moisture content

Table 1. pH, moisture and fat content of the fish species

Species	Latin name	pH of raw fillet	Moisture (%)	Fat (%)
Bluefish	*Pomatomus saltatrix*	6.2 ± 0.1*	71.7 ± 2.1†	5.4 ± 1.2†
Catfish	*Ictalurus catus*	6.5 ± 0.3	77.1 ± 1.1	5.2 ± 1.1
Cod	*Gadus morhua*	6.8 ± 0.1	82.2 ± 0.3	1.0 ± 0.2
Haddock	*Melanogrammus aeglefinus*	6.7 ± 0.1	81.2 ± 0.3	1.0 ± 0.2
Halibut	*Hippoglossus hippoglossus*	6.7 ± 0.1	79.5 ± 0.6	1.6 ± 0.3
Mackerel	*Scomber scombrus*	6.1 ± 0.1	73.4 ± 0.8	6.6 ± 0.4
Monkfish	*Lophius piscatorius*	6.9 ± 0.2	84.3 ± 0.2	1.0 ± 0.2
Sole	*Solea solea*	6.6 ± 0.1	81.9 ± 0.3	1.2 ± 0.2
White Sucker	*Catostomus commersonii*	6.5 ± 0.1	80.4 ± 0.2	1.8 ± 0.4
Turbot	*Athoresthes stomias*	7.0 ± 0.1	77.3 ± 0.8	9.4 ± 0.9

*Mean ± s.e. of the mean; determined on two batches of fish, two measurements taken in each experiment. For bluefish the number of batches was 15.

†Mean ± s.e. of the mean; determined on five batches, two to three measurements taken in each experiment. For bluefish the number of batches was 15.

from 77–81%. Bluefish had the lowest average moisture content (71.7%) and monkfish had the highest (84.2%). Lipid content varied from a low of 1.0% for cod, haddock and monkfish, to a high of 9.4% for turbot. However, the variability in these measurements was relatively greater in some species, especially in bluefish.

Tables 2 and 3 summarize the binding properties of the various fish species.

Table 2. The effect of fish species on the binding scores

Species	Control binding scores	+ 1.5% NaCl binding scores
Bluefish	1.1 ± 0.7d†	5.4 ± 1.1b*
Catfish	1.1 ± 0.9	3.8 ± 1.0b, c
Cod	2.0 ± 0.6	8.0 ± 0.8a
Haddock	3.3 ± 0.8	8.0 ± 0.8a
Halibut	2.6 ± 1.0	8.8 ± 1.0a
Mackerel	1.6 ± 0.8	3.8 ± 0.8b, c
Monkfish	1.9 ± 1.0	8.1 ± 1.0a
Sole	1.3 ± 0.7	5.4 ± 0.7b
White Sucker	1.0 ± 0.9	2.8 ± 1.0c
Turbot	1.0 ± 0.9	2.3 ± 1.0c

*Means with the same letter are not significantly different at $\alpha = 0.05$.

†Mean ± s.e. of the mean; determined on two to three batches of fish, two measurements taken in each experiment (for bluefish the number of batches was 5). This applies in both columns.

Table 3. The effect of fish species on tensile strength

Species	Tensile force (N)		Relative energies (mg)	
	Control	1.5% NaCl	Control	1.5% NaCl
Bluefish	54 ± 9.8*	117 ± 1	90 ± 6	278 ± 80
Catfish	24 ± 3.3	59 ± 6.9	47 ± 10	128 ± 11
Cod	8 ± 1.9	102 ± 6.1	22 ± 6	235 ± 46
Haddock	58 ± 10.2	137 ± 18	60 ± 10	178 ± 3
Halibut	64 ± 12.5	193 ± 38	102 ± 28	360 ± 57
Mackerel	74 ± 8.1	162 ± 14.3	124 ± 34	217 ± 26
Monkfish	39 ± 7.5	121 ± 18.4	78 ± 2.5	314 ± 36
Sole	10 ± 2.1	35 ± 4.2	46	42 ± 14
White Sucker	27 ± 2.1	53 ± 8.6	41 ± 6	106 ± 20
Turbot	—			

*Mean ± s.e. of the mean; determined on two batches of fish, two measurements taken in each experiment.

The corresponding total solids and protein contents in the liquor are found in Table 4.

The binding scores of all the control samples (i.e., without salt added) were low and ranged from 1.0 (for white-sucker and turbot) to 3.3 (for haddock). However, with 1.5% NaCl the binding scores of the various fish species varied from a low of 2.3 (for turbot) to a high of 8 (for haddock, halibut and monkfish). Such levels of salt resulted in good binding, as shown in the literature (Maesso *et al.*, 1970a).

The volumes of the liquor released on cooking of the control were higher in most cases than the salt-treated samples, except for turbot which resulted in relatively large volumes in both cases. The percentage of total solids in the liquor from the salted samples were slightly higher compared with the controls. This probably reflects the amount of salt present. The protein content was generally less in the liquor from salted samples, with a few exceptions however. The standard errors (s.e.) were very large in some cases.

The correlation matrix of the parameters that were studied on samples with 1.5% NaCl can be found in Table 5. The correlation coefficients between these parameters and the binding scores, although statistically significant, were low.

The pH of the raw fillets generally varied between pH 6 and pH 7, with variability up to 0.5 pH units within species. The greatest variability was observed in bluefish. The pH of fish fillets vary according to the freshness of the fish (Love, 1980) and differences in pH within species might indicate differences in freshness.

The binding properties of the fish correlated with their moisture and fat contents (see Tables 1, 2 and 3). The correlation coefficient between the binding scores and the lipid content of the fish species was $r=-0.724$. When the fat content of the fish species was high and the moisture content was low,

Table 4. The effect of fish species on cookout

Species	Liquor volume (ml/200g)		Total solids in liquor (%)		Protein concentration in liquor (mg/ml)		Protein content in liquor (mg)	
	Control	1.5% NaCl	Control	1.5% NaCl	Control	1.5% NaCl	Control	1.5% NaCl
Bluefish	20.8 ± 4.0a	16.8 ± 4.0	6.1 ± 0.5	8.9 ± 0.5	32.4 ± 2.1	31.7 ± 2.8	683.7 ± 71.5	646.8 ± 77.6
Catfish	22.4 ± 5.6	8.0 ± 5.6	5.4 ± 0.7	8.6 ± 0.7	33.2 ± 1.9	43.6 ± 4.2	760.8 ± 91	335 ± 121.5
Cod	25.0 ± 3.6	5.0 ± 4.6	5.7 ± 0.4	7.9 ± 0.6	38.1 ± 2.3	43.1 ± 4.8	937.2 ± 232.5	268.2 ± 57.0
Haddock	21.5 ± 4.6	3.9 ± 0.8	5.3 ± 0.6	8.6 ± 0.6	37.6 ± 2.7	42.0 ± 5.1	789.4 ± 136.9	156.1 ± 28.9
Halibut	6.8 ± 5.6	3.0 ± 0.8	6.1 ± 0.7	7.2 ± 1.1	44.1 ± 3.1	44.4 ± 0.4	280.5 ± 53.0	25.0 ± 27.3
Mackerel	13.0 ± 5.6	7.6 ± 5.6	6.5 ± 0.7	9.7 ± 0.7	23.4 ± 5.5	30.0 ± 7.3	289.5 ± 25.1	242.5 ± 60.2
Monkfish	53.3 ± 5.8	24.5 ± 5.6	5.6 ± 0.7	5.9 ± 0.7	26.2 ± 2.9	29.4 ± 1.6	1469 ± 154.6	719.8 ± 30.6
Sole	32.8 ± 4.0	17.0 ± 4.0	6.5 ± 0.6	8.0 ± 0.6	40.6 ± 3.3	48.5 ± 2.8	1176.2 ± 136.4	704.7 ± 125.1
White Sucker	16.8 ± 5.7	2.2 ± 0.6	6.8 ± 0.7	7.7 ± 0.7	33.5 ± 0.5	39.0 ± 3.7	563.5 ± 321.5	83.8 ± 31.7
Turbot	47.5 ± 6.1	49.8 ± 5.6	13.4 ± 0.7	17.0 ± 0.7	29.9 ± 0.9	32.7 ± 2.2	1398.3 ± 70.0	1588.7 ± 110.1

*Mean ± s.e. of the means. Two measurements were taken in each experiment; each experiment was repeated two to three times. The total protein content in the cookout fluids was computed as an average of the protein content of each individual cookout.

Table 5. Correlation coefficients

	Liquor volumes	Total solids (%)	Protein in liquor (mg)
Bindscores	−0.380 (0.006)*	−0.392 (0.0045)	−0.360 (0.0094)
Liquor volumes		0.625 (0.0001)	0.961 (0.0001)
Total solids (%)			0.621 (0.0001

* Figures in parentheses are the Prob > $|R|$ under H_0:$RH_0 = 0$.

the binding was poor, even with 1.5% NaCl added. Examples are catfish, mackerel and turbot, with a fat content above 5%. The moisture and fat contents are inter-related in most species of fish (Borgstorm, 1961). Bluefish with an average moisture content of 5.4% presents a special case and is discussed later.

Sole and white-sucker (white-sucker was the only freshwater species involved in this study) had a relatively low fat content (1.2% and 1.8% respectively), but exhibited poor binding properties. The reason has not been determined yet.

The tensile strength test (Table 3) appeared to agree fairly well with the subjective judgment of the binding. The correlation coefficient between the peak heights and the binding scores was r=0.784, and between the areas under the peaks and the binding scores, r was 0.722. However, in some cases the results of this test did not fully reflect the texture of the samples, and did not agree with the binding scores. For example, the mean binding score for salt-treated samples of mackerel was 3.8, with a tensile strength of 162 N, whereas cod with a binding score close to 8, had a lower tensile strength of 102 N. A possible explanation for this discrepancy is the failure of the tensile test to measure the cohesiveness and other textural characteristics of well-bound samples. Combination of this test with other tests such as relaxation could yield a more complete analysis of the textural properties of bound products.

The variability within the bluefish

The binding properties of most fish species studied, were similar from batch to batch. However, a large variability in binding properties between batches was observed in bluefish, ranging from very poor to excellent, and it was decided to examine this further.

The fillets that showed poor binding had a broken structure appearance known as gaping (Love, 1980). The fillets with good binding properties (i.e. with binding scores as high as 8) did not show gaping. In addition, the fillets

Table 6. The rheological properties of bluefish batters

Treatment	Cross head speed (cm/min)	Maximum peak height (N)	Area under the peaks (mg)	Slope = y/x
Back extrusion cell test				
Good control (no salt)	5	53.5 ± 1.5*	450.5 ± 23.7	1.35 ± 0.2
	50	35.0 ± 1.0	300.0 ± 23.2	0.83 ± 0.0
Poor control (no salt)	5	54.5 ± 1.5	400.5 ± 19.7	1.36 ± 0.4
	50	42.0 ± 4.0	266.5 ± 26.8	1.34 ± 0.2
Good—salt (1.5%)	5	30.0 ± 0.9	269.8 ± 40.4	0.47 ± 0.1
	50	56.8 ± 0.9	446.3 ± 52.1	1.00 ± 0.2
Poor—salt (1.5%)	5	25.3 ± 3.4	207.8 ± 40.4	0.45 ± 0.2
	50	25.8 ± 3.0	209.8 ± 39.4	0.51 ± 0.1

Tensile strength test	Tensile force (N)	Tensile energies (mg)
Good—control	66.5 ± 3.5†	64.8 ± 22.0
Poor—control	57.5 ± 6.6	30.5 ± 1.5
Good salt	144.0 ± 18.0	131.2 ± 34.3
Poor salt	31.5 ± 11.6	21.6 ± 10.1

*Mean ± s.e. of the mean; two measurements were taken in each experiment; each experiment was repeated on two different batches of fish.

†Mean ± s.e. of the mean; two measurements were taken in each experiment; the experiment was repeated twice.

with good binding properties had a consistent sticky mass in the mixer (with 1.5% NaCl), compared with a very mushy batter obtained with the poor binding fillets. The rheological properties measured with a back extrusion cell (BEC) of the Instron are summarized in Table 6. In the control samples (i.e. no salt added), the measured parameters with the BEC test for both the 'good' and 'poor' materials were similar. With samples containing 1.5% NaCl, when the cross-head speed was increased from 5 to 50 cm/min, in the case of good binding material, the measured parameters increased by 180% for maximal peak heights, 165% for areas under the peaks, and by 212% for the slope. With the poor binding batters (with 1.5% NaCl), these parameters changed very little with the increase in the cross-head's speed. Examples of peaks obtained by this test with the good and poor binding batters are shown in Fig. 1. The tensile strength tests performed on the cooked products also showed significant differences for the salt-treated samples (Table 6).

The pH, moisture and fat content, and the corresponding binding qualities of good and poor binding fillets of bluefish are shown in Table 7. The pH of the various bluefish fillets varied from 5.9 to a high of 6.3. This could reflect differences in glycogen reserves of the fish resulting from differences in nutrition or differences in degree of struggle when caught. At first it was thought that inferior binding was associated with low pH and a high degree of

Table 7. Chemical properties and binding quality of bluefish fillets

Batch	pH	Moisture (%)	Fat (%)	Binding score (with 1.5% NaCl)
1	6.0	74.4 ± 4.1*	—	1
2	6.3	76.3 ± 0.8	2.6 ± 0.5	7.3 ± 0.8†
3	6.1	73.3 ± 0.8	4.6 ± 0.4	4.5
4	6.2	79.7 ± 0.2	1.3 ± 0.0	6.5
5	6.0	67.5 ± 1.0	16.6 ± 0.2	0
6 Fish A	5.9	74.2 ± 0.2	—	0
Fish B	6.2	76.4 ± 0.5	—	6
7 Fish A	5.9	72.5 ± 0	—	0
Fish B	6.0	77.9 ± 0.1	—	9
8 Fish A	5.9	73.5 ± 0.5	9.3 ± 1.2	0
Fish B	6.0	78.0 ± 0	2.0	7
9 Fish A	—	66.3 ± 0.5	16.1 ± 0.5	0
Fish B	—	80.2 ± 0.1	1.8 ± 0.1	7
10 Fish A	—	59.1 ± 0.9	—	0
Fish B	—	66.4 ± 0.5	—	1

*Mean ± s.e. of the mean of duplicate sample.
†Mean ± s.e. of the mean, determined by three judges.

gaped structure. This agreed with the findings of Love (1980), who associated gaping in cod with seasonal decline in pH. However, a more careful analysis of the observations revealed that in some cases, fillets with similar pH values exhibited extreme behaviour with respect to their binding properties.

The moisture content of the various bluefish fillets studied ranged from a low of 59.1 to a high of 79.7%. The percentage of fat varied from 1.3 to 16.6%. The literature describes that variations in composition within fish species exist due to various physiological conditions, such as nutrient availability and spawning (Borgstorm, 1961; Love, 1980).

Table 7 reveals that in general raw fish with a high moisture content (above 76%) and a low fat content (below 2%) produced good binding, while fillets with a low moisture content, and a high percentage of fat (above 5%), resulted in poor binding.

There are several possibilities with regard to the mechanism by which high fat content might interfere with the heat-initiated binding in fish: (1) by decreasing the moisture content which may be crucial for good binding; (2) by imposing a physical obstacle for the individual particles binding together. This issue should be subject for further examination.

Conclusions

The fish species studied differed significantly in their binding properties. Bluefish exhibited variability of binding properties within species.

The volumes of liquor released on cooking, the percentage total solids and protein content of the liquor, as well as the pH of the raw fillets could not serve as indicators of binding quality.

High fat content and low moisture level appeared to be associated with poor binding properties. There is a potential for products from fish in which heat-induced binding is involved. However, the manufacturer should be aware of the factors influencing the binding phenomenon.

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Collaborative evaluation of universal calibrations for the measurement of protein and moisture in flour by near infrared reflectance

B. G. OSBORNE and T. FEARN

Summary

Calibrations which had been previously determined for the analysis of protein and moisture in U.K. and Irish milled straight run white flour were set into nine near infrared (NIR) reflectance instruments of the same model situated in different laboratories. One set of samples was circulated to adjust the bias (intercept) which has a component specific to each instrument then a second set was circulated 2 months later to evaluate the adjusted calibrations. Kjeldahl proteins were determined on the first set by seven of the laboratories and on the second set by one 'reference' laboratory for direct comparison of that method with the NIR method and in order to assess the accuracy of NIR as well as precision.

On the second set of samples the mean NIR results for the different laboratories varied from 10.92 to 11.30% compared with a mean for the Kjeldahl of 11.03%. The accuracy of NIR calculated separately for each laboratory as the standard deviation (s.d.) of differences between NIR and Kjeldahl from the reference laboratory was between 0.13–0.33% while the precision or repeatability (within-laboratory) was 0.07%. For comparison the 'accuracy' of Kjeldahl, calculated separately for each laboratory in the same way as for NIR, was 0.10%–0.27% and the precision (within-laboratory) was 0.04%.

In the case of moisture, the NIR results for the second set of samples had mean values ranging from 13.08 to 13.28% compared with a mean for oven drying of 13.13%. The accuracy of NIR was 0.20–0.29% with no significant biases and the precision was 0.05%.

Introduction

Near infrared (NIR) reflectance is a widely used method for the rapid analysis of oilseeds, grains and flour (Miller, 1980) and has gained acceptance

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as an approved method in at least one country (Anon, 1980). The prerequisite for the acceptance of a technique as an approved method is the establishment of its accuracy and precision in a number of laboratories but collaborative testing has so far only been carried out for the use of NIR in the measurement of protein in wheat (Hunt *et al.*, 1977; Miller *et al.*, 1978; Downey, Morrissey & Flynn, 1982) and barley (Baker, 1980) and protein and oil in soybeans (Hunt *et al.*, 1977). Furthermore, the results of these previous exercises were expressed in terms of within- and between-laboratory precision rather than the accuracy of each laboratory compared with the reference methods against which the NIR instruments were calibrated.

General calibrations, valid for the analysis of the population 'white U.K. and Irish flour', by NIR have been established for protein and moisture (Osborne, Douglas & Fearn, 1982) but it is necessary to confirm the expectation that these calibrations would be valid, subject to a bias adjustment, for all instruments of the same family of models (Day & Fearn, 1982).

The purpose of the work described in this paper, therefore, was to investigate the transferability of NIR calibrations and to establish the between-laboratory precision and, more importantly, the accuracy of NIR for the analysis of protein and moisture in flour.

Materials and methods

InfraAlyzer 300B instruments (Technicon Instrument Co. Ltd, U.K.) which were in routine use in nine separate laboratories were set up with universal calibrations for protein and moisture in flour (Osborne *et al.*, 1982). Forty samples of U.K. and Irish commercially milled white flour were collected and analysed by standard methods for protein (ICC 105/1) and moisture (ICC 110/1) then divided into two sets with approximately the same ranges and means: 1–20, protein 8.2–13.8%, moisture 11.5–14.8%; A–T, protein 7.7–14.2%, moisture 12.0–14.4%.

The first set of samples were analysed by each laboratory for protein and moisture by NIR using the published calibrations and seven of the laboratories also carried out Kjeldahl protein determinations but only one carried out oven moisture determinations by ICC standard method 110/1. Having established acceptable between-laboratory precision for the Kjeldahl method, all the NIR protein results were compared with Kjeldahl results obtained by one laboratory chosen to be the reference laboratory. Any biases between NIR and Kjeldahl were calculated and set into the appropriate instrument for the second part of the experiment and a similar procedure was carried out for NIR and oven moisture values. The Kjeldahl protein and oven moisture determinations used for the adjustment of the NIR instruments were carried out in the same reference laboratory.

The second set of flour samples were analysed about 2 months later by each

laboratory for protein and moisture by NIR using the adjusted calibrations and the reference laboratory carried out Kjeldahl protein and oven moisture determinations. Standard deviations of differences between NIR and manual methods were calculated separately for each instrument and a further check for significant biases was carried out. In addition within- and between-laboratory precision was assessed by an analysis of variance.

Results

Between- and within laboratory precision and accuracy of Kjeldahl protein determination

Table 1 shows the results of protein determination on twenty samples of white flour by the Kjeldahl method (N×5.7) expressed as mean protein content and standard deviation of differences from Laboratory 1, whose figures were used to compare with NIR. Although reproducibility is the more usual figure to quote for such data, the s.d. of differences were included for comparison with similar results for NIR. The overall range of the means was 10.90 to 11.14% with s.d. of differences varying between 0.10 and 0.27%. Small inter-laboratory biases exist and have not been removed in the calculation of these s.d. The largest s.d., not surprisingly, belongs to the laboratory whose mean is furthest from that of Laboratory 1. The within-laboratory precision or repeatability as assessed from duplicates carried out on different days by the same analyst was 0.04%. The between-laboratory precision or reproducibility as assessed by an analysis of variance was 0.14%.

Precision and accuracy of NIR protein determination

The within-laboratory and between-laboratory precision or repeatability and reproducibility of NIR protein determination were assessed by an analysis

Table 1. Kjeldahl protein determinations on twenty samples of white flour (1–20) in seven laboratories

Laboratory	Mean (%)	Standard deviation of differences from Laboratory 1 (%)
1	10.90	—
2	11.03	0.17
3	11.05	0.17
4	11.14	0.27
5	10.94	0.10
6	10.92	0.19
7	11.09	0.23

Overall mean 11.01.

Table 2. NIR protein determination on twenty samples of white flour (1–20) in nine laboratories

Laboratory	Mean (%)	Bias	Standard deviation of differences between NIR & ICC (corrected for bias)
A	10.97	+0.07	0.19
B	12.22	+1.32	0.20
C	10.98	+0.08	0.22
D	9.46	−1.44	0.19
E	10.75	−0.15	0.18
F	13.74	+2.84	0.30
G	11.17	+0.27	0.16
H	10.94	+0.04	0.23
I	13.20	+2.30	0.17
ICC 105/1	10.90		

of variance of values of twenty samples in nine laboratories. The within-laboratory precision (repeatability) was 0.07% and that between laboratories (reproducibility) was 0.17%.

The accuracy of NIR compared with Kjeldahl was assessed by using the first set of twenty samples to correct the bias individually for each instrument (Table 2). These bias adjustments were set in and about 2 months later (to test for drift in the calibrations) a second set of twenty samples was used to calculate the s.d. of differences between NIR and Kjeldahl for each instrument (Table 3). The s.d. were calculated separately for each instrument because this is a more rigorous test of inter-instrument performance than calculation of a single pooled s.d. The figures for the nine instruments were

Table 3. NIR protein determination on second twenty samples of white flour (A–T) in nine laboratories after adjustment for biases in Table 2

Laboratory	Mean (%)	Bias ($P < 0.01$)	Standard deviation of differences between NIR & ICC
A	11.29	+0.26	0.31
B	11.30	+0.27	0.33
C	10.95	—	0.21
D	11.04	—	0.15
E	11.12	—	0.17
F	11.10	—	0.17
G	11.24	+0.21	0.27
H	10.92	—	0.22
I	11.08	—	0.13
ICC 105/1	11.03		

0.15–0.33% and there was still a significant bias in the case of three out of nine. However, there were no significant skews (that is the line fitted to the data by least squares at an angle to the 45° line through the origin for a plot of NIR *versus* Kjeldahl). Therefore, the transferability of the calibration constants K_1, K_2 . . . is confirmed subject to an adjustment of the bias, K_0 although it appears that a single adjustment may be inadequate. The accuracy and the precision of NIR for measurement of protein in white flour were both quite acceptable: within-laboratory precision of 0.04% for Kjeldahl and 0.07% for NIR; between-laboratory precision of 0.14% for Kjeldahl and 0.17% for NIR and s.d. of differences between Kjeldahl in one laboratory and the other Kjedahl results 0.10–0.27% while in the case of NIR the figures were 0.15–0.33%.

Precision and accuracy of NIR moisture determination

The evaluation for moisture was carried out in exactly the same way as for protein except that inter-laboratory testing of the oven drying method was not carried out. It was originally intended to use the moisture results for correction of the protein figures only as it was expected that changes in moisture content may have occurred in transit and because all sets of samples were not analysed at the same time. In fact, the agreement between the seven laboratories who took part in the NIR moisture exercise was excellent and so the precision and accuracy were assessed. The within-laboratory precision was 0.05% and the between-laboratory precision was 0.16%. The accuracy of NIR compared with oven drying was assessed by using the first set of twenty samples to correct the bias individually for each instrument (Table 4). The bias adjustments were made and again 2 months later the second set of samples was used to calculate the s.d. of differences between NIR and oven drying for each instrument (Table 5). The figures for the seven instruments are all very

Table 4. NIR protein determination on twenty samples of white flour (1–20) in seven laboratories

Laboratory	Mean (%)	Bias	Standard deviation of differences between NIR & ICC (corrected for bias)
A	13.38	−0.12	0.21
B	13.18	−0.32	0.20
D	13.67	+0.17	0.21
E	13.25	−0.25	0.15
F	12.87	−0.63	0.21
H	13.65	+0.15	0.16
I	12.77	−0.73	0.21
ICC 110/1	13.50		

Table 5. NIR moisture determination on second twenty samples of white flour (A–T) in seven laboratories after adjustment for biases in Table 5

Laboratory	Mean (%)	Bias ($P < 0.01$)	Standard deviation of differences between NIR & ICC
A	13.08	—	0.20
B	13.28	—	0.24
D	13.28	—	0.29
E	13.16	—	0.20
F	13.22	—	0.28
H	13.18	—	0.20
I	13.18	—	0.27
ICC 110/1	13.13		

close (0.20–0.29%) and there were no significant biases or skews. The range of the mean values for the samples was 13.08–13.28% compared with a mean for the oven method of 13.13%.

Discussion

The principle of near infrared reflectance analysis is that a background corrected log reflectance measurement (log $1/R$) from a powdered sample at a wavelength characteristic of the analyte is empirically related to concentration (C) by the equation:

$$\log \frac{1}{R} = \frac{aC}{s}$$

where a is the absorbtivity and s is the scattering coefficient. The absorbtivity for a particular band is in principle measurable, but the amount of scattering varies from sample to sample and thus s becomes a new unknown with each measurement.

The purpose of calibration, therefore, is to determine a constant (K_1) which includes the absorbtivity and an average value for the scattering coefficient. The greater the number of different samples that are used, the more reliable that constant will be, and, because the constant is a property of the samples and not of the instrument, it is not expected to vary between instruments of the same family of models (for instruments which use the same optical and detection system). However, due to arbitrary scaling of the log reflectance values, and due to the fact that NIR and reference methods are measuring different things, an intercept or bias constant (K_0) is also needed part of which will be characteristic for each instrument:

$$C = K_0 + K_1 \left(\log \frac{1}{R}\right)$$

In practise, in order to achieve background correction, log reflectance measurements at several wavelengths are used and the equation is expanded into the more familiar form which is entered into the instrument:

$$C = K_0 + K_1\left(\log\frac{1}{R}\right)_1 + K_2\left(\log\frac{1}{R}\right)_2 + \ldots$$

In the case of protein for example, three readings are used and the background corrected log reflectance (log $1/R$) consists of the height of the protein peak at 2180 nm (referred to the level of the spectrum at 1680 nm) minus 65% of the height of the overlapping starch peak at 2100 nm (referred to the level of the spectrum at 1680 nm).

Because it is accepted practice to determine the constants K_0, K_1, K_2 . . . with reference to another method the NIR analysis becomes instrinsically linked to that reference method and therefore assessment of performance of NIR in terms only of within- and between-laboratory precision is not adequate; an assessment of the accuracy of NIR as s.d. of differences relative to the reference methods is also required in order to complete the evaluation.

The results of the collaborative exercise show that the slopes of the NIR *versus* Kjeldahl protein and NIR *versus* oven moisture lines were not significantly different between the nine instruments but the intercepts were. Even after bias adjustment three of the nine instruments had erroneous intercept settings in the second part of the evaluation and, assuming that the instruments had not been adjusted in the intermediate period, this can only have been caused by drift in the readings possibly due to dust build up on the optical components. The fact that none of the moisture results showed a significant bias in the second part of the experiment is not surprising; the protein biases were quite small (0.2–0.3%) and protein is by far a more demanding application in terms of signal to noise requirements.

It is clear that despite the remaining protein biases, the precision and accuracy of NIR are quite acceptable taking into account that the experiment was designed to represent the worst conditions in terms of time interval between calibration adjustment and prediction and that no data whatever were excluded from the analysis so that these results represent a realistic and genuine assessment of the performance of the NIR method for the analysis of protein and moisture in flour.

Furthermore, this exercise represents a test not merely of the transferability of two specific equations but of any equations which have been shown to be robust for one instrument. This follows because in order for the constants to be transferable, the log reflectance readings of the nine instruments must have been sufficiently similar once the intercepts were set and therefore if these readings were used in any other equation it too would be transferable. There is also no reason to suppose that this transferability would not be valid in the future as there is a theoretical justification both in optical (Day & Fearn, 1982) and chemical terms (above) for this type of instrument. There is an indication, however, that the bias may change with time but this does not

appear to be a sample-related phenomenon so that the annual calibration adjustment at harvest for wheat may not always be due to changes in sample characteristics.

Conclusions

(1) Calibrations for the determination of protein and moisture in white flour by near infrared reflectance were transferable between nine individual instruments of the same model, subject to a bias adjustment.
(2) The precision and accuracy of NIR protein determination proved, in this realistic assessment, to be quite acceptable for routine use.

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Effect of salting and roasting on the lipids of Iranian pistachio kernels

GHASSEM G. KASHANI and L. R. G. VALADON

Summary

The simple lipids which make up 95% total lipids of Iranian pistachio kernels were identified as sterols and their esters; mono-, di- and tri-glycerides; and free fatty acids while the complex lipids were sterolglycosides; mono- and di-galactosyldiglycerides; cardiolipin, phosphatidyl-choline, -ethanolamine, -serine and -inositol; sphingolipid and phosphatidic acid. After salting and roasting, there was an increase in free fatty acids and in phosphatidic acid. Total fatty acids made up of C14:0, C16:0, C18:1, C18:2, C18:3 and C20:0 were not significantly altered after roasting, nor was the iodine value, nor the malonaldehyde content. However, the peroxide value was increased.

Introduction

Roasting alters and substantially improves the flavour, texture, colour and appearance, and is one of the most common forms of processing for pistachio nuts. These nuts contain large amounts of lipids which may undergo a number of degradative changes during heat processing and may have therefore important effects on their palatibility and wholesomeness. Heated lipids are unavoidably exposed to adverse conditions during the heat processing which may produce certain undesirable changes. The polyunsaturated fatty acids and possibly the nutritive value of such lipids are decreased (Morton, 1977). There is also the added problem that the undesirable changes which occur in heated lipids may have a deleterious effect on human health (Crampton, Common & Farmer, 1956; Kaunitz, 1956).

Although the composition of pistachio kernels is known to a certain extent (Clarke, Brar & Procopiou, 1976; Kamangar, Farrohi & Mehran, 1975), no thorough investigations on lipids have been carried out to date. The purpose

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of this study was therefore to investigate the effect of roasting on the lipids of Iranian pistachio kernels. There are several cultivars of pistachio grown in Iran and therefore varietal differences may be expected. This will also be investigated, as will certain botanical features of these nuts.

Materials and methods

Six samples of pistachio nuts from three locations were kindly provided by the Institute of Modification and Preparation of Seeds, Karadj, Iran. After drying in an oven at 70±3°C, the pericarps were removed and the nuts were examined.

From each sample, the weight of 100 nuts, the percentage of kernel to nut, and the moisture content were obtained (AOCS, 1956).

Lipid extraction

Ten grams of pistachio kernels were homogenized twice in 200 ml chloroform and 100 ml methanol in a Waring blender for 2 min and then washed with 0.2% of its volume of 0.73% NaCl according to Folch, Lees & Stanley (1957). Total lipids were stored at −20°C in benzene : amyl alcohol : chloroform (1 : 1 : 1, v/v) with 0.01% butylated hydroxytoluene added to prevent oxidation (Deven & Manocha, 1975).

Separation and quantitative determination of lipid classes

The separation into simple and complex lipids by thin-layer chromatography (TLC) and the identification of lipid classes using various sprays were as described by Farhangi & Valadon (1982).

For the quantitative determination of lipid classes, the developed TLC plates were sprayed with 3% cupric acetate in 8% phosphoric acid heated at 180°C for 25 min (Fewster *et al.*, 1949) and the resulting dark colour estimated by using a Joyce–Loebl Chromoscan densitometer (Gasbarro, 1972). Results with the densitometer scans were generally reliable as they compared favourably with those of the weighing method.

Lipid saponification

A solution of 5% KOH in a mixture of methanol : benzene (80 : 20, v/v) was prepared and 2 ml of this was added to 50 mg lipid extract, in addition to 10 ml of a methanol : benzene (80 : 20, v/v) mixture. This was refluxed for 2 hr. After being acidified to pH 3–4, the fatty acids were extracted by diethylether and were washed with distilled water until the pH was 8 (Asselineau & Montrozier, 1976); this yielded total fatty acids. Free fatty acids were extracted by the method of Draper (1969). Fatty acid methyl esters were

prepared by using BF_3–methanol reagent according to Metcalfe & Schmitz (1961).

Gas–liquid chromatography (GLC) of fatty acid methyl esters

The fatty acid methyl esters were analysed on a 10% PEGA column using a PYE gas chromatograph as already described (Farhangi & Valadon, 1982).

Iodine value, peroxide value and malonaldehyde content

Iodine value (I.V.) and peroxide value were determined according to AOAC (1970). The malonaldehyde content was estimated by the method of Shamberger, Shamberger & Wills (1977).

Roasting

Five hundred grams pistachio nuts were soaked in 15% NaCl (99.998% pure) in deionized water (w/v) for 5 hr with slow stirring. The salted nuts were then dried in a rotary drier at 70°C for 1 hr until the moisture taken up by the salting was removed. The temperature was then raised to 145°C over a 30 min period and held for 20 min (Bloch & Brekke, 1960). After roasting samples were cooled at room temperature. They were put in polythene bags separately and were stored in the deep-freeze (−10°C).

The solvents were from AR stock, redistilled where necessary.

All the experiments were repeated several times and the results where appropriate are given as the average ±standard error (s.e.).

Results and discussion

The local Iranian names of six cultivars and some characteristics of the pistachio nuts are given in Table 1. The weight of 100 nuts varied greatly from 90.5 (Sample 2) to 135.5 g (Sample 3). The ratio of kernels to whole nuts did

Table 1. Characteristics of six varieties of Iranian pistachio nuts

Sample no.	Local name	Origin	Wt of 100 nuts (g)	Kernel/ nut (%)	Moisture kernel (%)	Oil/ kernel (%)
1	Akhavan salari-Darbari	Rafsanjan	135.2	54.8	5.8	52.5 ± 0.5
2	Badami	Rafsanjan	90.5	51.9	5.3	56.6 ± 0.5
3	Kaleghochi	Kerman	135.5	56.7	6.0	55.1 ± 0.5
4	Momtaz	Rafsanjan	134.6	57.2	5.7	53.5 ± 0.5
5	Ohadi	Rafsanjan	102.9	57.1	5.4	54.4 ± 0.5
6	Shasti	Damghan	128.0	54.1	5.0	54.1 ± 0.4

not vary greatly (54–57%), while moisture content was low (5–6%). The total lipid contents ranged from 52.5–56.6% and were not very different from results obtained by Kamangar *et al.* (1975) and by Clarke *et al.* (1976). So, although these cultivars were from different regions and varied somewhat in their sizes, the total lipid contents did not vary very widely. Kashani (1982) has studied these six cultivars in detail but did not observe very wide differences in their lipid components. In fact the differences between the six cultivars were so small that although the effect of roasting was studied on all six, the only one to be discussed in great detail in this presentation will be variety Badami (Sample 2), the smallest in size but with most total lipids.

It can be seen in Table 2 that simple lipids make up the bulk of the lipids (95%) and therefore complex lipids are only 5% total lipids. Roasting has no effect on total lipids, total simple lipids or on total complex lipids. The individual simple lipids identified were sterols and their esters; mono-, di- and tri-glycerides; and free fatty acids; while the complex lipids were sterolglycosides; mono- and di-galactosyldiglycerides; cardiolipin; phosphatidyl-choline,

Table 2. The effect of heat treatment (roasting) on various lipids of the pistachio variety Badami (the results are expressed as mg/g fresh weight ± s.e. where appropriate)

Lipids	Controls	Roasted
Simple		
Sterol esters	3.1	3.1
Triglycerides	437.6	434.9
Free fatty acids	2.7	5.5
Sterols	10.6	10.0
1,2-Diglycerides	48.4	48.3
1,3-Diglycerides	32.9	31.7
Monoglycerides	+	+
Unidentified	3.7	4.0
Total simple lipids	539.0 ± 4.2	537.5 ± 6.5
Complex		
Sterol glycosides	4.0	4.1
Monogalactosyl diglycerides	3.4	3.5
Cardiolipin	0.8	0.9
Phosphatidyl ethanolamine	2.4	2.2
Sphingolipid	1.8	1.8
Digalactosyldiglycerides	1.1	1.1
Phosphatidyl choline	6.7	5.9
Lysophosphatidyl choline	5.6	4.9
Phosphatidyl serine	+	+
Phosphatidyl inositol	+	+
Phosphatidic acid	0.8	2.7
Total complex lipids	26.6 ± 2.0	27.1 ± 1.9
Total lipids	565.6 ± 4.6	564.6 ± 5.0

+, Indicates a trace.

-ethanolamine, -serine and -inositol; sphingolipid and phosphatidic acid. After roasting, there was an obvious increase in fatty acids and in phosphatidic acid and a slight decrease in triglycerides and in the choline compounds, while most of the other lipid components stayed the same. These results were not altogether unexpected for when various oils were heated at 160°C or at 180°C, Fleischman *et al.* (1963) and Yuki (1967) observed huge increases in free fatty acids. It seems that hydrolysis and therefore the increased production of free fatty acids is an early reaction in heat processing of lipids and of lipid containing foods. Similarly hydrolysis of certain of the phospholipids would give rise to increases in phosphatidic acid which was very obvious after roasting in the present study.

The free fatty acids of the pistachio kernels investigated were: palmitic acid (16:0), oleic (18:1) and linoleic acid (18:2) while total fatty acids were the above together with smaller amounts of lauric (12:0), myristic (14:0), stearic (18:0), linolenic (18:3) and arachidic acid (20:0) (Table 3). These results are in agreement with those of Kamangar *et al.* (1975) who in addition also identified minute amounts of palmitoleic in other varieties of Iranian pistachio oils.

The results of roasting on the major free fatty acids and total fatty acids of the pistachio var. Badami are given in Table 3. There was an increase in unsaturatedness of the free fatty acids while the percentages of fatty acids making up total fatty acids hardly changed. Since free fatty acids were only a small part of total fatty acids and since the percentages of total fatty acids did not change, then roasting does not seem to affect total fatty acids all that much.

To identify further possible effects of roasting on the quality of pistachio oil, measurements of iodine and peroxide values and of malonaldehyde contents were carried out.

Roasting does not have any significant effect on the iodine value or the malonaldehyde contents of the pistachios used (Table 4). However, there was

Table 3. The effect of heat processing (roasting) on the free (FFA) and total fatty acids (TFA) of the pistachio variety Badami (the results are expressed as percentage total)

	FFA (% total FFA)		TFA (% total TFA)	
Fatty acids	Controls	Roasted	Controls	Roasted
C14:0	0	0	0.9	1.2
C16:0	23.1	14.5	16.8	17.1
C18:1	50.0	47.3	46.3	46.9
C18:2	26.9	38.2	29.2	28.1
C18:3	0	0	4.1	2.8
C20:0	0	0	2.7	3.0

Table 4. The effect of heat processing (roasting) on (a) iodine value, (b) peroxide value and (c) malonaldehyde content (MA) of the pistachio var. Badami (the results are expressed as: (a) centigrams of iodine absorbed per gram of oil; (b) milli-equivalents of peroxide per 100 g of oil; and (c) mg of free MA per 100 g fresh weight; within each case ± s.e.).

Treatment	(a) Iodine value	(b) Peroxide value	(c) MA content
Controls	128.8 ± 2.4	7.3 ± 0.4	1.7 ± 0.2
Roasted	124.3 ± 3.1	10.4 ± 0.5	2.0 ± 0.2

a significant effect on the peroxide value after roasting (Table 4). These results were not altogether unexpected since oils have to be treated to fairly high temperatures (180°C) for some time (1 hr or more) before iodine values show a significant fall (Rao & Rao, 1968). Furthermore, the malonaldehyde (MA) content stayed fairly constant presumably as the rate of MA loss was the same as that of its formation (Newburg & Concon, 1980).

However, the increase in peroxide value after roasting suggests that some degradation of the oil has taken place. So although peroxide formation is significantly higher than in controls, yet it is not very high. This is no doubt due to the mild heating and to the presence of natural oxidants in the kernels.

In conclusion then, the common method of roasting pistachio nuts does cause an increase in free fatty acids and in phosphatidic acid, and also an increase in the unsaturatedness of the free fatty acids. Furthermore, the peroxide value is significantly increased after roasting. However, roasting does not have any effect on total lipids, or on total simple or total complex lipids of the six Iranian cultivars under study. It does not affect the iodine value or the malonaldehyde content. Though it can be taken that roasting does cause some degradation of the oil, this is very slight.

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Physical and chemical changes during roasting of semolina (suji)

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Summary

A simple laboratory method for uniform roasting of semolina under controlled conditions and for isolation of volatile compounds formed during roasting is described. The changes in sugars, proteins, lipids, gelatinization behaviour and carbonyl compounds formed during roasting of wheat semolina at 160, 175 and 190°C are discussed.

Introduction

In India, a large number of sweet and savoury preparations are made from wheat semolina. In all these preparations semolina is invariably roasted in some form or other which not only provides desirable consistency for the pasta products, but is also essential for their characteristic flavour. Though considerable information is available about the chemical changes that take place during roasting of cocoa (Rohan & Stewart, 1966a, 1966b; Pinto & Chichester, 1966), peanuts (Newell, Mason & Matlock, 1967; Neucere, Ory & Carney, 1969; Neucere, 1972; Thomas & Neucere, 1973), coffee seeds (Stoffelsma *et al.*, 1968) and barley (Underwood & Deatherge, 1952), surprisingly no study seems to have been undertaken on the extent of physical and chemical changes taking place during semolina roasting and volatile compounds formed therein. The present paper describes a simple and reproducible procedure for uniform roasting under controlled conditions, isolation of volatile compounds and physico-chemical changes that take place during roasting processes.

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Materials and methods

Semolina

Good quality wheat semolina of small particle grade conforming to IS Specification (IS 1010-1968) was procured from the local market. On sieving, the entire quantity of semolina passed through a 1.18 mm IS sieve but only 10% and 1.5% of the total semolina passed through 710 μm and 250 μm IS sieves respectively.

Roasting conditions

In the conventional method semolina is roasted in an iron or aluminium pan with continuous mixing. In this method, temperature control and trapping of volatiles that are generated during roasting is very difficult. Initial attempts to roast the samples in an ordinary round bottomed or flat-bottomed flask using a rotary evaporator were not successful mainly because of inadequate mixing of semolina and thereby non-uniform roasting. To overcome this difficulty two glass pieces (2×2 cm) were fused perpendicularly to the inner walls of a flat-bottomed 1 l pyrex glass flask at a height of 2.5 cm from the bottom. The two pieces were exactly on opposite sides facing each other and served as mixing blades in the rotary flask. All the roasting operations in the present study were conducted in this specially fabricated flask. Weighed quantities of semolina (150 g) were taken in the above flask, connected to a rotary evaporator (Tempo) and heated in a thermostatically controlled bath at 160, 175 and 190°C for 10–15 min. The outlet of the evaporator was connected to two flasks, connected in series, containing 0.5% 2,4-dinitrophenyl hydrazine (DNP) solution in 2 N hydrochloric acid, which in turn were connected to the suction pump. The inlet tube of the vacuum evaporator was also connected to traps having a saturated solution of DNP in dilute sulphuric acid for eliminating any chances of contamination from the atmosphere. The DNP solution was kept overnight at room temperature and dinitrophenyl hydrazones (DNPHs) were extracted with two 100 ml aliquots of carbonyl free chloroform. The combined chloroform extracts were washed with 2 N hydrochloric acid repeatedly to remove DNP and with distilled water to remove traces of acid. The chloroform extract containing DNPHs was evaporated under vacuum, dried over anhydrous sodium sulphate and made up to standard volume (25 ml). The DNPHs were separated by TLC as described earlier (Arya, Premavalli & Parihar, 1976); carbonyls were also isolated from roasted samples as DNPHs and examined similarly.

Changes in proteins were followed by determining their solubility in 0.1 N acetic acid, 70% alcohol, water and 3 M urea-phosphate buffer. Two gram semolina samples were treated with 50 ml of the respective extractant and kept in a refrigerator. After 2 hr the mixture was homogenized in a blender for 4 min and the homogenates were centrifuged at 6000 rev/min for 30 min. The concentration of proteins in the supernatant fractions were measured by the

procedure of Lowry *et al.* (1951). The acetic acid soluble fraction was also fractionated on a Sephadex G-150 column. Changes in reducing and total sugars (Cereal Laboratory Methods, 1957), free and bound lipids (Daftary & Pomeranz, 1965), browning index (Kannur, Ramanuja & Parihar, 1973), gelatinization viscosity (Yesunaga, Bushuk & Irvine, 1968) and free amino groups (Satake *et al.*, 1960) were measured before and after roasting.

Results and discussion

A decrease in water soluble free amino nitrogenous compounds is one of the most prominent changes taking place during roasting of semolina (Table 1). Based on this criterion maximum variations among different batches of semolina roasted under similar conditions did not vary more than ±0.5 mg leucine/100 g semolina indicating adequate reproducibility in this procedure. Maximum variation in other parameters like 0.1 N acetic acid soluble proteins, reducing and total sugars were also less than ±2%. Though in the present study only carbonyls were investigated, other compounds like pyrazines, acids, esters and hydrocarbons may also be investigated by replacing DNP traps with other suitable reagent traps or with a cold finger trap.

Carbonyl compounds

Besides pyrazines, carbonyl compounds have been reported to be formed during roasting operations in cocoa (Vanpragg, Stein & Tibbetts, 1968), coffee (Stoffelsma *et al.*, 1968) and ground nut and are known to influence their flavour. The composition of carbonyl compounds present in raw and roasted semolina is given in Table 2. Unroasted semolina contained only pyruvic aldehyde, formaldehyde, acetaldehyde and acetone besides two unidentified bands having R_f values of 0.32 and 0.41 having absorption maxima at 374 and 390 nm respectively. McWilliams & Mackey (1969) have reported that whole wheat contained acetaldehyde, isobutyraldehyde, butyraldehyde, hexanal, heptanal, octanal, isovaleraldehyde, isopentanone and diacetyl, but the majority of these compounds except a few could not be detected in semolina. It is most likely that the major volatile compounds in whole wheat are generated from the germ portion of the grain containing active lipoxygenases and other enzymes. Removal of germ during milling of semolina may have resulted in the altered carbonyl profile. Roasted samples contained pyruvic aldehyde, diacetyl, formaldehyde, acetaldehyde, acetone, propanal, crotonaldehyde, 2,4-hexadienal and 2,4-hexadecadienal besides six unidentified bands (Table 3). Except 2,4-hexadecadienal, all the other carbonyls identified in roasted semolina are known to be formed from sugar–amino acid interactions and thermal degradation of lipids. Hexadecadienal, on the other hand, may have been formed from aldol type

Table 1. Changes in free amino groups, sugars, lipids, browning index and amylograph peak viscosity in roasted semolina

Semolina treatment		Free amino groups*	Reducing sugars†	Non-reducing sugars‡	Total sugars‡	Browning index§	Free lipids (%)	Lipid acidity ‖	Amylograph peak viscosity (B.U.)
Temperature (°C)	Time (min)								
No treatment (Raw semolina)		22.7	54.7	241.2	296.0	0.14	1.70	76.98	640
160	10	15.5	46.7	232.5	279.2	0.15	1.73	74.56	880
160	15	11.3	45.3	232.3	277.6	0.16	1.91	70.52	590
175	10	8.3	46.1	225.5	271.6	0.175	1.84	70.86	630
175	15	7.2	45.0	225.1	270.1	0.18	1.99	68.42	500
190	10	5.7	60.1	194.5	254.6	0.20	2.03	67.03	580
190	15	3.3	59.1	112.1	171.2	0.39	2.19	67.08	160

* (mg leucine/100 g sample).
† (mg maltose/10 g flour).
‡ (mg sucrose/10 g flour).
§ Absorbance at 420 nm.
‖ (mg of KOH/100 g sample).

Table 2. TLC R_f values, absorption maxima and tentative identification of carbonyls of semolina samples

Band no.	R_f values		Absorption maxima (nm)	Tentative identification
	Raw semolina	Roasted semolina		
1	0.43	0.43	383	Pyruvic aldehyde
2	—	0.48	390	Diacetyl
3	0.58	0.58	353	Formaldehyde
4	0.60	0.61	355	Acetaldehyde
5	0.65	0.65	364	Acetone
6	—	0.67	357	Propanal
7	—	0.81	392	—
8	Not sharp	Not sharp	—	A broad band

Thin layer: Silica gel G.
Solvent: Carbon tetrachloride : hexane : ethylacetate (10 : 2 : 1).

condensation of lower chain aldehydes which has been reported to take place during cooking or roasting of meat (Nakanishi & Suyama, 1970).

Qualitatively there were not significant differences in the samples roasted at 160, 175 and 190°C (Table 4). The total amount of volatile carbonyls formed was, however, significantly higher at 190°C (99.9 mmol) than at 175°C (55.9 mmol) and 160°C (38.0 mmol). Also the proportion of acetaldehyde which has been reported to be associated with the harsh flavour notes increased with rise in temperature from 160°C to 190°C (Table 4).

Table 3. Further separation of eighth band of Table 2

Band no.	R_f values		Absorption maxima (nm)	Tentative identification
	Raw semolina	Roasted semolina		
1	0.32	0.24	368	—
2	0.32	0.33	374	—
3	—	0.39	368	—
4	0.41	—	388	—
5	—	0.43	374	Crotonaldehyde
6	—	0.56	389	2,4-hexadienal
7	—	0.73	373	—
8	—	0.78	390	2,4-hexadecadienal
9	0.81	0.82	390	—

Thin layer: Silica gel G.
Solvent: Petroleum ether : Diethyl ether (70 : 30).

Table 4. TLC R_f values and tentative identification of volatile carbonyls formed during roasting of semolina

Band no.	R_f value	Total volatile carbonyls (%) I	II	III	Tentative identification
1	—	27.6	30.5	83.5	Dicarbonyls
2	0.35	4.0	3.5	3.0	—
3	0.40	27.6	33.9	41.0	Acetaldehyde
4	0.48	2.3	4.9	4.1	Acetone
5	0.50	4.6	5.3	5.5	Propanal
6	0.59	8.8	5.8	7.3	—
7	0.67	10.8	5.3	10.2	Valeraldehyde
8	0.69	5.2	4.0	5.0	Hexanal
9	0.71	4.0	4.0	2.2	—

Thin layer: Silica gel G.
Solvent: Carbon tetrachloride : hexane : ethyl acetate (10:2:1).
I, II, III: Volatile carbonyls formed during roasting at 160, 175 and 190°C respectively.

Changes in sugars

The effect of roasting on reducing, non-reducing and total sugars is shown in Table 1. There was a slight, but significant decrease in reducing, non-reducing and total sugars during roasting at 160 and 175°C. At 190°C, however, reducing sugars increased slightly but non-reducing and total sugars decreased considerably. This is in contrast to the results reported by Rohan & Stewart (1966a, 1966b), who found a complete destruction of reducing sugars and a considerable decrease in non-reducing sugars during roasting of cocoa beans. Pinto & Chichester (1966) have also observed a decrease in non-reducing sugars during roasting of cocoa beans. A decrease in reducing sugars may result from their interaction with amino compounds in browning reactions whereas a decrease in non-reducing sugars may be due to the hydrolytic dissociation of higher molecular weight oligosaccharides into smaller fragments and their subsequent utilization in browning reactions. Alternatively, it may also be argued that the decrease in non-reducing sugars may be caused by the condensation reactions resulting in the decrease in the solubility of this fraction, whereas an apparent increase in reducing sugars at 190°C may have resulted because of the formation of highly reducing substances during the course of browning reactions. Whichever the mechanism, it is apparent that at 160 and 175°C, the rate of consumption of reducing sugars in non-enzymic browning reactions is higher than formation of total reducing substances either from the dissociation of non-reducing sugars or during the course of browning reactions. But at 190°C, the rate of formation of reducing substances exceeds their consumption, resulting in a slight increase

in the apparent reducing sugar level in the roasted semolina. An overall decrease in total sugars (Table 1) during roasting at all the three temperatures indicates their involvement in browning reaction and thereby in the formation of volatile carbonyl compounds by Strecker's degradation. This is also supported by a decrease in the concentration of water soluble amino compounds (Table 1) and a significant increase in the browning intensity of the 70% alcohol solubles of roasted semolina (Table 1).

Table 5. Free lipids, bound lipids and lipid acidity of roasted semolina samples

Samples	Free lipid (%)	Bound lipid (%)	Free lipid acidity	Bound lipid acidity
Raw semolina	1.32	0.57	25.46	14.4
Roasted semolina (at 175°C for 10 min)	1.41	0.50	21.87	14.0
Roasted semolina (conventional method)	1.42	0.48	23.05	13.8

Lipid acidity is expressed as mg KOH/100 g sample.

Changes in lipids

Wheat lipids play an important role in the texture and flavour of pasta products. Roasting at high temperatures may bring about changes in the proportion of free and bound lipids while their thermal degradation may release a large number of volatile compounds and thereby decrease in the weight of total lipids. Accordingly, changes in free and bound lipids as a result of roasting were investigated and the results are given in Tables 1 and 5. The initial free lipids of the two lots of commercially available semolina samples were 1.32 and 1.7%. On roasting the proportion of free lipids significantly increased with a corresponding decrease in the proportion of bound lipids. The percentage of total lipids, however, remained practically constant. The extent of increase in free lipids as a result of roasting depended on the severity of heating (Table 1); the changes being least at 160 and highest at 190°C. Since in the native state difficultly extractable lipids are bound to proteins and starch with hydrogen and hydrophilic bondings, probably these bonds are destroyed during roasting making these lipids more easily extractable. In contrast, Kowsalya & Urs (1979) have reported that dry roasting of bengal gram decreases the proportion of free lipids with a corresponding increase in bound lipids. In the present study, the percentage of total lipids or lipid acidity did not change to a significant extent indicating only minor alterations as a result of roasting. Sekhon, Ahuja & Sandhu (1971) have claimed that fatty acid composition of ground nuts is not altered significantly as a result of roasting.

Changes in proteins

The effect of roasting on semolina proteins was determined by following their solubility in water, alcohol (70%), 0.1 N acetic acid and 3 M urea-phosphate buffer and also by the Sephadex G-150 gel filtration behaviour of 0.1 N acetic acid soluble proteins. There was a gradual decrease in the solubility of semolina proteins (Figs 1, 2 and 3) in all the four major solvents as a result of roasting. The highly dissociating 3 M urea-phosphate buffer which solubilized about 90% of toal unroasted semolina proteins extracted only 20% of total proteins in samples roasted at 190°C for 15 min, indicating irreversible denaturation of proteins during roasting operations. The decrease in solubility of proteins as a result of roasting increased with the roasting temperature and period of roasting. The changes were much less at 160 and 175, compared to 190°C even at the same intensity of roasted flavour.

A typical gel filtration behaviour of acetic acid soluble proteins from raw and roasted semolina is given in Fig. 4. It may be observed that acetic acid soluble proteins separated into four peaks corresponding to (1) glutenins, (2) gliadins, (3) albumins and (4) polypeptides. During roasting, heights of Peaks (1) to (3) decreased significantly but that of Peak (4) slightly increased. The

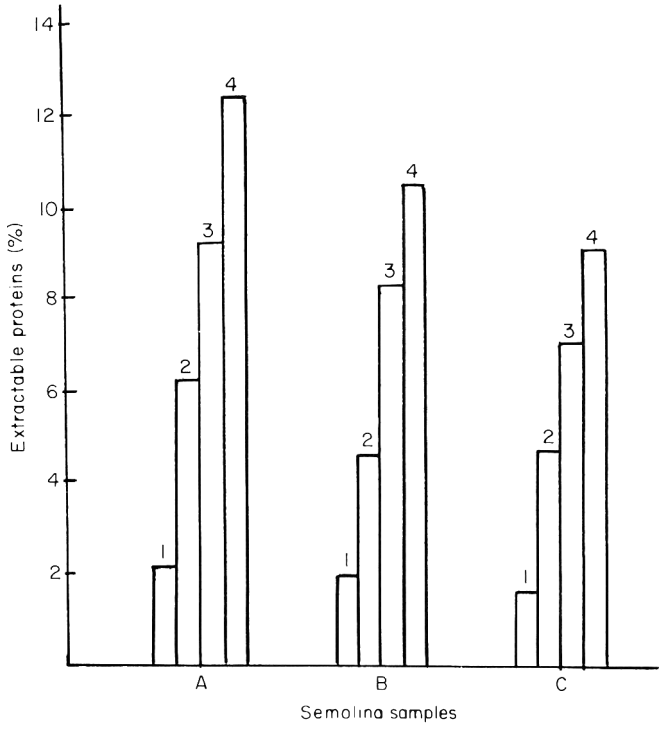

Figure 1. Changes in solubility of semolina proteins in various solvents, semolina roasted at 160°C. A, raw semolina; B, semolina roasted for 10 min; C, semolina roasted for 15 min; 1, water extractable proteins; 2, alcohol extractable proteins; 3, acetic acid extractable proteins; 4, 3 M urea extractable proteins.

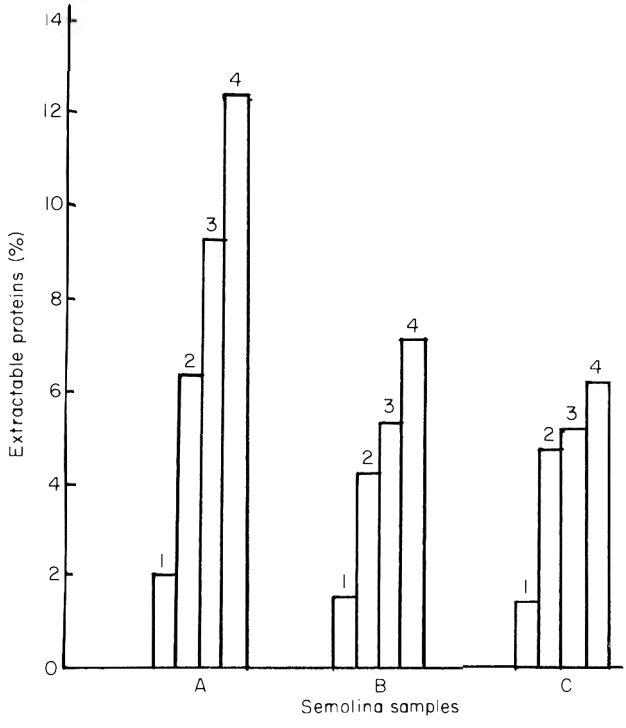

Figure 2. Changes in solubility of semolina proteins in various solvents, semolina roasted at 175°C. A, raw semolina; B, semolina roasted for 10 min; C, semolina roasted for 15 min; 1, water extractable proteins; 2, alcohol extractable proteins; 3, acetic acid extractable proteins; 4, 3 M urea extractable proteins.

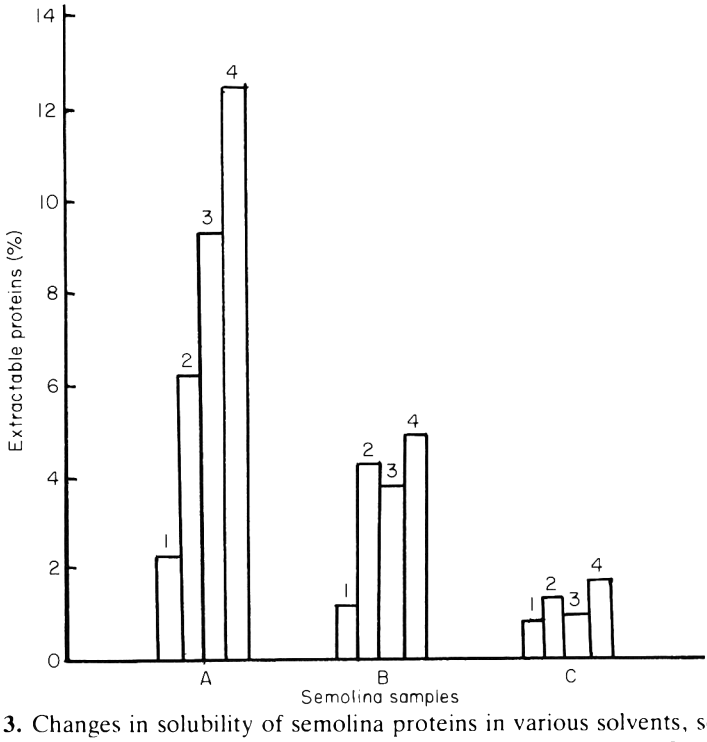

Figure 3. Changes in solubility of semolina proteins in various solvents, semolina roasted at 190°C. A, raw semolina; B, semolina roasted for 10 min; C, semolina roasted for 15 min; 1, water extractable proteins; 2, alcohol extractable proteins; 3, acetic acid extractable proteins; 4, 3 M urea extractable proteins.

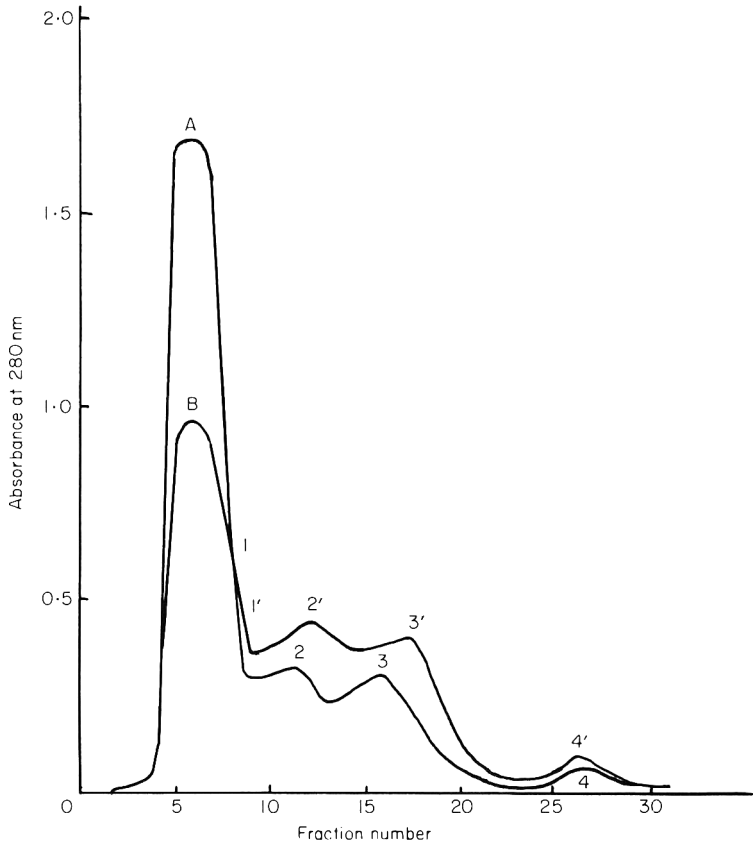

Figure 4. Profile of 0.1 N acetic acid soluble protein extracts of semolina samples from Sephadex G-150 column with 0.1 N acetic acid. A, raw semolina; B, semolina roasted at 175°C for 10 min; Peak 1, glutenin; Peak 2, gliadin; Peak 3, globulin and albumins; Peak 4, low molecular weight peptides.

positions of the four peaks, however, remained unchanged. A slight increase in polypeptide fraction (4) indicates an increase in their concentration during roasting as a result of scission of peptide linkages. This has also been observed by Hansen, Johnston & Ferrel (1975) and Hayase, Kato & Fujimaki (1975) at temperatures above 160°C.

Effect of roasting on gelatinization viscosity

The gelatinization behaviour of various starches greatly influence the consistency and texture of various pasta products and in turn their acceptability. It may be observed that roasting of semolina considerably affects its gelatinization behaviour (Table 1). At 160°C, initial roasting for 10 min significantly increased the gelatinization viscosity, but subsequent heating at this temperature lowered the gelatinization viscosity. The initial increase in gelatinization may have resulted from the inactivation of native

amylases whereas the subsequent decrease in gelatinization viscosity must have been due to the inadequate water imbibing capacity of the starch granules as a result of excessive heating. This is further supported from the data on gelatinization viscosity of samples roasted at 175 and 190°C. It may be seen that at 175 and 190°C, even during first 10 min of roasting, the gelatinization viscosity of roasted samples decreased slightly. The effect of inactivation of amylase activity was suppressed by the very rapid loss in water imbibing capacity and swelling of granules as a result of roasting.

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Rabbit meat for manufacturing

The effect of different post-slaughter cooling treatments

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Summary

The effects of extremes of post-mortem cooling on properties relevant to the inclusion of rabbit meat in products have been investigated. Covered and uncovered carcasses were cooled post-mortem by slow chilling (+12°C), rapid chilling (0°C) or rapid freezing (−30°C).

There was little difference in effect on quality between slow chilling and rapid freezing. Rapid chilling produced the shortest sarcomere lengths in *Musculus longissimus dorsi,* and this is attributed to indirectly induced shortening. Rapid chilling reduced significantly the water holding capacity, increased the resistance to compression and extrusion of the raw comminutes, and reduced the shear stress of the heated fine comminute. It is suggested that the decrease in WHC with rapid chilling results not from cold shortening but from the better integrity of the Z line.

Introduction

The ease with which rabbits may be kept and bred attracts considerable interest from both developing and developed countries. Compared with other meats, rabbit is low in fat and cholesterol, high in protein and several vitamins (MAFF, 1978; Paul & Southgate, 1978). For these reasons, several projects on rabbit production are being pursued in Africa, South America, Asia and Indo-Pacific countries (Owen, 1977).

Scientific reports on rabbit have concentrated on improvements in husbandry or carcass quality; the possibility of using rabbit for meat products has received little attention. Not only do meat products offer the consumer more choice, but they can also allow more economic storage of animal protein

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when compared to meat left on the carcass. Using rabbit for meat products might therefore help to even-out the marked seasonal variation in price of rabbit carcasses which is characteristic of this part of the U.K. meat industry.

The development and acceptance of processed poultry products suggests that there may be a market for similar products from rabbit meat. This was recognized in the U.S.A. by Whiting & Jenkins (1981) who compared some functional properties of rabbit meat with those of beef and chicken. Sensory evaluation of frankfurters made from all three meats demonstrated that those from rabbit were as acceptable as those from beef and slightly superior to those from chicken, although there was little difference in functional properties. Baker, Darfler & Vadehra (1972) had earlier found that frankfurters made with rabbit meat as the main meat or in association with an equal quantity of chicken were very well accepted. Rabbit products may also be attractive in urban markets of developing countries (Owen *et al.*, 1979).

Because of their small size and low fat cover, rabbit carcasses could be affected more than other meat species by different temperature treatments applied post slaughter. If so, this could represent an important source of variability in the quality of products made from rabbit meat. We have therefore examined the effects of rapid post-mortem chilling and rapid post-mortem freezing of freshly slaughtered rabbit carcasses on the quality of comminuted meat products.

Materials and methods

Materials

Thirty-two New Zealand White rabbits, approximately 86 days old and weighing about 2.2 kg, were obtained in batches of four (two males, two females) from the Rabbit Unit, University of Bristol. They were killed by a blow to the back of the neck, dressed and skinned. Samples (approximately 1 g) of *M. longissimus dorsi* (l.d.) from the mid-back and *M. biceps femoris* (b.f.) were removed for determination of initial pH (pH_i). Carcasses were suspended by their hind legs, weighed, and transferred to constant temperature rooms at the desired temperature within 1 hr of exsanguination.

Procedure

Post-slaughter cooling. Three post-slaughter temperature treatments were used: slow chilling, rapid chilling, and rapid freezing. In each treatment some carcasses were covered individually with loosely fitting polythene bags.

Slow chilling. A temperature of 12°C was chosen to represent a slow chilling regime. At this temperature the rate of glycolysis is liable to be minimal (Jeacocke, 1977; Jolley, Honikel & Hamm, 1980–81) and cold shortening absent (Bendall, 1973a). Two batches of four animals were chilled uncovered, and a third batch chilled covered. All twelve carcasses were held at 12°C for 24 hr with an air speed of 3 ± 0.3 m/sec.

Rapid chilling. Carcasses were placed in a room at 0°C for 24 hr (air speed 3 m/sec). Two batches of four animals were chilled uncovered, a third batch covered.

Rapid freezing. Carcasses were placed in a room at −30°C with air speed 3 m/sec for 24 hr. One batch was frozen uncovered, a second covered. Both batches were held at −20°C in a domestic deep-freeze for 21 days.

The uncovered batch was thawed for 8 hr at +1°C and then stored at 15°C for 15 hr. The covered batch was thawed by holding at 15°C for 15 hr.

Carcasses were weighed at the end of each cooling and storage treatment and further samples of l.d. and b.f. removed for estimation of ultimate pH (pH_u). An additional sample of l.d. was taken for determination of sarcomere length by diffraction (Voyle, 1971). The remainder of the l.d. and the musculature of the hindleg were removed from both sides of the carcass. This material was minced once through a 10 mm plate using a small kitchen mincer (Moulinex, France). Samples of the minced lean were taken for estimation of dry weight and WHC.

Preparation of comminutes

Two types of test material were manufactured from the minced rabbit lean; the first a fine comminute of rabbit meat, salt, and water, the second a coarser comminute formulated as a burger.

Fine comminute

These were produced in a similar manner to that of Jolley *et al.* (1980–81). Sixty-six grams minced lean was mixed by hand with 2 g NaCl. Thirty-three grams iced water was mixed into the salted mince and the mass finely comminuted in four bursts of 3 sec duration in a Moulinette chopper (Moulinex, France). One fine comminute was produced from the minced lean of each rabbit.

Burger mix

This was produced from the minced lean of each rabbit using a 'mince–mix–mince' method frequently used in the U.K. A mixture of 1.6 g NaCl and 0.2 g white pepper was added to 70 g minced lean and mixed by hand. Ten grams pork back fat (pre-minced through a 10 mm plate) was added and mixed, and finally 13.2 g iced water and 5 g dried yeastless rusk (DYR, T. Lucas & Co., U.K.) were mixed in. The mass was passed through the 10 mm plate and held overnight at 0°C until assessed for resistance to compression and extrusion.

Determination of pH

Samples for pH determination were homogenized in 10 ml 150 mM KCl/5 mM iodoacetic acid (neutralized to pH 7.0) using a laboratory mixer-

emulsifier (Silverson Machines Ltd, Bucks). The pH of each extract was determined with a pH M63 Digital pH meter (Radiometer, Copenhagen) using a combined glass electrode (Russell pH Ltd).

Determination of dry weight

Dry weight was determined on the minced lean by drying an accurately weighed sample of approximately 15 g in an oven at 105°C for 24 hr.

Assessment of water holding capacity

Water holding capacity (WHC) was assessed on 0.3 g samples of minced lean by the filter paper press method of Grau & Hamm (1952, 1957) and expressed as bound water related to meat.

The meat's ability to hold its own and added water in the presence of NaCl was determined on 0.3 g of the fine comminute by the same method, and also expressed as bound water related to meat.

Assessment of mechanical properties of the comminutes

Mechanical measurements were made using a materials testing instrument (Instron, model TM-SM type 1102). The unheated fine comminute and the burger mix were assessed for resistance to compression and back extrusion.

Duplicate samples of 8–9 g which filled the inside of a cylindrical steel cup (18 mm i.d., 34 mm depth) were mounted on the compression table of the

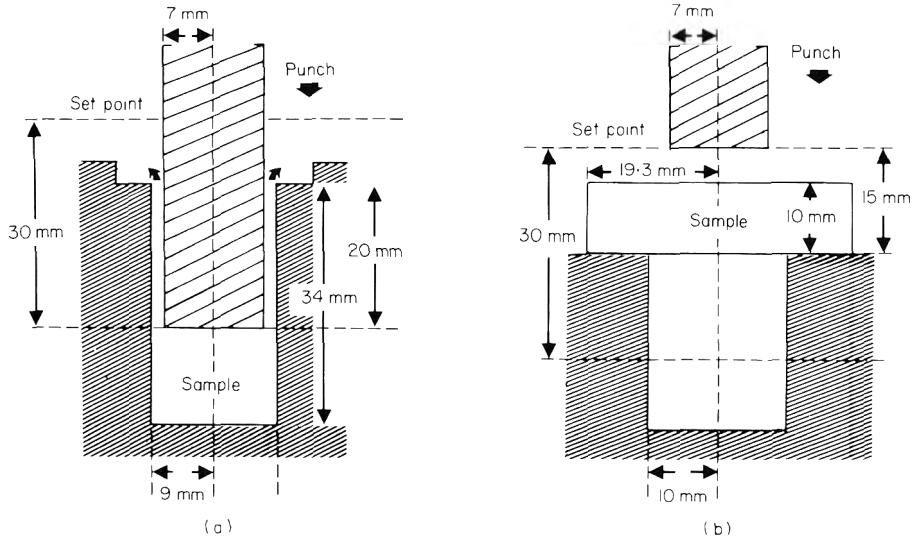

Figure 1. Dimensions of Instron punch travel and sample holders for assessment of mechanical properties of the comminutes. (a) Assessment of resistance to compression and back extrusion of unheated fine comminute and burger mix. (b) Assessment of shear stress of fine comminute after heat treatment.

load measuring cell. Each sample was compressed and back extruded with a cylindrical steel punch (14 mm diameter) attached to the moveable crosshead directly above the sample holder. The punch was driven 20 mm at 50 mm/min and returned to the starting position at the same speed (Fig. 1a). Continuous force–time records were taken and the work done up to withdrawal of punch was calculated from the area under the force–time curves measured with a planimeter.

Shear stress (resistance to compression and shear) was assessed on samples of the fine comminute after heat treatment. Accurately weighed samples of approximately 33 g were placed in identical 50 ml Pyrex beakers, compacted to exclude air. The beakers were covered in aluminium foil and placed in a water bath at 82°C for 30 min. Juice released during heating was drained off after the sample had cooled, and the sample was lightly blotted before re-weighing. Heating losses were calculated as percentage of the raw weight. Heated samples were held overnight at 0°C before assessment.

Shear stress was measured on two 10 mm thick discs of 38.6 mm diameter cut from the circular face of each heated sample. Each disc was placed above and concentric with a hole (Fig. 1b) in a steel block on the load cell. A punch (14 mm diameter) was driven down 30 mm at a speed of 50 mm/min shearing a cylindrical plug from the sample disc. The punch returned to its initial position at a speed of 125 mm/min. Load-deformation (time) curves were recorded continuously. As shearing proceeded the force increased progressively reaching a maximum at about the exit of the punch from the sample disc. Force decreased rapidly to zero as the disc core was pressed out. The maximum force was determined and expressed as shear stress by dividing by 4.4×10^{-4} m^2 (the area of newly exposed surface of the core sample).

Statistical analysis of results

Unless otherwise stated, all data were analysed by a standard two-way analysis of variance with post-mortem temperature treatment and covering as the main effects. Their interaction was also calculated.

Results

Effect of cooling and covering on carcass characteristics and WHC

Overall (n=32) mean carcass weight before cooling was 1.304 kg, with a standard deviation (s.d.) of 0.075 kg.

Cooling and covering had a very highly significant ($P<0.001$) interactive effect on carcass weight losses (Table 1). Uncovered carcasses had an overall 4.2% weight loss (n=20) and covered carcasses only 0.9% (n=12). Decreasing the temperature from 12 to −30°C with uncovered carcasses reduced weight loss from 7.6 to 1.3% but did not affect covered carcasses.

Table 1. Weight losses, expressed as percentage of initial carcass weight, arising from treatment

	Slow chill (+12°C)	Rapid chill (0°C)	Rapid freezing (− 30°C)
Uncovered	7.75 c	2.05 b	1.29 a
Covered	0.56 a	0.91 a	1.34 a

Means with the same subscript are not significantly different at the 5% level. Least significant differences (LSD) between uncovered carcasses slow chilled and uncovered carcasses rapid chilled (maximum replicates, eight animals in either treatment) 0.57%, between minimum replicates (all other treatments) 0.81%, and between maximum and minimum replicates 0.70%.

The greater loss of moisture from uncovered carcasses was reflected in their significantly higher ($P<0.001$) dry weight (means for minced leans: 25.97 compared with 24.35% for covered carcasses). Assuming this arises from evaporation, which is a surface phenomenon, then this result suggests marked surface desiccation with uncovered carcasses. Cooling had no effect on dry weight.

Sarcomere length was influenced by an interactive effect of cooling and covering ($P<0.01$; Table 2). With both covered and uncovered carcasses, rapid chilling produced shorter sarcomeres than slow chilling. Sarcomeres were longer in the uncovered carcasses when slowly chilled but shorter with freezing and thawing.

The mean pH_i in the l.d. was 6.74 (s.d.=0.18) and 6.73 (s.d.=0.20) in the b.f. Respective values for pH_u were 6.00 (s.d.=0.24) and 6.04 (s.d.=0.28).

Because pH_i and pH_u affect WHC, all pH values were included as covariates in the analysis of variance of WHC of the minced lean, and WHC and heating losses of the fine comminute. The values for WHC adjusted for pH_i and pH_u are shown in Table 3. Neither cooling nor covering affected WHC of the minced lean, but cooling had a highly significant effect ($P<0.001$)

Table 2. Mean sarcomere lengths (μm) by treatment

	Slow chill (+ 12°C)	Rapid chill (0°C)	Rapid freezing (− 30°C)
Uncovered	2.00 d	1.68 a, b	1.73 a, b
Covered	1.76 b, c	1.58 a	1.91 c, d

Means with the same subscript are not significantly different at the 5% level. LSD between maximum replicates = 0.11 μm, between minimum replicates = 0.17 μm, and between maximum–minimum replicates 0.14 μm.

Table 3. Water holding capacity (%) of minced lean and fine comminute

	Slow chill (+ 12°C)	Rapid chill (0°C)	Rapid freezing (− 30°C)
Minced lean	52.9	48.9	46.9
Fine comminute	87.9 b	72.7 a	96.4 b

All values are corrected for pH_i and pH_u as covariates (see text).

There were no significant differences for WHC of minced lean. LSD between slow chill and rapid chill 6.6%, and between rapid freezing and either slow chill or rapid chill 7.4%.

Means of the fine comminute with the same subscript are not significantly different at the 5% level. LSD between slow chill and rapid chill 12.7%, and between rapid freezing and either slow chill or rapid chill 14.2%.

on the WHC of the fine comminute, rapid chilling producing the lowest WHC, 15% less than slow chilled and 24% less than freezing and thawing. Whilst there was not a similar significant effect on heating loss (with mean values for slow chilling, rapid chilling, and rapid freezing of 22.1, 26.0 and 23.9% respectively) there was a highly significant ($P<0.001$) linear correlation between heating loss and WHC of the fine comminute, but with a low coefficient ($r=-0.59$). There was no relationship between heating loss of the fine comminute and WHC of the minced lean ($r=0.04$).

Effect of post-mortem cooling and covering on mechanical properties of comminutes

There was a highly significant interactive effect ($P<0.001$) between cooling and covering on the work done in compressing and extruding the unheated burger mix (Table 4). By far the greatest work was applied to the mix from the uncovered rapidly chilled group, about one third greater than the next highest

Table 4. Work done in compressing and extruding unheated burger mix ($Nm \times 10^{-2}$)

	Slow chill (+ 12°C)	Rapid chill (0°C)	Rapid freezing (− 30°C)
Uncovered	11.1 a, b	19.3 d	9.8 a
Covered	14.3 c	12.6 b, c	12.9 c

Means with the same subscript are not significantly different at the 5% level. LSD between maximum replicates = $1.24 Nm \times 10^{-2}$, between minimum replicates = $1.75 Nm \times 10^{-2}$, and between maximum–minimum replicates = $1.52 Nm \times 10^{-2}$.

Table 5. Work done in compressing and extruding the fine comminute ($Nm \times 10^{-2}$)

	Slow chill (+ 12°C)	Rapid chill (0°C)	Rapid freezing (− 30°C)
Uncovered	4.7	6.3	4.8
Covered	3.9	4.6	4.4

Covering and cooling both had significant effects ($P < 0.01$ and 0.001 respectively).

Overall mean ($n = 20$) of comminutes from uncovered carcasses was $5.3\,Nm \times 10^{-2}$ and $4.3\,Nm \times 10^{-2}$ ($n = 12$) for comminutes from covered carcasses; least significant difference (5%) = $0.6\,Nm \times 10^{-2}$.

Overall mean ($n = 12$) for comminutes from slow chilling was $4.3\ Nm \times 10^{-2}$ and $4.7\ Nm \times 10^{-2}$ ($n = 8$) for rapid freezing; least significant difference (5%) = $0.72\ Nm \times 10^{-2}$ (n.s.). Overall mean ($n = 12$) for comminutes from rapid chilling was $5.6\ Nm \times 10^{-2}$; least significant difference between this and (a) slow chilling = $0.65\,Nm \times 10^{-2}$ (b) rapid freezing = $0.72\,Nm \times 10^{-2}$.

mean. Least work was applied to the mix from the uncovered rapidly frozen group.

Cooling and carcass covering had separate significant influences on the work done in compressing and extruding the unheated fine comminute ($P < 0.001$ and 0.01 respectively; Table 5). Fine comminutes from uncovered carcasses required more work for compression and extrusion than those from covered carcasses. Similarly, more work was required to compress and extrude fine comminutes from the rapidly chilled groups than for either slowly chilled or rapidly frozen.

Work done and peak force were highly correlated ($P < 0.001$) in fine comminute ($r = 0.96$) and burger mix ($r = 0.97$).

There was a weakly interactive effect ($P < 0.05$) between cooling and covering on shear stress in the heated fine comminute (Table 6). Least shear

Table 6. Shear stress of heat treated fine comminute ($Nm^{-2} \times 10^4$)

	Slow chill (+ 12°C)	Rapid chill (0°C)	Rapid freezing (− 30°C)
Uncovered	2.07 b	1.55 a	2.83 c
Covered	2.14 b	1.44 a	2.15 b

Means with the same subscript are not significantly different at the 5% level. LSD between maximum replicates = 0.33 $Nm^{-2} \times 10^4$, between minimum replicates = $0.47\,Nm^{-2} \times 10^4$, and between maximum–minimum replicates = $0.41\,Nm^{-2} \times 10^4$.

stress was shown by the rapidly chilled groups, the highest by the uncovered group that was rapidly frozen.

Discussion

This study was designed to see if extremes of post-mortem cooling effect quality changes in rabbit meat products and to identify areas for future investigation. A high air speed (3 m/sec) was used to achieve efficient cooling but inevitably produced large evaporative losses. Carcass covering was therefore also included to reduce these losses.

Several parameters showed significant interactions between covering and cooling, probably because air trapped by covering acted as insulation and maintained a higher muscle temperature for longer. With slow chilling, the higher temperature and lower pH resulting from covering is likely to be detrimental, by analogy with PSE pork, but with rapid chilling covering may be beneficial in lessening the risk of cold shortening. Conversely, during rapid freezing, covering increases the risk of cold shortening by delaying the rate of freezing, the slowness of which *per se* might also be detrimental.

The detrimental effect of rapid chilling: a cold shortening effect in rabbit l.d.?

Several authors, particularly Bendall (1973b), Henderson, Goll & Stromer (1970), Lawrie (1968) and Locker & Hagyard (1963) have shown that rabbit l.d. or psoas do not cold shorten. In this work, sarcomere lengths in the l.d. of the rapidly chilled carcasses were significantly shorter than the corresponding slowly chilled carcasses (Table 2). Because of the volume and quality of literature showing that rabbit l.d. does not cold shorten, our results need to be explained, assuming that they are genuine and not a statistically unlikely coincidence. As the results are unexpected they are discussed in detail.

Cold shortening may have been induced in this experiment by using more severe chilling conditions and achieving lower temperatures earlier post mortem than in studies where muscle was held at 2°C (Bendall, 1973b; Henderson *et al.* 1970; Lockyer & Hagyard, 1963), although Lawrie (1968), who should have achieved very rapid cooling with strips of *M. psoas major* suspended in moist air at 0°C, observed no cold shortening. Nevertheless, rabbit psoas and l.d. can generate tension in response to lowering of temperature (R. E. Jeacocke, pers. comm.) and this suggests that some cold induced shortening of sarcomeres can occur in these muscles. The earlier studies were carried out with muscles excised immediately after slaughter, and it is common experience that removal of restraint frequently produces shortening. Initial muscle lengths are usually measured at slack length after excision, by which time the muscle may already be shortened to some extent. Perhaps excision shortening in rabbit l.d. is greater than that induced by lowering of temperature.

Another, and more satisfactory, explanation is that the cold induced sarcomere shortening in the l.d. was brought about indirectly. Rabbit red muscles have been shown to cold shorten (Bendall, 1973b; Lawrie, 1968; Newbold, 1979). When a rabbit carcass is rapidly chilled, the muscles that can cold shorten will tend to do so and create localized tension within the carcass. If this is great enough, and sufficient ATP is present in the l.d. to allow relaxation, the sarcomeres in the l.d. will be forced to shorten. The spine need not show any curvature since the orientation of the fibres in the l.d. allows marked sarcomere shortening without changing muscle length (Locker *et al.*, 1975, p. 175). Alternatively, areas of localized indirect shortening could be matched by others of stretch (*cf.* Marsh & Leet, 1966; Voyle, 1979).

Indirect cold induced shortening of rabbit l.d. would be a complex phenomenon. The final sarcomere length at rigor would be the result of tensions generated by several muscles responding to cold at different rates as a function of physiological role (*cf.* Bendall, 1975) and depth on the carcass.

If we assume that cold shortening has occurred, why does it produce inferior WHC in the fine comminute? Greater interdigitation between thick and thin filaments in cold shortening implies proportionally more actomyosin and less myosin than in non-shortened muscle. Since myosin is a better binding agent in the presence of NaCl than actomyosin (MacFarlane, Schmidt & Turner, 1977) a change in their proportions might reduce ability to hold water. Another factor may arise from skeletal restraint during cold shortening; muscle held at fixed length during cooling has a different and more varied morphology from that of freely suspended muscle (Voyle, 1979). This could lead to differences in size and uniformity of fibre fragments produced on comminution, and this in turn could affect product quality.

If the detrimental effect of rapid chilling on WHC is due to either skeletal restraint or increased filament interdigitation it should also occur in other meats. But, in fact, sarcomere length seems to have no effect on WHC of hot boned beef, comminuted and salted post rigor (Honikel, Fischer, & Hamm, 1980) although there are no comparable data for muscle chilled on the carcass. The differences in WHC may not result directly from longitudinal shortening but may reflect instead the relative integrity of Z lines. The integrity of the Z line in rabbit is particularly affected by post-mortem storage temperature whilst beef is not (Henderson *et al.*, 1970). Rapidly chilled rabbit could have less WHC because the Z lines impose greater restraint on transverse myofibrillar swelling (Offer & Trinick, 1983); this would explain differences between the species in response to rapid chilling.

The effect of pre-rigor freezing and subsequent thawing

Although rabbit white muscle is generally believed not to cold shorten, it does show thaw contracture (Lawrie, 1968; Bendall, 1973a). If it occurred in this study, it was not evidenced by l.d. sarcomere length (Table 2). In only one of the other parameters (shear stress of the heated fine comminute; Table 6)

did the meat from the rapidly frozen carcasses differ significantly from the corresponding slow chilled group. The apparent absence of thaw rigor in the uncovered group probably resulted from a combination of skeletal restraint and comparatively gentle thawing regimes, since there was sufficient ATP present at the time of thawing to allow contraction (R. L. T. Lopes, unpubl.). Because of this lack of effect, the holding period at +1°C was omitted for the covered group and the carcasses placed immediately at 15°C. Meat from these carcasses behaved differently to that from the uncovered group (Tables 4 and 6). Although it is not possible to attribute this solely to either covering or thawing it clearly demonstrates that varying the conditions of pre-rigor freezing and subsequent thawing will greatly affect quality.

Relationship between WHC of minced lean and WHC of the fine comminute

Post-mortem cooling treatments had no effect on the WHC of minced lean, but had an appreciable effect on the WHC of the fine comminute (Table 3). There was no relationship between the two parameters (r=0.1). The results for the fine comminute are far more relevant to the present investigation, since sodium chloride and additional water are typically present in meat products.

The literature frequently shows instances when the ability of meat to hold its own water bears no relationship to its ability to hold added water in the presence of NaCl, although it is only rarely commented upon. Sometimes the distinction is made between the two attributes by referring to the former as 'water holding capacity' and the latter as 'water binding capacity (Ranken, 1976) and this suggests that many workers are aware of the lack of relationship. Nevertheless, the two terms are so frequently used interchangeably that the distinction has lost its value. Major differences can and do exist.

Mechanical properties of the comminutes

Assessment of resistance to compression and extrusion is relevant to meat products because these types of deformation are inevitably applied at some stage of manufacture. These types of deformation vary tremendously according to process and machinery, and many processors believe that such differences affect the texture of the end product. Rapid chilling without covering greatly affected resistance to compression and extrusion of the fine comminute (Table 4) and the burger mix (Table 5), strongly suggesting that rapid chilling of rabbit could affect product texture over a wide range of the degrees of comminution and formulation found in commerce.

Since most meat products are cooked before consumption, we tested the texture of the heated fine comminute using a shear test. This measures the cohesiveness of a sample, an important attribute of texture of meat products. Rapid chilling reduced cohesiveness.

It is interesting that rapid chilling produced raw comminutes with greatest resistance to compression and back extrusion but lowest cohesiveness after heating. This is consistent with a larger particle size. Salt penetration and/or protein solubilisation may be less complete with larger particles and this would explain reduction in WHC.

Conclusions

This preliminary investigation has established that rabbit meat for use in products is greatly influenced by post mortem cooling conditions. In particular, rapid chilling is detrimental to product water holding capacity and cohesiveness, and produces more variable texture. How these changes have been effected warrants study to establish whether rapid chilling of the carcass post mortem can induce similar quality changes in meat products from the more economically important meat species.

Regardless of the mechanism, rapid chilling shortened sarcomeres in the l.d. and this would be expected to lead to tougher unprocessed meat. More work needs to be done to determine the magnitude of this induced toughness as it is clearly an important factor in determining consumer acceptance of rabbit meat.

In this investigation, rapid pre-rigor freezing, in conjunction with thawing at 15°C, had little detrimental effect on quality. Whether this would be found under the more variable conditions likely to be encountered in the factory is not certain and this should be taken into consideration in processing design.

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Synergistic effects of various lipases and phospholipase C on milk fat

M. W. GRIFFITHS

Summary

The enzyme, phospholipase C, from a number of psychrotrophs isolated from milk was shown to survive HTST pasteurization and UHT sterilization processes. The phospholipase C of *Bacillus cereus* was able to increase lipolytic activity of a commercial lipase preparation and native milk lipoprotein lipase in raw milk by making the substrate more susceptible to hydrolysis: phospholipase C had no effect on the lipase activity of a fluorescent *Pseudomonas* spp. The enhancement of lipolysis by phospholipase C was affected by pronase and papain, but not by trypsin. Homogenization of the raw milk had the same effect on lipolysis by lipoprotein lipase as addition of phospholipase C.

Introduction

Fat exists in milk as minute globules surrounded by a coating or membrane. This membrane of protein and phospholipid serves as a physical barrier to prevent agglomeration of the globules during agitation, and it also may offer protection against lipolysis. Its removal may allow easy accessibility of lipases from milk or micro-organisms to the glycerides of the fat globule (Law *et al.*, 1973). Since the membrane is essential to the integrity of the milk emulsion, any factor capable of disrupting or modifying it may be deleterious to the emulsion itself. Mechanical damage of the membrane during milking is fairly common, but the possibility of enzymatic degradation of the membrane also exists. This could be achieved by the action of phospholipase. Although milk contains no natural phospholipase, several of the bacteria commonly found in milk produce this enzyme (Fox, Chrisope & Marshall, 1976; Owens, 1978). In the survey of factors affecting shelf life of double cream, over 50% of the

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organisms isolated were capable of producing phospholipase (Phillips, Griffiths & Muir, 1981). Among the organisms capable of synthesizing phospholipase C when growing in milk are *Bacillus* sp., *Pseudomonas* sp., *Alcaligenes* sp., *Acinetobacter* sp., *Enterobacter* sp. and several others (Fox *et al.*, 1976; Owens, 1978). The effect that these enzymes have on the induction of lipolysis in dairy products has received very little attention.

It has been known for several years that *B. cereus* and *B. mycoides* are responsible for 'bitty' or broken cream in pasteurized milk due to the production of phospholipases capable of hydrolysing lecithin present in the milk fat globule membrane (Stone, 1952), although other enzymes, probably proteases, may also be implicated (Stone & Rowlands, 1952). It is also known that phospholipase can suppress the development of oxidized flavours in milk, possibly due to the migration of diglycerides formed in hydrolysis to the hydrophobic centre of the milk fat globule (O'Mahoney & Shipe, 1972). It has also been shown that the phospholipase C of *Ps. fluorescens* hydrolysed phospholipids adsorbed to fat globules in a model emulsion composed of butter oil emulsified with crude soy lecithin (Chrisope & Marshall, 1976). Modification of the membrane by this enzyme enhanced lipolysis when steapsin, a lipase, was introduced to the emulsion. Phospholipase C also enhanced the activity of milk lipase in raw milk. This paper investigates further the enhancement of lipolysis in milk by the presence of phospholipase, and also investigates the thermostability of the enzyme molecule. Some evidence already exists which suggests that phospholipases synthesized by psychrotrophic bacteria may be capable of surviving pasteurization procedures (Fox & Marshall, 1975).

Methods and materials

Growth of organisms

The bacteria used in this study were isolates obtained from milk or pasteurized double cream and identified as described previously (Griffiths, Phillips & Muir, 1981). Bacteria were grown in Pope & Skerman's mineral salts medium according to Griffiths *et al.* (1981). For the lipase purification the organism (*Ps. fluorescens* 1467) was grown in Pope & Skerman's medium in 500 ml conical flasks containing 100 ml of medium. The flasks were incubated at 21°C with shaking in a New Brunswick G24 environmental incubator shaker fitted with a cooling coil (New Brunswick Scientific Co. Inc.).

Preparation of enzyme samples

Cells were removed by centrifugation at 19 000 ***g*** for 10 min using an MSE Hi-spin 21 centrifuge (MSE Scientific Instrument Ltd). The cell-free supernatant liquids were concentrated about five-fold using an ultrafiltration membrane (Amicon PM 30 Diaflo ultrafiltration membrane) in a Chemlab

C50 ultrafiltration cell. The concentrated cell-free supernatant liquid was used as the source of phospholipase C activity in the thermostability trials.

The cell-free supernatant liquid from *Ps. fluorescens* 1467 was used to obtain a lipase activity free from protease and phospholipase activity. This was achieved by addition of solid ammonium sulphate to 25% saturation. The precipitate was removed by centrifugation and more ammonium sulphate was added to 45% saturation. The precipitate was obtained, again by centrifugation. The precipitate was dissolved in a small volume of 50 mM sodium phosphate buffer, pH 7.0 and applied to a column containing Sephadex G-200 and eluted with phosphate buffer containing 0.02% sodium azide. Five millilitre fractions were collected using a Gilson model VL linear fractionator (Gilson Medical Electronics, Inc.) and the absorption at 260 nm and 280 nm monitored with a Uvicord III 2089 UV absorptiometer combined with an LKB 6520 recorder (LKB-Produkter AB). The eluate was concentrated with an Amicon model CEC 1 on-line column eluate concentrator fitted with a PM 10 ultrafiltration membrane (Amicon Ltd). Fractions were assayed for lipase, protease and phospholipase, and fractions containing lipase activity were pooled.

The pooled fractions were applied to a DEAE Sepharose CL-6B column and eluted with a linear gradient of 0 to 1 M NaCl in phosphate buffer containing 0.02% sodium azide. The gradient was formed with a GM-1 gradient mixer (Pharmacia (Great Britain) Ltd.). Fractions were concentrated, monitored and collected as described above, and assayed for enzymic activity. The fractions containing lipase activity free from contaminating protease and phospholipase activities were pooled and concentrated further using the Chemlab C50 ultrafiltration cell fitted with a PM 30 ultrafiltration membrane.

Assay of enzyme activities

Lipase and protease activity were assayed using agar plate techniques as described previously (Griffiths *et al.*, 1981).

Phospholipase was also assayed using a plate technique. Blood agar base (Oxoid) was sterilized at 121°C for 15 min in an Agarmatic (New Brunswick Scientific Co. Inc.) and dispensed in 200 ml volumes into sterile bottles. When required, the blood agar base was melted and cooled to 45°C, and 10 ml of egg yolk emulsion (Oxoid) and 2 ml of a 1% solution of thiomersal were added to 200 ml of blood agar base. These were thoroughly mixed and poured on to a 30×30 cm glass plate equipped with a frame of 6 mm depth, or poured into Petri dishes.

Assays were performed by cutting wells in the agar, and adding 20 μl of the sample to be tested to the well. Phospholipase C activity can be detected as an opaque zone caused by the hydrolysis of phosphatidylcholine (Owens, 1974). The concentration of phospholipase present was directly proportional to the square of the true zone radius, r^2 (i.e. allowing for the diameter of the well).

This was verified using different concentrations of the commercial phospholipase C preparation from *Bacillus cereus*.

One unit of phospholipase activity was defined as the amount of enzyme producing an opaque zone with r^2 of 17.0 after 5 hr incubation at 30°C in egg yolk agar of 3.5 mm thickness.

One unit of lipase activity was defined as the amount of enzyme producing a clear zone with r^2 of 0.35 after 5 hr incubation at 30°C in tributyrin agar (Oxoid) of 3.5 mm thickness.

Estimation of extent of lipolysis in milk

The method of Deeth, Fitz-Gerald & Wood (1975) was used.

Heat treatment of enzyme samples

Enzyme samples were heated in capillary tubes (Griffiths *et al.*, 1981). The two treatments used were 77°C for 17 sec holding time (HTST treatment) and 140°C for 5 sec holding time (UHT treatment).

Preparation of milk substrate for enzyme tests

Fresh raw milk was obtained from the bulk tank of the Institute farm and heat-treated by immersion in a water bath at 74°C for 5 min to inactivate the native milk lipoprotein lipase (Shipe & Senyk, 1981). After cooling, sodium azide was added to a final concentration of 0.02% to prevent microbial growth. The milks used in these experiments had initial counts between 9.9×10^2 and 8.7×10^3 cfu/g (30°C for 3 days incubation).

In certain experiments where the effect of milk lipoprotein lipase was studied, no heat treatment was given to the milk, but sodium azide was added to the same concentration as above.

For experiments to investigate the effects of homogenization on lipolysis, the milk was homogenized at 55°C using a Manton–Gaulin pump homogenizer at 3000 psi. The milk was then heat-treated and azide added as described above.

Enzyme additions were made to aliquots of the milk in conical flasks and incubated at 30°C in a Hearson water-jacketed incubator (Astell Hearson). Samples were removed at intervals and assayed to determine the extent of lipolysis.

Source of enzymes

Papain was from Sigma Chemical Company. Phospholipase C from *B. cereus*, lipase from *Rhizopus arrhizus*, pronase from *Streptomyces griseus* and trypsin from porcine pancreas were obtained from Boehringer Corporation

Table 1. Stability of extracellular phospholipases produced by Gram-negative psychrotrophs

Isolate	Activity remaining after (%)	
	HTST	UHT
(A) *Pseudomonas* spp.		
(1) *Fluorescent*		
Ps. aureofaciens G6/9	61	57
Ps. fluorescens B3/4	38	31
Ps. paucimobilis K1/18	43	37
Ps. putida B4/10	26	31
(2) *Non-fluorescent*		
Ps. stutzeri P5/6	13	5
(B) *Alcaligenes* spp.		
Al. faecalis P3/9	29	22
Al. odorans P1/4	32	40
(C) *Aeromonas* spp.		
Aer. hydrophila 1058	0	0

(London) Ltd. The lipase from *Ps. fluorescens* was purified in the laboratory as preparations of this enzyme were not available commercially.

Results and discussion

Effect of heat treatment on phospholipase C activity on a number of organisms

The phospholipases produced by a number of Gram negative psychrotrophs were capable of surviving both HTST and UHT pasteurization (Table 1). The enzyme produced by the fluorescent pseudonomads was the most stable whereas the enzyme produced by *Ps. stutzeri* P5/6 and *Aeromonas hydrophila* 1058 were markedly less stable. The enzymic activity of the latter was completely destroyed by both HTST and UHT treatments. Similar differences in the heat stability of phospholipases have been reported previously (Fox & Marshall, 1975) but in this case the phospholipases were heated at 63°C for 30 min in the presence of 5% glycerol.

The data suggest that the extracellular phospholipases produced by psychrotrophs are similar to the proteases and lipases in their ability to survive pasteurization and sterilization procedures (Griffiths *et al.*, 1981). Evidence has been presented (Barach, Adams & Speck, 1976) which indicates that proteases are more stable in skim-milk than if dissolved in buffer. This is probably the case for other extracellular enzymes such as phospholipase C in which case phospholipase C would be more resistant to heat denaturation in milk than in cell-free supernatant liquids.

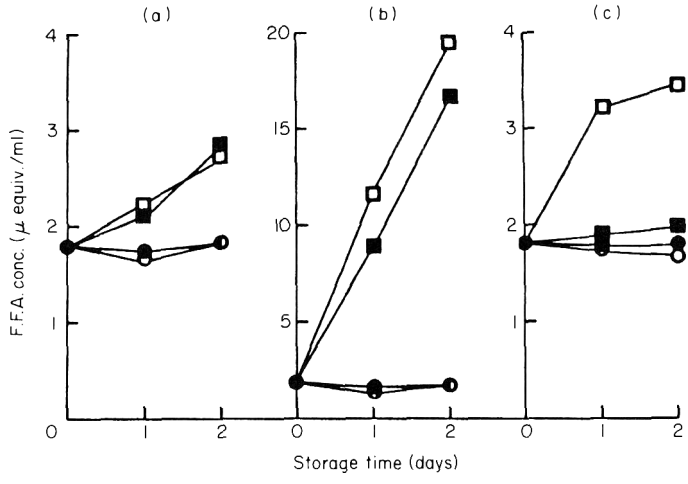

Figure 1. Effect of phospholipase C from *Bacillus cereus* on the lipolytic activity of lipase from *Pseudomonas fluorescens* (a), *Rhizopus arrhizus* (b) and milk (c) during storage at 30°C. For 1(a) and (b), free fatty acid (FFA) concentrations were determined in milk to which no enzyme additions had been made (●); in milk in the presence of phospholipase C (0.5 u/ml) alone (○); in milk containing lipase (0.5 u/ml) alone (■) and in milk containing both phospholipase C and lipase (□). For 1(c), free fatty acid concentrations were determined in heat-treated (74°C for 5 min) milk (●); in heat-treated milk containing phospholipase C (0.5 u/ml) (○); in raw milk which had not undergone heat treatment (■) and in non-heat-treated raw milk containing phospholipase C (□).

Enhancement of lipolysis in the presence of phospholipase C

The addition of phospholipase C from *B. cereus* to milk (0.5 u/ml) containing lipases from different sources had varying effects (Fig. 1). In all experiments, storage of heat treated milk containing azide at 30°C for up to 2 days had no effect on lipolysis. The free fatty acid concentration, on average, for the milks at the beginning of the storage period was 1.72 μequiv/ml and after 2 days at 30°C it was 1.68 μequiv/ml.

Phospholipase C added to milk containing lipase purified from a strain of *Ps. fluorescens* did not increase the extent of lipolysis above that seen with lipase alone. However, when phospholipase C was added to milk containing a commercially available lipase from *Rhizopus arrhizus,* there was a marked enhancement of lipolysis. This was similar to the findings of Chrisope & Marshall (1976) using model fat globules, and was due to an increased susceptibility of the substrate to hydrolysis by lipase.

Phospholipase C also greatly enhanced lipolysis by native milk lipoprotein lipase. The source of the lipase was milk which had not been subjected to heat treatment at 74°C for 5 min. The rate of lipolysis in the absence of added phospholipase was low, but addition of phospholipase to the unheated milk produced a dramatic increase in free fatty acid concentration after 24 hr storage at 30°C. Again this effect is similar to that found by Chrisope &

Marshall (1976) on adding phospholipase C from *Ps. fluorescens* to raw milk. In none of the experiments did phospholipase C added by itself have any effect on the extent of lipolysis.

The enhancement of lipolysis by phospholipase can be explained by its action on the phospholipid fraction of the milk fat globule membrane. The degradation of this membrane allowed easier access to the substrate for the lipase. What is not clear, however, is why the lipolytic activity of the lipase from *Ps. fluorescens* is not enhanced by the addition of the phospholipase. Presumably the difference must lie in the properties of the lipases, and not the phospholipase.

Effect of protease addition on the enhancement of lipolysis by phospholipase C

In addition to phospholipids, the milk fat globule membrane contains proteins. If these proteins are degraded as well as the phospholipids, there will be complete destruction of the fat globule membrane with a concomitant release of fat contained therein. Recent evidence (Phipps & Temple, 1982) supports the idea of a two layer membrane (Prentice, 1969; Mulder & Walstra, 1974) with one layer being composed of interface protein (glycoprotein) which is resistant to disruption by normal processing conditions. These proteins have different susceptibilities to proteases (Shimizu, Yamauchi & Kanno, 1979). The addition of trypsin produced a small change in the

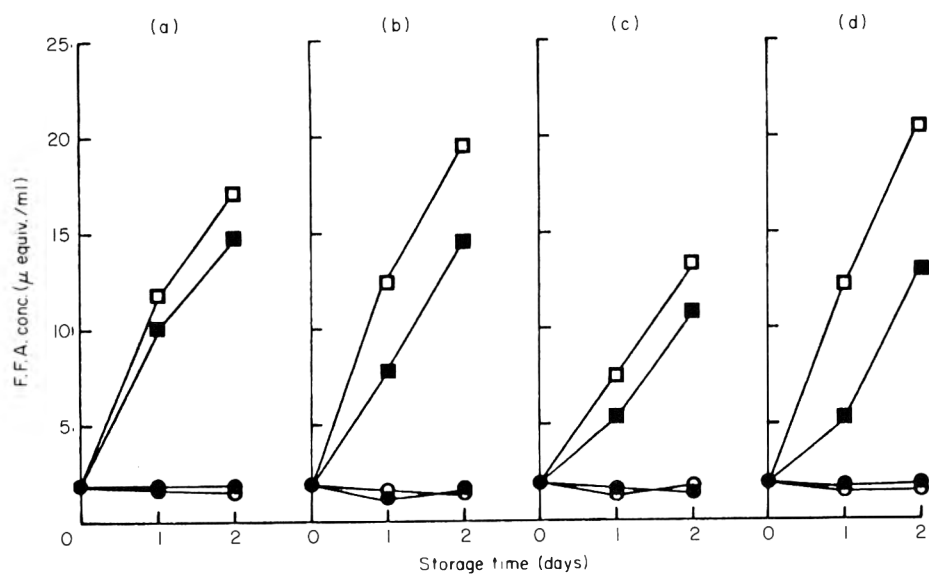

Figure 2. Effect of protease addition on the enhancement of lipase activity of *Rhizopus arrhizus* in the presence of phospholipase C. during storage at 30°C. Graphs show the effect of no enzyme addition (●); addition of phospholipase C (0.5 u/ml) (○); addition of lipase (0.5 u/ml) (■) and addition of both phospholipase C and lipase (□) on the free fatty acid (FFA) levels in milk containing no protease (A), pronase (0.5 u/ml) (B), trypsin (0.5 u/ml) (C) and papain (0.5 u/ml) (D).

emulsion stability of washed cream; however, a much larger decrease in stability was obtained on addition of papain (Shimizu, Yamauchi & Kanno, 1980). These results suggested that these glycoproteins are important for the stabilization of milk fat emulsion. Shimizu *et al.* (1980) also showed that treatment of washed cream with phospholipase C caused a dramatic decrease in emulsion stability.

A similar pattern emerged when proteases were added to milk containing lipase and phospholipase C (Fig. 2). When papain and pronase, which are capable of degrading glycoproteins in the milk fat globule membrane (Shimizu *et al.*, 1980) were added to milk the increase in lipolysis in the presence of phospholipase C was significantly greater than that obtained when no protease was present. However, addition of trypsin brought about no such increase in lipolysis. In fact lipolysis was decreased in the presence of trypsin when compared with the levels achieved in the control milks. Thus, even when the phospholipids of the milk fat globule membrane were broken down by phospholipase C, the membrane may retain some integrity due to the glycoproteins, but when these were removed by proteolysis more fat was released for degradation by lipases.

Effect of homogenization on enhancement of lipolysis by milk lipoprotein lipase in the presence of phospholipase C

It is well known that excessive mechanical agitation and turbulence of milk causes induced lipolysis (e.g. Downey, 1980). Similarly homogenization renders milk more susceptible to the action of lipoprotein lipase (Gould & Trout, 1936; Nilsson & Willart, 1960) and this was confirmed by the results shown in Fig. 3. Homogenization acts by increasing numbers of small fat globules which are not surrounded by the normal milk fat globule membrane, but which are covered by a film of protein which is more permeable to lipoprotein lipase (Mulder & Walstra, 1974). This greatly increased number of fat globules and their much greater surface area makes homogenized milk very susceptible to the action of lipase. As the fat globule membrane was disrupted by the homogenization procedure to be replaced by a protein film, phospholipase C can exert no effect on these small fat globules. Thus the enzyme cannot enhance lipolysis in homogenized milks (Fig. 3). The results shown in Fig. 3a confirmed the ability of phospholipase C to increase the susceptibility of milk fat to hydrolysis by native milk lipoprotein lipase (Fig. 1c).

Conclusions

Many of the psychrotrophs commonly found in milk and milk products are capable of producing phospholipase C (Fox *et al.*, 1976; Owens, 1978; Phillips *et al.*, 1981), and a number of these organisms synthesise a phospholipase C

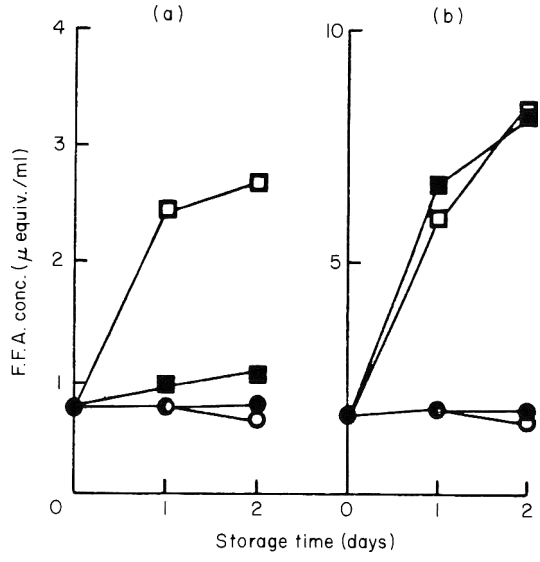

Figure 3. Effect of homogenization on lipolytic activity of native milk lipoprotein lipase in the presence of phospholipase C during storage at 30°C. Milk was treated at 74°C for 5 min to destroy natural milk lipase activity (●). Unheated milk (■) retained lipoprotein lipase activity and phospholipase C (0.5 u/ml) was added to both heat treated (○) and unheated milk (□). Raw milk (A) and milk homogenized at 3000 p.s.i. (B) were used as substrates.

molecule which can withstand HTST pasteurization and UHT sterilization procedures.

It has also been shown in this study that the phospholipase C from *B. cereus* can accentuate lipolysis in milk in the presence of certain lipolytic enzymes including milk lipoprotein lipase. This results as a consequence of the phospholipase increasing the susceptibility of the substrate to hydrolysis by lipase. The conditions of storage of the milk in these experiments (*viz* 30°C for up to 48 hr) is not representative of those encountered in the dairy industry, however, the enzyme concentrations used were low. There are no data available on the actual levels of phospholipase C activity encountered in milk. In one experiment carried out at the Institute (M. W. Griffiths, unpublished results), milk stored at 6°C for 2 days contained 1.1 u/ml of phospholipase C. The milk had a psychrotroph count of 4.6×10^7 cfu/ml and of the isolates obtained from this milk, only 10% were able to produce phospholipase C. During studies on raw milk from farm bulk tank and creamery silos, J. D. Phillips & M. W. Griffiths (unpublished results) have shown that on average 57% of isolates obtained from seven samples of raw milk were capable of synthesizing phospholipase C with the range being from 10 to 85%. Thus in the example shown, the level of phospholipase C in milk could be at the lower limit, and it is likely that the level of the enzyme in milk after storage for 2–3 days at 6°C could be over 10 u/ml (about twenty times the value used in these

experiments). The enzyme was 3.4 times more active at 30°C than at 6°C, so even at 6°C enough phospholipase activity could remain to degrade the milk fat globule membrane.

Similarly lipase activity in raw milk stored at 6°C for 2 days was estimated at 20 u/ml (M. W. Griffiths, unpublished results), with much of the activity being due to native milk lipoprotein lipase. Thus, during prolonged storage of milk at 6°C, there may be enhancement of lipolysis due to the action of phospholipase C on the milk fat globule membrane. The extent of this synergetic effect will vary with raw milk samples due to the difference in microbial flora.

The importance of the phospholipase C molecule being able to survive heat treatments such as HTST and UHT procedures is unclear. The native milk lipoprotein lipase activity will be affected by the heat treatment. Although the lipases from psychrotrophic organisms present in milk can survive these processes (Griffiths et al., 1981), it is unclear whether the action of these lipases is enhanced by phospholipase C as the presence of phospholipase C does not enhance lipolysis due to the lipase of a psychrotrophic pseudomonad (Fig. 1a). It is likely that the presence of organisms capable of synthesising phospholipase C in the raw milk supply can enhance lipolysis due to native milk lipoprotein lipase during prolonged storage at 6°C, but lipolysis caused by bacterial lipase may be unaffected.

Acknowledgments

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Thin-layer air drying of soybeans and white beans

D. HUTCHINSON and L. OTTEN

Summary

A thin-layer air drying apparatus was designed and constructed to determine the drying characteristics of various agricultural and food products. The equipment was designed to monitor the weight loss during drying and to operate over a practical range of air temperatures and humidities. This paper presents a description of the apparatus and an evaluation of its performance. The results of studies conducted to determine the thin-layer drying behaviour of soybeans and white beans are included.

Introduction

A wide variety of foods and animal feeds are dried in order to reduce spoilage during storage. Many products, particularly corn and other grains, are commonly dried artificially using forced heated air. Others, such as soybeans and white beans, are normally allowed to dry in the field to moisture contents that are low enough for safe storage. A number of benefits may be gained by artificially drying even those products that are traditionally field dried (Rodda, 1974). For example, losses due to weathering may be reduced or eliminated. In addition, shatter losses due to contact with the combine reel and cutter bar are reduced for many crops, including soybeans, when harvested at higher moisture contents. High energy costs tend to offset the potential economic advantage that may be gained by artificial drying. However, dryer efficiency may be increased by using simulation models in the development of improved dryer designs and in the operation of drying systems.

Morey *et al.* (1978) has summarized existing models that have been used in grain drying simulation. The more comprehensive of these simulation models

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include a thin-layer equation to predict local drying rates within a deep bed. In general, the parameters of the thin-layer equation depend on the material being dried and the drying conditions. Parameter values must be experimentally determined.

This paper describes an experimental thin-layer dryer that was designed to obtain meaningful and accurate data for use in the development of thin-layer drying models. The performance of the dryer is evaluated. In addition, the results of studies to determine the thin-layer drying behaviour of soybeans and white beans are presented.

Literature review

Review of existing drying theories have been presented by Fortes & Okos (1980), Bruin & Luyben (1980) and van Brakel (1980). The following mechanisms have been proposed to account for moisture movement in capillary-porous solids (Van Brakel 1980; Brooker, Bakker-Arkema & Hall, 1974): (1) liquid diffusion due to concentration gradient; (2) vapour diffusion due to partial vapour pressure gradients; (3) liquid movement due to capillary forces; (4) liquid or vapour flow due to differences in total pressure; and (5) Knudsen flow.

Liquid and/or vapour diffusion have been assumed to be the primary mass transfer mechanisms in drying studies on cereal grains and similar products including soybeans. The most widely investigated theoretical model in the thin-layer drying literature is the diffusion model, given by Fick's second law.

$$\frac{\partial M}{\partial t} = \nabla \cdot (D \nabla M) \tag{1}$$

The assumptions that are inherent in the diffusion model are as follows: (1) the primary mechanism for moisture transport is diffusion; (2) the principal driving force for mass transport is the internal moisture content gradient; (3) temperature within the kernel is assumed constant; (4) either vapour or liquid diffusion predominates. If both occur simultaneously, the diffusion coefficient must represent the combined effects of liquid and vapour diffusion.

Solutions to the diffusion equation, developed for several different geometries after making a number of simplifying assumptions about material properties, initial conditions and boundary conditions, have been applied in a number of thin-layer drying studies. Whitaker & Young (1972), Henderson (1974) and Wang & Singh (1978) used this approach in their studies on the drying behaviour of peanuts, corn and rough rice respectively. Examples of solutions to the diffusion equation may be found in Crank (1974).

Sharaf-Eldeen *et al.* (1979) applied the general solution to the diffusion equation which is given by the infinite series

$$MR = \frac{\bar{M} - M_e}{M_0 - M_e} = A_0 \exp(-k_0 t) + A_1 \exp(-k_1 t) + \ldots \tag{2}$$

in their thin-layer study of shelled corn. This solution applies regardless of a particle's geometry or boundary conditions. A constant diffusivity is assumed.

While excellent fits of experimental data have been obtained using the diffusion equation, it is unlikely that the assumptions made in adopting the diffusion equation are valid. Most researchers agree that the diffusivity is probably moisture dependent (Sharaf-Eldeen *et al.*, 1979; Singh, Barre & Hamdy, 1972). Similarly the assumption of homogeneity that is normally made does not provide an accurate description of most biological materials. For example, Mensah *et al.* (1979) found that the resistance of soybean seedcoats to moisture transfer was approximately six times that of the cotyledons. The fact that the solutions to the diffusion equation decay exponentially with time in a manner similar to experimental drying curves may account for the good fits obtained with experimental data.

An alternative approach to the analysis of thin-layer drying data has been to use purely empirical relationships. Very good fits of experimental data have been reported by researchers using empirical relationship (Morey *et al.*, 1978; Brooker *et al.*, 1974). One equation that has been widely used in thin-layer studies of a variety of materials (Wang & Singh, 1978; White, Ross & Westerman, 1973; Misra & Brooker, 1979; Agrawal & Singh, 1977) is Page's equation:

$$MR=\exp(-Kt^{N}) \tag{3}$$

White *et al.* (1973) and Overhults *et al.* (1973) used Page's equation to describe the thin-layer drying behaviour of soybeans.

Wang & Singh (1978) used quadratic equations in the analysis of shelled corn and rough-rice, respectively. Roa *et al.* (1977) used the following empirical expression to predict the drying behaviour of soybeans:

$$MR=\exp(-m(P_{vs}-P_{v})^{n}t^{q}). \tag{4}$$

The drying conditions which are accepted as influencing drying rates include temperature, humidity and airflow rate. Therefore, the parameters used in both empirical and theoretical thin-layer equations are normally expressed as functions of one or more of the drying variables mentioned above.

The effect of temperature on drying rate is well documented in the literature (Henderson & Pabis, 1961). Most of the material properties that are relevant to drying, for example, mass diffusivity, thermal conductivity and latent heat of evaporation, are temperature dependent (Singh *et al.*, 1972).

Airflow rate influences convective mass transport (Hamdy & Barre, 1969). A number of researchers have chosen to neglect the effect of airflow rates in the analysis of thin-layer drying data, citing the conclusion of Henderson & Pabis (1962) that resistance to moisture movement at the surface is negligible compared to internal resistance.

The humidity of the drying air has a significant impact on the final or equilibrium moisture content that is achieved as a result of drying. In addition,

the driving force for convective transport at the surface of the drying material depends on the difference between the partial pressure of water vapour at the surface and that of the drying air (Fortes & Okos, 1981).

Traditionally, thin-layer drying data have been obtained by forcing conditioned air through a single layer sample and monitoring the loss of mass during drying. Drying conditions are normally held constant during each experiment in order to facilitate the development of functions relating the value of the thin-layer drying parameters to the drying variables. Experimental thin-layer dryers have been described by a number of authors (Misra & Brooker, 1979; Agrawal & Singh, 1977; Fortes & Okos, 1981). Adequate performances have been reported for experimental dryers employing a variety of weighing and air-conditioning systems. However, most dryer designs reported in the literature have not included a fully acceptable solution to the problem of how to obtain accurate values of airflow through the sample without interfering with the accuracy of measurements of the sample mass. A physical seal between the sample tray and the side walls of the drying chamber ensures that all the air flows through the sample but interferes with weighing. Conversely, if a gap is left between the sample tray and the side walls, the airflow through the sample may be reduced significantly.

Materials and methods

A schematic diagram of the experimental thin-layer dryer that was developed for use in this study is illustrated in Fig. 1. The dryer was designed with the following two broad objectives: (1) measurement and control of the drying environment over a range of conditions expected in a deep bed bean dryer; (2) a procedure for determining moisture loss from the sample having negligible effect on the drying process.

Measurement and control of the drying environment

A portable air compressor was used as an air supply for the dryer. Two pressure regulators connected in series were required to maintain constant flow conditions because of the inlet pressure fluctuations resulting from compressor cycling. Airflow rate was monitored using a rotameter.

When required, humidification was achieved by passing the air through a packed bed of wetted gravel. Water was circulated from the reservoir and sprayed through a nozzle over the gravel surface. The amount of moisture added to the air was controlled by setting the temperature of the air entering the humidification bed and the temperature of the water being sprayed over the bed.

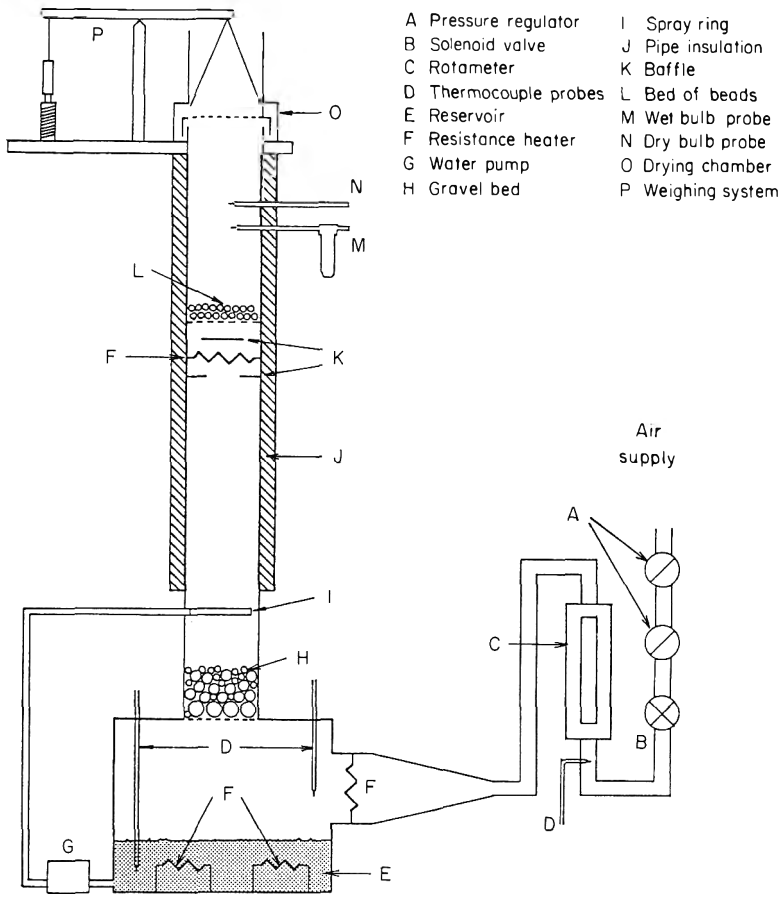

Figure 1. The thin-layer drying apparatus.

An electrical resistance heater connected to a variable voltage source was used to control the temperature of the pre-heated air. Water temperature was thermostatically controlled.

The final dry bulb temperature was controlled using a second resistance heater connected to a variable voltage supply. Baffles before and after the final heating element were provided to ensure thorough mixing of the heated air. Insulation was included to reduce heat loss through the walls of the supply pipe and prevent the development of a significant temperature profile in the air entering the drying chamber.

The bed of glass beads located immediately following the final heater served as a flow straightener. It was desirable to obtain a uniform velocity and temperature profile as the air enters the drying chamber. Velocity profile determinations during development of the equipment showed that the deviations from the means were less than 10%, while the temperatures were within 1°C of the means.

All temperatures were measured using copper-constantan thermocouples with soldered junctions. The locations of various temperature probes are shown in Fig. 1. Wet and dry bulb temperature measurements were used to determine humidity.

Drying chamber and sample tray

A sectional view of the drying chamber and the 19.7 cm diameter sample tray is provided in Fig. 2. A liquid seal of mineral oil was used to prevent leakage of air around the sample. The disruption of air flow through the sample could be minimized by aligning the inlet and outlet pipes with the sample tray. An exit pipe was placed after the experimental chamber in order to reduce exit effects on the flow profile. The chamber was constructed of clear acrylic to allow for visual inspection of the chamber which was required to ensure that the sample tray was properly aligned and that there was no contact between the sample tray and chamber's walls. The drying chamber and sample tray, complete with a thin-layer sample are shown in Fig. 3.

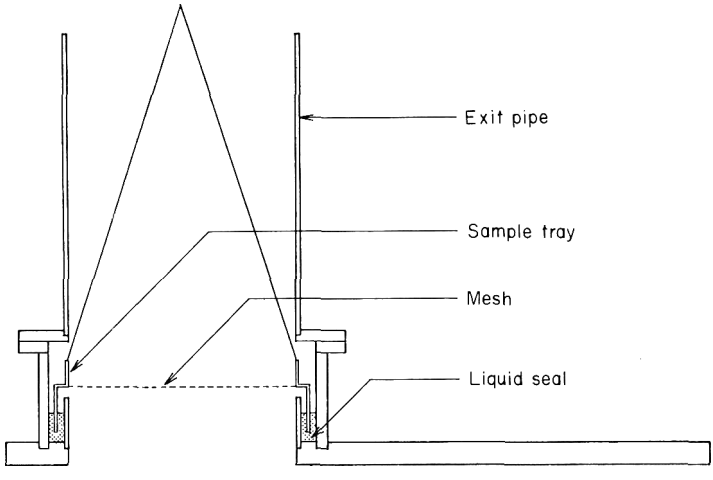

Figure 2. Sectional view of the drying chamber and sample tray.

Weighing system

The system used to weigh the sample during drying may be seen in Fig. 4. An equal arm balance with knife edge and agate bearing pivots was employed. The balancing force was provided by the interaction between a permanent magnet suspended from one end of the balance arm and the magnetic field generated by passing a current through a copper-wire coil. Because the strength of the generated magnetic field depended on the coil current, it was possible to determine the relationship giving the sample mass as a function of coil current.

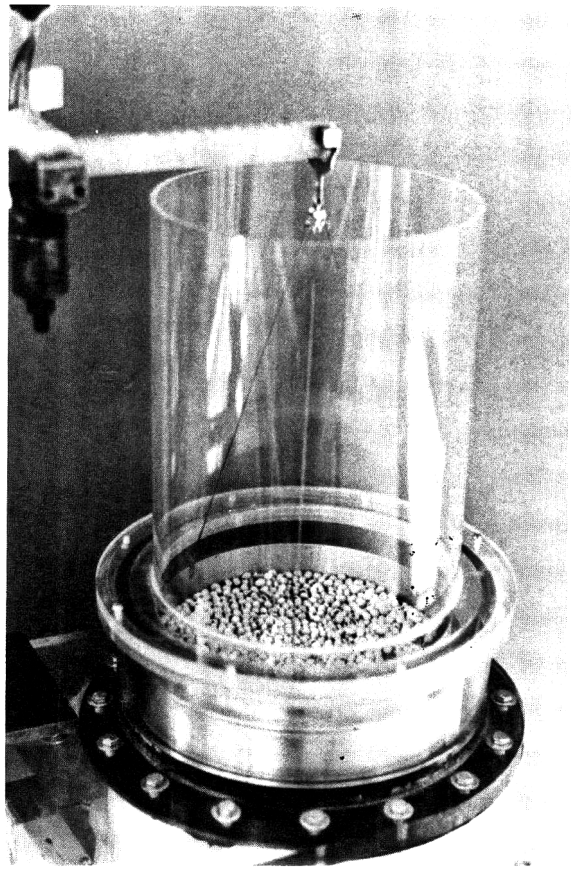

Figure 3. Drying chamber with a thin-layer sample.

The coil current was determined by measuring the voltage drop across a standard one-ohm resistor (Rubicon Co.) connected in series with the coil. A HP3476A digital multimeter was used to measure voltage. Readings were recorded manually.

Procedure

A sample of test material was removed from storage and allowed to thaw overnight in a sealed plastic bag. After the drying equipment had reached the desired steady-state, an experimental run was started.

A sample of 150.0 g was weighed out with an electronic laboratory balance. The sample was spread in a thin-layer over the sample tray and an initial measurement of mass was then taken using the coil balance. The air flow, which was shut off to spread the sample and take the initial mass measurement, was resumed. The sample was weighed every 10 min for the first 100 min. It was then weighed at 120 min and subsequently at 30 min

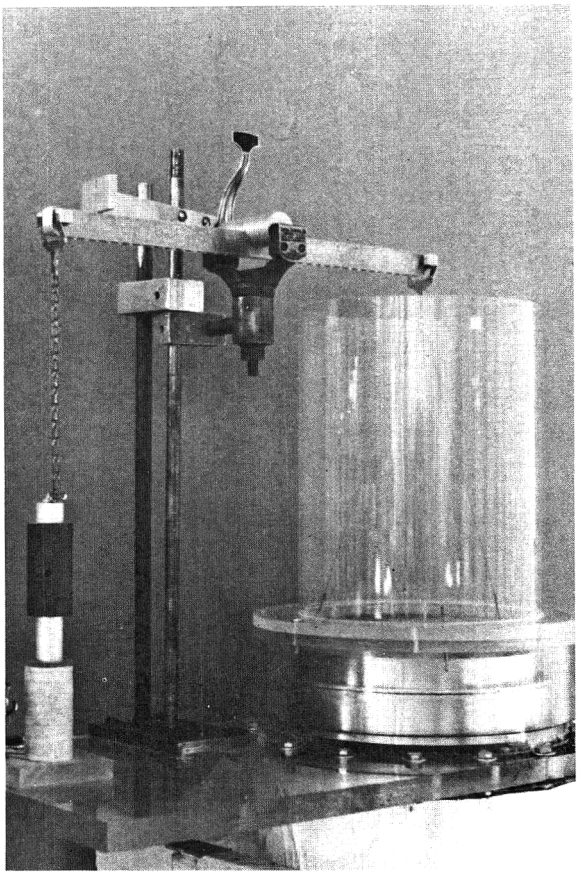

Figure 4. Experimental balance.

intervals until the experiment was completed. Samples were dried for at least 5 hr.

Upon completion of a drying test the sample was removed immediately and weighed using a laboratory balance in order to check the accuracy of the experimental balance.

Evaluation of dryer performance

The dryer was able to deliver air over a limited, but practical range of drying condition. Because no dehumidification system had been included, low temperature and low humidity environments that were achievable were limited by ambient conditions. Good control of airflow rate could be maintained over a wide range of flow rates. The manual control of drying temperature and humidity was found to be adequate. Once equilibrium had been achieved, very little control action was required over the course of a 5-hr experiment provided that there were no major changes in ambient conditions.

A sufficiently uniform temperature profile was observed at the drying chamber entrance. At the highest drying temperature, 58°C, the temperature 0.6 cm from the wall was within 1°C of the midpoint temperature. A wall temperature of 56°C was measured.

The dry and wet bulb temperatures could be maintained to within ±0.6°C of the desired values. For the range of conditions considered the error in wet and dry bulb measurements may be translated into a maximum error of about ±5.0% in the value determined for relative humidity.

The performance of the experimental balance was satisfactory. Measurements taken using the experimental balance normally differed by 0.1 g or less from results obtained using the laboratory balance. An error of 0.1 g in the measurement of sample mass translates into an error of less than 0.1% in the dry basis moisture content determined for the samples in this study.

Mathematical procedure

A two-term approximation of the general series solution to the diffusion equation (eqn 2) and Page's equation (eqn 3) were examined as potential thin-layer drying models for soybeans and white beans. Data for soybeans and white beans were analysed separately. Equilibrium moisture content for soybeans was calculated using Henderson's equilibrium moisture content equation with parameter values developed for soybeans by Alam & Shove (1972). Equilibrium moisture contents for white beans were calculated using Henderson's equilibrium moisture content model with parameter values which were determined from experiments performed as part of the overall project.

$$M_e=\left[-\frac{\ln\,(1-rh)}{F(T+G)}\right]^{1/E}, \tag{5}$$

where $G=190.62+10.632T$, $F=0.08855-0.002414T+0.0000224T^2$, and $E=1.8033-0.00728T$.

Regression analyses were performed on data using the statistical analysis system (SAS) (Helwig & Council, 1979). The procedure GLM (general linear modelling system) was used where an equation could be expressed in a linear form. Where this was not possible, the SAS procedure NLIN was used to perform a non-linear least-squares regression.

The method for determining an appropriate regression model based on Page's equation is outlined in detail below. The analysis, based on the two-term exponential model was identical with several exceptions that will be noted after the discussion that follows.

Page's equation was expressed in linear form (eqn 6) and a linear regression was performed to determine values of the parameters K and N for each drying test.

$$\ln\,[-\ln\,MR]=\ln\,K+N\ln\,t. \tag{6}$$

The SAS procedure RSQUARE was employed in order to select potential linear expressions for use in describing the dependence of the drying parameters K and N on the drying conditions. The RSQUARE procedure performs linear regressions on all possible combinations of the specified independent variables. The results give all combinations sorted by number of variables in the model and goodness of fit. The independent variables considered in this analysis were T, T^2, T^{-1}, rh, rh^2, rh^{-1}, log rh, $T \cdot rh^{-1}$, $T^{-1} \cdot rh$, v and v^2.

The most promising models for the parameters K and N were selected on the basis of goodness of fit as indicated by the coefficient of determination. The linear functions selected for K and N were then substituted into the original equation and a non-linear least-squares regression was performed over the entire set of drying data to determine values for the required regression coefficients. A number of different equations were developed based on different linear models of K and N.

The two-term exponential model could not be expressed in linear form. As a result, the initial regression performed was nonlinear. Values of the parameters A, B and D given in eqn (7) were obtained for each drying test.

$$MR = A \exp(-DT) + (1-A) \exp(-BDT). \tag{7}$$

The mean values of A and B were computed and substituted into the original eqn (7) as constants. The RSQUARE procedure was then invoked to determine a series of suitable linear models for the parameter D.

Results and discussion

Recommended models

Figures 5 and 6 show the results of two typical drying tests conducted for soybeans and white beans respectively. Fitted drying curves based on Page's equation (solid line) and on the two-term exponential model (dashed line) are also included. The results shown in Figs 5 and 6 are typical of the results obtained over the entire range of drying conditions examined. Both equations were found to provide excellent fits of the experimental data for both soybeans and white beans.

Very little improvement, measured in terms of goodness of fit, was achieved by increasing the complexity of the expressions used for the drying parameters K, N and D. Consequently, the more complex models were eliminated. Page's equation was chosen over the two-term exponential model on the basis of its computational efficiency. Therefore, the recommended thin-layer equation for white beans and soybeans is

$$MR = \exp(-Kt^N)$$

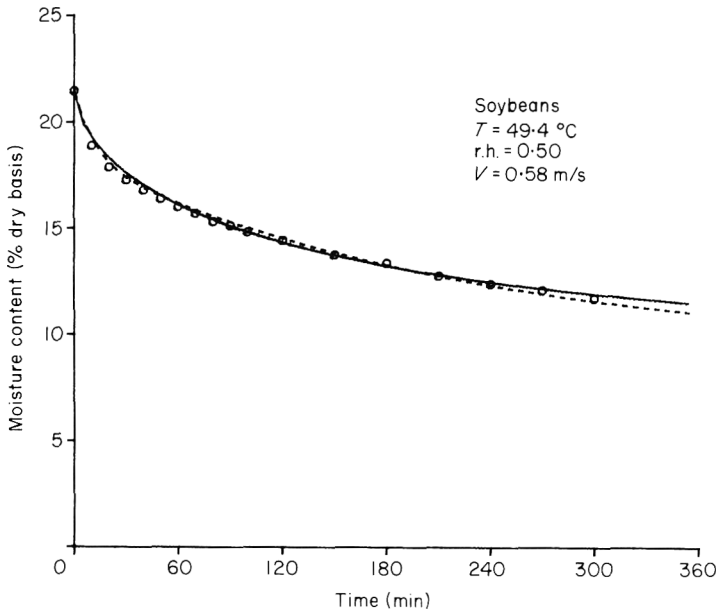

Figure 5. Observed results and fitted curves for a typical soybean drying test. —— Page's Equation; – – – two-term exponential.

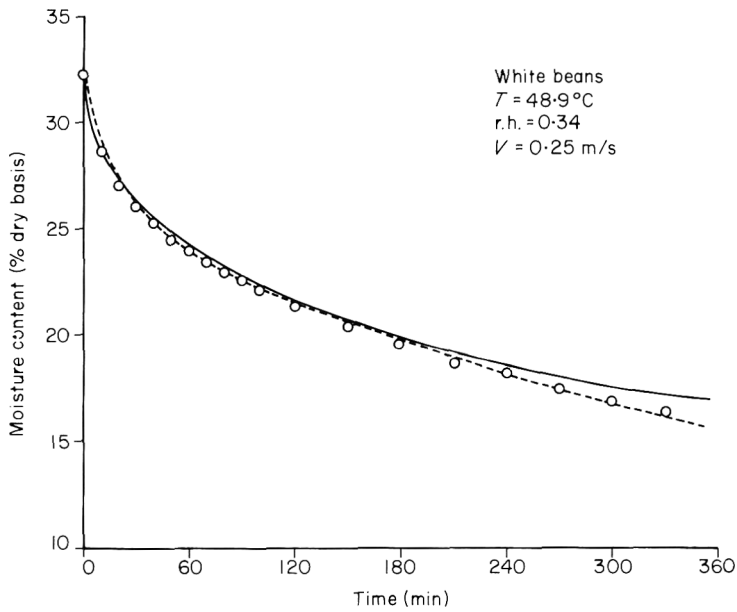

Figure 6. Observed results and fitted curves for a typical white bean drying test. —— Page's Equation; – – – two-term exponential.

The drying parameters for white beans are given by

$$K=0.0466-0.0104\times rh^2 \qquad (8)$$
$$N=0.4002+0.00728\times T\times rh.$$

The drying parameters for soybeans are given by

$$K=0.0333+0.0003\times T$$
$$N=0.3744+0.00916\times T\times rh$$

It should be noted that the above thin-layer drying equations for soybeans and white beans were generated from data obtained over a limited range of drying conditions. Specifically, temperature was varied from 32 to 49°C while relative humidity ranged from 34 to 65%.

Effect of drying conditions on thin-layer drying rates

As was expected, thin-layer drying rates increased with increasing temperature and with decreasing relative humidity. The effect of temperature, as illustrated in Fig. 7, was most dramatic. Changes in relative humidity had only a small impact on drying rate. Changes in air flow had no observable effect on the drying rate.

Figure 8 illustrates the apparent independence of drying rate and air velocity. Results of three drying tests conducted on soybeans are plotted. Humidity and temperature were maintained constant while airflow rates were varied. As can be seen, more than doubling the nominal air velocity had no appreciable effect on drying rate.

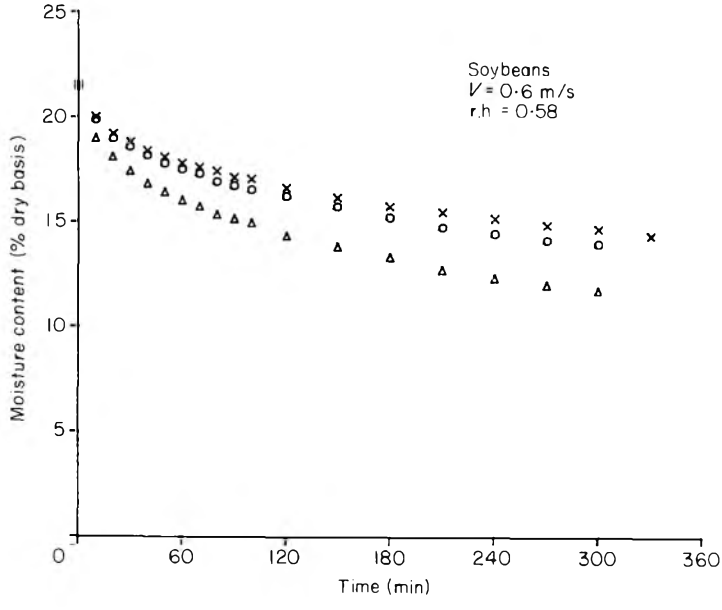

Figure 7. Effect of air temperature on drying rate of soybeans. ×: T=32.8°C; ○: T=38.3°C; △: T=49.4°C.

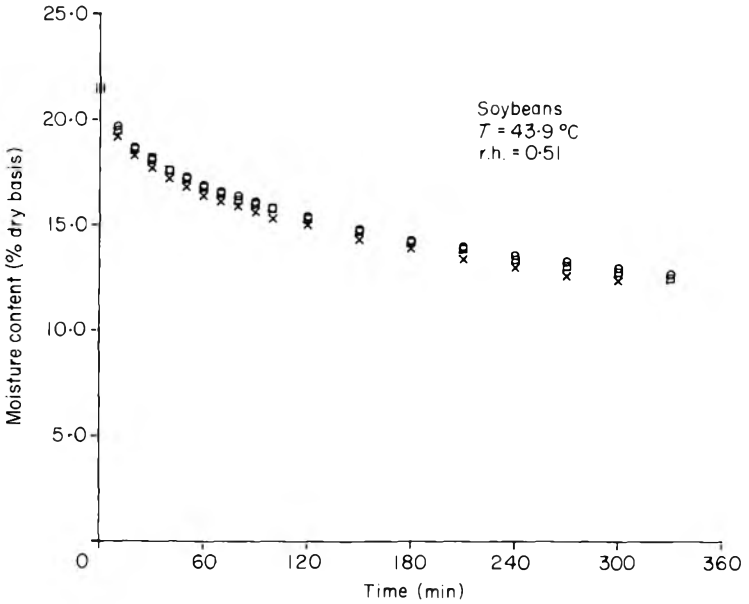

Figure 8. Effect of airflow rate on drying rate of soybeans. ×: v=0.58 m/sec; ○: v=0.36 m/sec; □: v=0.25 m/sec.

No reductions in drying rates were observed in white beans for nominal velocities as low as 0.14 m/sec. While no attempt was made to determine the point at which flow rate becomes significant, the results are in agreement with the cut-off figure of 0.102 m/sec suggested by Henderson & Pabis (1962).

Comparison of the drying characteristics of soybeans and white beans

The drying behaviours of soybeans and white beans were found to be similar. The expressions developed for both parameters, K and N, for soybeans (eqn 9) closely resemble those developed for white beans (eqn 8). A comparison of the drying behaviour of the two products is presented in Fig. 9. Predicted drying curves for soybeans and white beans, expressed as the moisture ratio plotted against time, are given. The similarity in drying behaviour illustrated in Fig. 9 was found to be typical for the range of drying conditions examined in this study.

The results summarized above suggest that the thin-layer equation developed for soybeans can be used to approximate the thin-layer drying behaviour of white beans.

Conclusions

The thin-layer drying apparatus developed in this study was found to be capable of producing accurate and meaningful thin-layer drying data. The

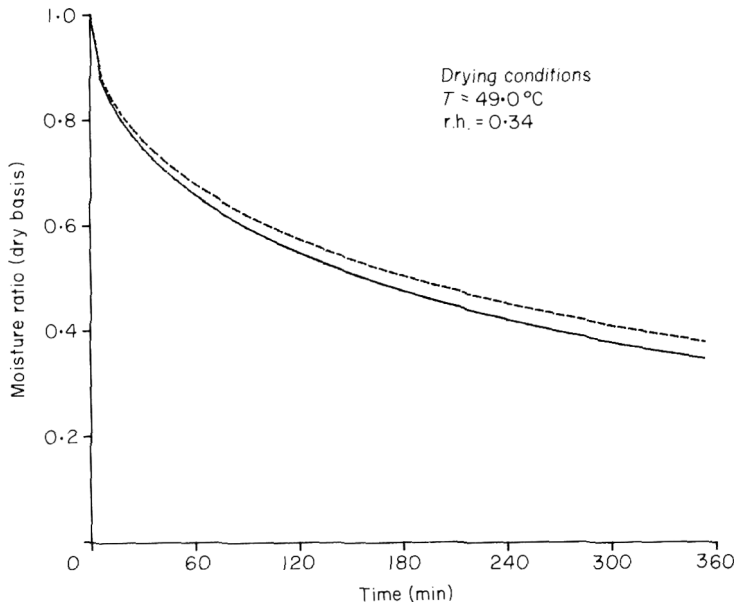

Figure 9. Comparison of predicted drying rates of soybeans and white beans. —— Soybeans; – – – white beans.

weighing system was able to provide measurements which could be used to determine sample moisture content to within 0.1 percentage point. In general, the performance of the air conditioning system was adequate in that the equipment was capable of maintaining a constant drying environment. The operating range of the dryer could be increased by including a dehumidification system.

Empirical thin-layer drying equations based on Page's equation were developed to describe thin-layer drying of soybeans and white beans. Excellent fits of experimental data were obtained where the drying parameters K and N were expressed as functions of temperature and relative humidity. Drying rates appeared not to depend on air velocity for the range of velocities considered in this study.

Acknowledgments

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Appendix

A Parameter in two term exponential model
A_0, A_1 Parameters in series solution of diffusion equation

B Parameter in two-term exponential model
D Diffusion coefficient (m^2/min) or parameter in two-term exponential model (min^{-1})
E Parameter, Henderson's EMC equation
F Parameter, Henderson's EMC equation
G Parameter, Henderson's EMC equation (°C)
K Appropriately dimensioned parameter in Page's equation
k_0, k_1 Parameters in series solution to diffusion equation (min^{-1})
m Appropriately dimensioned constant in Roa thin-layer equation
M Moisture content (dry basis) (decimal)
$\bar{M}$ Average dry basis moisture content (decimal)
M_0 Initial dry basis moisture (decimal)
M_e Equilibrium moisture content (dry basis) (decimal)
MR $(\bar{M}-M_e)/(M_0-M_e)$=moisture ratio
n Constant in Roa thin-layer equation
N Parameter in Page's equation
P_v Water vapour pressure (N/m^2)
P_{vs} Saturated vapour pressure (N/m^2)
q Constant in Roa thin-layer equation
rh Relative humidity (decimal)
t Time (min)
T Temperature (°C)

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Evaluation of a process for obtaining partially concentrated distilled lime oil

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Summary

In this paper we discuss the evaluation of a new process for obtaining partially concentrated oil from distilled lime oil. A qualitative and quantitative analysis was carried out with the aid of gas–liquid chromatography and the physical, chemical and sensory characteristics were also determined in the processed oil. The results indicated that the partially concentrated oil has a good quality and greater stability compared with the natural oil.

Introduction

The distilled essential oil from limes (*Citrus aurantifolia* Swingle) is broadly used as flavouring for food products and drinks. This essential oil is a complex mixture of chemical compounds that can be classified in a general way in two groups: the terpene hydrocarbons, which represent the larger quantity in the oil, and the oxygenated compounds including alcohols, aldehydes and esters, some of which impart the aroma and flavour qualities (Tápanes, 1975; Shaw, 1979).

The terpene hydrocarbons are unsaturated and can be oxidized readily in air with the development of unpleasant odours and flavours. These changes constitute a great problem in the citrus processing industry and numerous attempts have been made to reduce these oxidative changes (Littlejohn, 1940; Lasztity, Nedelkovits & Bogyo, 1962; Waginaire, 1968; Pino, 1976).

However, efforts in this direction have failed because the proposed treatments have always resulted in the removal of some important lime flavouring compounds. For example, vacuum fractional distillation is not very effective because at the low pressures required the boiling points of the terpene hydrocarbons and some oxygenated compounds are so close together

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that separations are difficult. It had been reported that *p*-cymene produced from γ-terpinene is one of the main degradation reactions which develop unpleasant odours and flavours (Ikeda, 1966) and a process has been patented to remove the γ-terpinene from natural lemon oil by vacuum fractional distillation under suitable conditions in the presence of water (Platt, 1967).

The object of this paper is to evaluate a new process on a laboratory scale for obtaining a partially concentrated distilled lime oil with the natural aroma and flavour in which the γ-terpinene and *p*-cymene have been removed and which does not develop any objectionable odour or flavour over a period of storage.

Materials and methods

Material

Distilled lime oil produced in Banes, Cuba was used in this investigation.

Experimental procedure

Figure 1 is a diagrammatic flow sheet of the patented process (Pino & Tápanes, 1980). In this process, natural distilled lime oil (1 kg) is separated into the oxygenated compounds from the terpene hydrocarbons and also some volatile compounds by vacuum fractional distillation (5–15 mm Hg) in the presence of 8 kg water (Distillation I). A second vacuum fractional distillation at 5–15 mmHg (Distillation II) of the terpene hydrocarbon fraction is achieved to separate the γ-terpinene and *p*-cymene. In this second distillation, which is also carried out in the presence of water (twice the quantity of oil), the γ-terpinene and *p*-cymene with fewer terpene hydrocarbons distil over leaving a residue which consists essentially of oxygenated compounds and terpene hydrocarbons with lesser quantities of γ-terpinene and *p*-cymene. The first distillation which is continued until the oxygenated compounds begin to decrease, and the second distillation are monitored by gas–liquid chromatography. Finally, the first distillation residue is mixed with the second distillation residue, producing 0.5 kg of a flavour-stable, partially concentrated distilled lime oil. The complete process requires 3 hr when carried out on a laboratory scale.

The vacuum fractional distillation equipment used consisted of a flask heated with an electric mantle and equipped with a fractionating column packed with Raschig rings. A still head was provided with means for controlling the reflux:distillate ratio to 1:2 (v/v), and a cold finger condenser were attached to the equipment.

The samples of oil produced with the process described and a sample of natural distilled lime oil were compared periodically during 2 years' storage at 10°C by sensory test and gas–liquid chromatography.

Physical and chemical characteristics

Relative density, specific rotation, refractive index, aldehydes and ester contents and colour were determined in the essential oils (Anon, 1967).

Liquid adsorption column chromatography

The oil samples were separated by this technique to determine the weight ratios of oxygenated compounds. The column was packed with silica gel 60 (60/230 mesh, Merck) in a weight ratio oil : adsorbent of 1 : 10. The oils were eluted with *n*-hexane to separate the hydrocarbons from oxygenated compounds which were eluted with diethyl ether. Solvents were removed from each fraction at room temperature under vacuum in a rotary evaporator and were then weighed.

Gas–liquid chromatography

Analyses were performed using a Packard–Becker 419 gas chromatograph with a flame ionization detector. Separation was on 2 m×0.4 cm O.D. stainless steel columns with a 7% polyethyleneglycol adipate stationary phase on Porolita 60/80 mesh or a SE-30 stationary phase on Supasorb 60/80 mesh. Nitrogen at a flow rate of 25 ml/min was the carrier gas and the oven temperature was 80–210°C at 4°C/min, then isothermic at 210°C for 10 min. The detector and injector temperatures were 250°C, respectively.

Major peaks were identified by comparing retention data of pure substances on polar and non-polar columns and by enrichment technique. Quantitative peak area measurements were made with an Autolab 6300 digital integrator

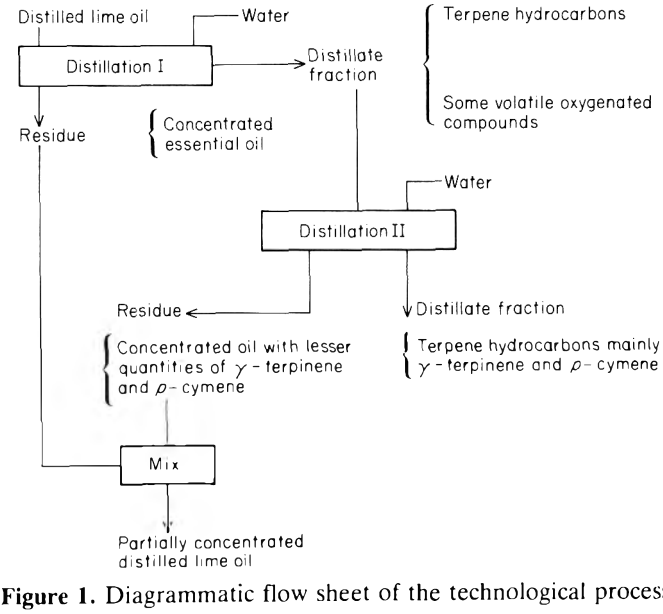

Figure 1. Diagrammatic flow sheet of the technological process.

and the response factors of the main compounds were calculated to determine the percentage concentration of each compound (Keulemans, 1959).

Sensory test

The essential oils were evaluated twice by five well-trained members of a sensory test panel using a duo-trio difference analysis for odour. The panellists were asked to identify the odd sample and then were asked to choose the most acceptable sample (Larmond, 1970). The essential oils were also evaluated by a highly trained expert with 10 years' experience in citrus aroma using a three-scale preference scale for odour.

Results and discussion

Table 1 lists the physical, chemical and sensory characteristics of the natural distilled lime oil and the partially concentrated oil obtained by the new process described. The physical and chemical characteristics of the processed oil are typical of a concentrated distilled lime oil. The sensory test indicated that the process had not caused adverse chemical changes in the constituents of the concentrated oil.

The results of the gas–liquid chromatography analysis of the natural oil and the processed oil are summarized in Table 2 in order of their retention times on polyethyleneglycol adipate stationary phase. The analysis of the data reflects the difference among the oils of the main compounds, particularly the oxygenated compounds which are concentrated three times in the processed oil due to distillations. It is seen that the new process produces a partially concentrated oil with a low quantity of γ-terpinene and *p*-cymene.

The stability of the partially concentrated oil compared with the natural oil was determined in storage for 2 years at 10°C. The results of this study are shown in Fig. 2, where the greater stability of the partially concentrated oil

Table 1. Physical, chemical and sensory characteristics of the natural distilled lime oil and partially concentrated oil

	Natural distilled lime oil	Partially concentrated oil
Relative density (d_{20}^{20})	0.8660	0.8962
Specific rotation (α_{20}^{20})	+40.20	+8.40
Refractive index (n_D^{20})	1.4750	1.4838
Aldehydes content (g %)	0.65	1.85
Ester content (g %)	0.98	2.90
Oxygenated compound (g %)	20.0	60.0
Colour	Light yellow	Light yellow
Sensory test	Excellent	Excellent

Table 2. Analysis by gas–liquid chromatography of the natural distilled lime oil and partially concentrated oil

Peak No.	Compound	Relative concentration (%)	
		Natural lime oil	Partially concentrated oil
1	—	*T**	—
2	—	*T*	—
3	—	*T*	—
4	—	*T*	—
5	—	*T*	—
6	—	*T*	—
7	—	*T*	—
8	α-pinene	2.0	*T*
9	—	*T*	—
10	Camphene	1.0	—
11 + 12	β-pinene + sabinene	3.6	1.0
13	Myrcene	2.0	*T*
14 + 15	α-terpinene + limonene	32.0	24.2
16	1,8-cineol	1.4	3.2
17	γ-terpinene	11.3	2.8
18	Terpinolene,	9.2	6.0
	p-cymene†	6.8	2.0
19 + 20	—	*T*	—
21	α-*p*-dimethylstyrene	2.0	1.5
22–23	—	*T*	*T*
24	*n*-octanol	*T*	*T*
25 + 26	Decanal + linalool	1.0	1.3
27	Terpinene-l-ol, fenchol	2.9	3.8
28	α-bergamotene	2.0	*T*
29 + 30	Terpinene-4-ol + β-terpineol	3.7	10.0
31	—	*T*	*T*
32 + 33	Borneol + α-terpineol	12.8	21.1
34	Neral, dodecanal	2.3	11.0
35 + 36	Neryl acetate + geranial, geranyl acetate	3.8	12.0

* *T* means less than 1% of the total composition.
† Separated on SE-30 stationary phase column.

can be observed. At the end of the storage, the processed oil has not more than 4% of *p*-cymene against a 16% in natural oil.

The sensory difference test at this time indicated that the oils have different odours (nine correct judgments from ten judgments, which are significant at the 0.1% level). The sensorial preference test indicated that the partially concentrated oil has an excellent aroma, while the natural oil has an unpleasant odour (nine correct judgments from nine judgments, which are significant at the 0.1% level).

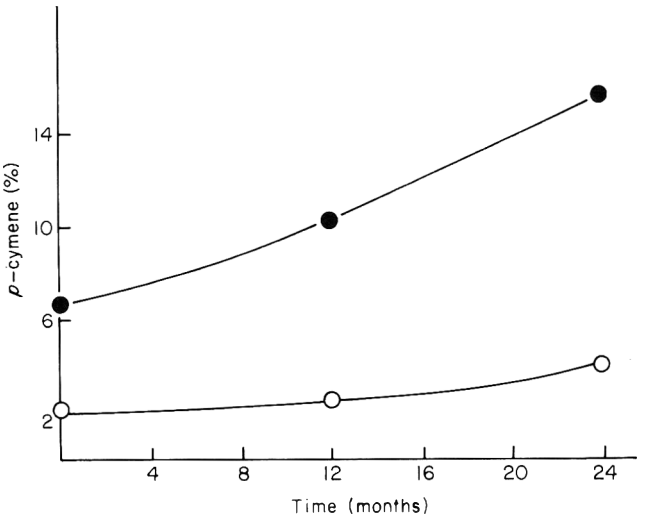

Figure 2. Changes in *p*-cymene content during the storage at 10°C of natural lime oil and partially concentrated oil. •, Natural distilled lime oil; ○, partially concentrated oil.

Conclusions

Summarizing our results we may say that with the new process based on vacuum fractional distillation in the presence of water it is possible to obtain a partially concentrated distilled lime oil with the natural aroma and flavour, in which the concentrations of γ-terpinene and *p*-cymene have been reduced and which does not develop any objectionable odour and flavour over a period of 2 years at 10°C. Experiments at a pilot-scale level are in progress.

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Technical note: Determination of ammonium ion concentration in collagenous films by a modified Kjeldahl method

J. PETERS, G. GEORGE* and A. TONER

Introduction

Methods for the detection of ammonia are limited to colorimetry (Fleck, 1974) and electrochemical techniques (Carr & Bowers, 1980). Colorimetric determination of ammonia includes the ninhydrin reaction, modified Nessler's reaction to produce a colloid, Berthelot reaction in which ammonia and phenol are oxidatively linked by hypochlorite, coupling to NAD/NADH through α-ketoglutarate and the release ot ammonia at high pH (Annino & Giese, 1976), by a Kjeldahl method which involves the destruction of organic matter.

The ninhydrin reaction gives positive results for amino acids and other amino compounds, and thus, non specific for ammonia; an intermediary distillation step could be included to avoid interferences (Richterich, 1969).

Nessler's method has the disadvantage of being temperature dependent and that the colloid formation is a very fast reaction.

The Berthelot reaction experiences some interferences from co-existing metal ions; however, when phenol and hypochlorite are replaced by salicylate and dichloroisocyanurate respectively, most interference is overcome (Pym & Milham, 1976).

The Kjeldahl method for the determination of total nitrogen is widely used due to its high sensitivity and simplicity. Since its introduction in 1883, many modifications have been applied, including instrumentation and automation (Deschreider & Maes, 1968; Ewart, 1967; Schultz, 1967; MacLeod, 1973; Wall *et al.*, 1974). Most attempts were focused upon two categories—reduction of digestion time using modified catalysis (Florence & Milner, 1979; Tingvall, 1978), and/or increasing sensitivity for distilled ammonia determination (Nkonge & Ballance, 1982).

However, the Kjeldahl method is valid for total nitrogen determination and

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could not, in its present state, be used selectively for the determination of ammonium ion concentration in the presence of other nitrogen-containing compounds.

Ammonia can also be detected with glass pH type electrodes, and selectively by microdiffusion methods which require pre-concentration at high pH (Deschreider & Maes, 1968). Furthermore, certain cations such as Na^+ and K^+ cause high interference when using the NH_4^+ sensors, thus requiring the removal of such cations from the media and restricting the use of buffers.

In the present investigation, a determination of inorganic ammonium ion concentration in a collagenous sample was made enabling the use of this method in the determination of ammonia in collagenous films.

Materials and methods

Materials

Ammonium chloride (Analar)—a stock solution containing 1 g NH_4Cl/5 ml distilled water; Sodium hydroxide solution—0.1, 1.0, 5.0 and 10.0M solutions; Boric acid/indicator reagent—4% w/v boric acid solution in distilled water containing BDH 4′5′ indicator; Bovine serum albumin (BSA)—accurately weighed 1 g samples; Hydrochloric acid reagent—0.1M HCl was prepared and standardized against a standard Na_2CO_3 solution.

Methods

Blanks contained 1 g BSA in a final volume of 10 ml. Standards contained 1 g ammonium chloride in a final volume of 10 ml. Intermediary standards composed of 1 g BSA and 1 g NH_4Cl in a final volume of 10 ml.

Samples were placed in Kjeldahl tubes (Tecator), 10 ml of the relevant concentration of NaOH was added and tubes were instantly attached to a Buchi 320 N_2 distillation unit. Distillation commenced for a total of 3 min into 25 ml of the boric acid/indicator reagent. This was titrated with 0.1M HCl in a Mettler DL-40 memotitrator.

Results and discussion

Results were expressed as percentages of the theoretical nitrogen yield by NH_4Cl and BSA. Values significantly higher than 100% indicated that the BSA is attacked by the alkali in intermediary standards which served as means of comparison among the different concentrations of NaOH used.

Figure 1 shows that NaOH concentrations below 1 M caused the under-estimation of nitrogen in NH_4Cl, whereas the recovery of nitrogen from NH_4Cl was consistent at alkali concentration 1 M.

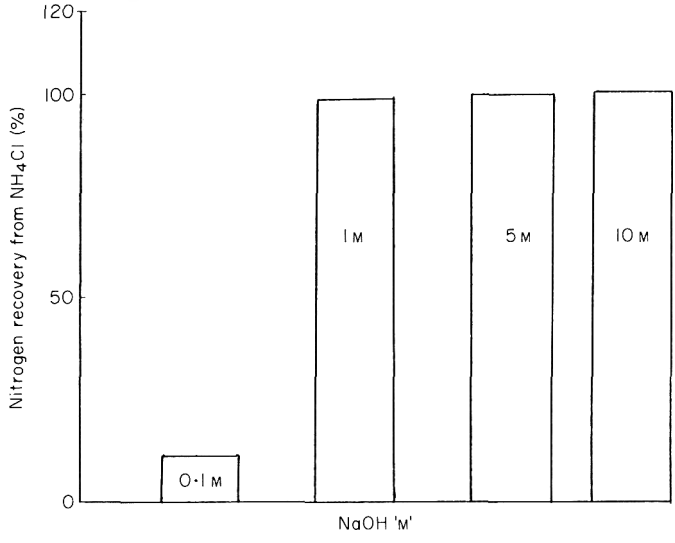

Figure 1. Recovery of nitrogen from ammonium chloride standards using different sodium hydroxide concentrations.

The effect of the different alkali concentrations on the breakdown of the protein in blanks is illustrated in Fig. 2, assuming that the conversion factor from nitrogen to BSA, is the conventional 6.25. It was clear that use of 10 M NaOH caused the release of 5.5% of the total BSA nitrogen compared with only 0.18% when a 1 M NaOH solution was used.

It was further demonstrated in Fig. 3 that 1 M NaOH was the most suitable alkali concentration for the release of ammonia from inorganic ammonium salts without a significant degradation of associated sample protein.

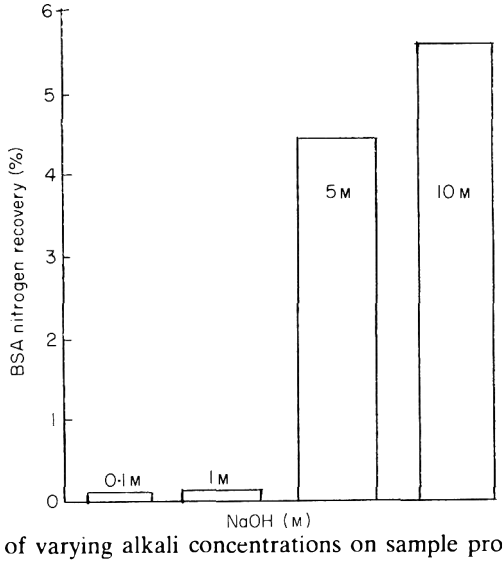

Figure 2. Effect of varying alkali concentrations on sample protein expressed as percentage of BSA, nitrogen recovered.

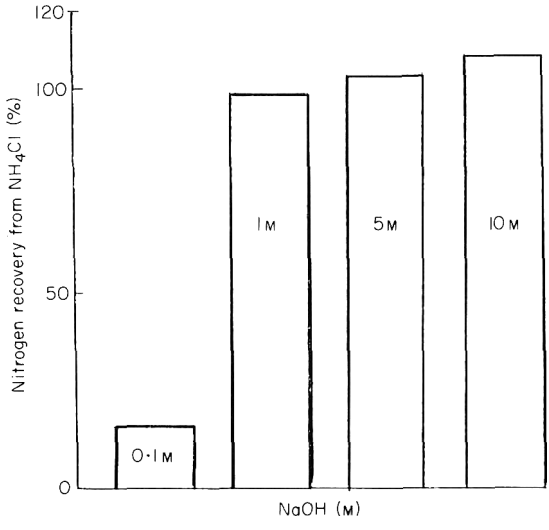

Figure 3. Effect of different sodium hydroxide concentrations on the recovery of nitrogen from ammonium chloride in a sample containing protein (NH_4Cl+BSA).

Trials to compare results with NH_3-electrode and Nessler's method showed good agreement in standard solutions; however, increasing interference was observed with both methods when analysing aqueous extracts of collagenous films, compared with negligible interference with the method under investigation.

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Book reviews

Canned Foods: Thermal Processing and Microbiology. By A. C. Hersom and E. D. Hulland.
Edinburgh: Churchill Livingstone, 1980. Pp. 392. ISBN 0443 021228. £16.00.

The seventh edition offers a major revision of this well-established reference book. The previous edition appeared in 1968 and contained 319 pages. The revised version is similar in style and layout but contains 392 pages, reflecting recent advances, particularly with regard to aseptic processing and packaging, new types of containers and the use of ionizing radiations.

Chapters 1 and 2 contain an introduction to bacteria and true fungi.

Chapter 3 describes the control of spoilage organisms and gives a concise review of methods such as refrigeration, gas storage, moisture limitation, salt, acids, curing, smoking and the use of antibiotics.

Chapters 4 to 9 deal more specifically with canning operations. They cover containers, an outline of canning operations, sources and control of contamination, principal spoilage organisms in canned foods, effects of heat on micro-organisms and principles of thermal processing. The latter chapter includes useful practical advice on obtaining heat penetration data and evaluating thermal processes from such data. A worked example, based on Ball's method is provided in the appendix. However, it is a pity that insufficient information is provided to apply this method to one's own heat penetration data, without needing to refer to Stumbo's text.

Chapter 10 on continuous flow sterilization deals with the advantages of high temperature short time (HTST) processes. Measurement of residence time distribution, the use of aseptic packaging systems and chemical sterilizing agents are discussed. Little is mentioned of direct steam injection, improved nutrient retention and optimization of HTST processes.

Chapter 11 gives a tidy review of the use of ionizing radiations, including their effects on micro-organisms, enzymes and chemical reaction rates; it ends with a few comments on the legal position, and a brief section on the use of ultraviolet radiation.

Chapters 12 and 13 return to canned foods and deal with the types of spoilage that can occur in canned foods, and the microbiology of sound canned foods respectively. The latter covers the important concept of commercial sterility. The majority of references date from before 1970. Perhaps this is a sign that we are not now so much concerned about searching for micro-organisms in sound cans.

Chapter 14 is a general chapter on bacterial food poisoning. Following the chapter on sound canned foods it is maybe a warning not to become too

complacent over the present low incidence of food poisoning attributed to canned foods.

The final four chapters deal with the laboratory analysis of canned foods, raw materials, processing plant and containers, and complete a comprehensive account of canning operations.

The book deals very competently with a wide range of applied microbiological topics, particularly the production of commercially sterile products and the control of spoilage organisms. The title of the book does not fully reflect these contents. In addition to dealing with 'canned foods', the book offers a very practical account of the factors affecting microbiological quality together with methods for assessment and suggestions for improvement of that quality. Therefore the book would be an invaluable reference source to any student or practitioner in the field of applied food microbiology.

Each chapter ends with a comprehensive list of references and the book is very well indexed. The appendices, which are largely unchanged from the previous edition, include steam tables and lethality tables but may well have usefully included additional tables for evaluation of thermal processing by Ball's formula method.

In conclusion this is a very readable, informative and well-presented text, which at £16.00 represents excellent value for money.

M. J. Lewis

Thermal Properties of Foods and Agricultural Materials. By N. N. Mohsenin.
New York: Gordon and Breach, 1980. Pp. vii+407. ISBN 0 677 05450 5. US$53.00.

The book covers three major areas: (1) the basic concepts of heat transfer together with heating and cooling data for different foods; (2) methods for determining the major thermal properties, particularly specific heat and thermal conductivity; and (3) a compilation of data for a wide variety of food and agricultural materials. These areas are covered in six chapters and a long appendix.

The first chapter deals with the basic concepts of heat transfer. The second chapter titled 'specific heat' describes methods for determining specific heats of a wide range of materials, and considerable data are presented throughout. Problems specific to frozen foods, dehydrated foods and oils and fats are also mentioned. The chapter ends with a section on latent heat of fusion. Chapter 3 deals in practical and theoretical terms with the measurement of thermal conductivity, thermal diffusivity and surface conductance. Chapter 4 reviews methods available and data obtained for an assortment of materials, such as seeds and grains, forage materials, fruits, vegetables and nuts, sugarbeet, tobacco, woods, soils, red meat, poultry and fish, beef manure, dairy products

and soya bean meal. Additional data on specific heats and thermal conductivity is supplied in the appendices

Chapters 5 and 6 are concerned more with applications, rather than with the properties *per se* and concentrate on unsteady state heat transfer situations. Chapter 5 deals with cooling and freezing operations and presents cooling data for a wide range of commodities. The chapter appears rather unbalanced, as only 3 of the 47 pages are devoted to freezing. Chapter 6 deals with heating operations and covers hot air drying, freeze drying and thermal processing. The chapter ends with sections on heat of respiration and thermal expansion.

The appendices occupy a further 120 pages. Considerable data are presented either in the form of tables (48 pages) or figures (36 pages) to supplement that presented in the main text. The tables and figures are presented separately and are numbered in such a way as to relate to the material in the six main chapters. The cited references from the six chapters form the final appendix.

The units used are mainly Imperial but conversion factors (S.I.) appear on pages 292 and 374 (373 in the index). Many worked examples are presented throughout the book. There are a few omissions; there are few data on surface conductance values, no mention of heat transfer by radiation or emissivity values of foods, despite a promise of this in the introduction to Chapter 3, and nothing on the use of microwave heating. There are the usual editorial problems of occasional ambiguous remarks, inconsistent use of symbols, typing errors and at least one reference to a table that does not exist. It is not the easiest book to find ones way around, particularly if being used as a data source; the index provided is not detailed enough always to be helpful in this respect.

However, despite these small moans, the book is quite well presented and the diagrams and tables are all very clear. The book contains much sound practical advice and a wealth of data, and for this alone warrants serious investigation by practising food technologists and engineers.

M. J. Lewis

Introduction to Bacteria for Students in the Biological Sciences. By P. Singleton and D. Sainsbury.
Chichester: John Wiley, 1981. Pp. vii+167. ISBN 0 471 10035 8. £4.95 (paper).

This book provides a good introduction to bacteriology. Although it is suitable for those going on to study the subject in depth, it will be of greatest value to students taking courses in which microbiology or bacteriology may play a relatively small part—agriculturalists, applied biologists, food scientists, food technologists, pharmacists etc.—for it gives a thorough grounding without being bulky. The book has a modern approach and important principles often

missing from elementary texts are clearly presented. The same precision that makes the authors' *Dictionary of Microbiology* such a useful book is evident throughout this work. Chapters deal with structure; growth and differentiation; aseptic technique, cultivation and counting; metabolism; genetics, including gene transfer and genetic engineering; bacteriophages; roles of bacteria in cycling of the elements; bacteria in medicine; identification procedures; descriptions of selected bacteria.

You will gather that I think highly of this book; but there are some minor criticisms. A little more could have been said about continuous cultivation, introducing through the Monod equation the concept of the effect of substrate concentration on growth rate. Students should be helped to realize that in many situations bacteria grow at less than their maximal rate. I am unhappy with the simplistic equating of the time for the completion of the cell cycle with doubling time—overlaps of, for example, DNA replication should have been considered. The procedure for determining whether a bacterium is an aerobe or an anaerobe is not described sufficiently. The choice of medium, particularly the incorporation of a fermentable substrate, should have been mentioned; though I accept that recent developments in our understanding of anaerobic growth make a simple statement difficult to produce. The survey of selected types of bacteria made me rather breathless! It is important that an idea of various types should be given in an introductory text but this section seemed less interestingly presented than the rest of the book. We sometimes forget that, although many medically important bacteria may be morphologically similar to one another, there are many distinct types. A few more line drawings showing and naming some of the types of bacteria that can be seen in a hay infusion or on a slide that has been immersed in water might have made the book more useful for introducing students to bacteriology. The complete absence of guidance on further reading is regrettable. Readers, having been introduced to bacteriology in such a stimulating way, should have been helped, even encouraged, to make a move to the next level of the subject.

I am confident that this book will find favour with many teachers and their students.

R. W. A. Park

A Manual of Recommended Methods for the Microbiological Examination of Poultry and Poultry Products. Ed. by R. T. Parry, L. Haysom, N. L. Thomas and R. Davis.
London: British Poultry Meat Association, 1982. Pp. vii+96. £4.95 incl. p & p.

At a time when discussion on microbiological criteria continues to increase, the need for standardization of methodology becomes of increasing importance. Although national and international organizations are seeking to set up

acceptable standard methods the rate of progress is somewhat slow in many areas. The British Poultry Meat Association and the Editors of this monograph have 'grasped the nettle' and prepared a manual of methods suitable for a range of microbiological examinations used in routine quality testing.

The manual provides helpful guidance on laboratory safety, equipment, laboratory procedures and sampling together with clear descriptions of the various test methods. The manual also has a section on calculation and expression of results and useful appendices.

The manual is clearly set out and even the tyro technician should have no difficulty in following the procedures for particular tests. The manual should also be of value to those in industry and control authorities who need to understand something of the mechanistics, but not necessarily be bench workers themselves.

My only quibble relates to the section on calculation wherein the concept of deriving weighted means is indicated for *some* purposes only. The expression of this concept is somewhat clumsy and there is no explanation or justification for suggesting its use other than that it yields the 'soundest statistical estimate'. Since this is so it would have been preferable to specify its use in all circumstances.

However, the use of this manual should be recommended to all concerned with poultry meat analysis. Hopefully, if everyone standardizes media and methods we may get to a situation where microbiological data from different laboratories become more comparable than is often the case.

Congratulations to the Editors and their Committee for the initiative shown on behalf of their industry.

B. Jarvis

Vitamin C (Ascorbic Acid). Edited by J. N. Counsell and D. H. Hornig. London: Applied Science, 1981. Pp. xiii+383. ISBN 0 85334 109 5. £32.00.

The rapid appearance of a reprint of this book, first published in 1981, is perhaps some indication of the general level of interest in the topic with which it deals, as well as a reflection of the absence until recently of competing volumes. Vitamin C (ascorbic acid) is a mixture of reviews and original contributions based on papers presented at a symposium held at the University of Warwick in 1981.

Fundamental considerations such as the function and metabolism of vitamin C in man, human requirements and recommended intakes, together with the vitamin C status of various population groups are dealt with at the outset. These four chapters set the scene for a volume which is throughout well arranged, well presented and exhaustively referenced. There follow a series of chapters of particular interest to the food scientist, discussing the use of

ascorbic acid in bread-making; meat processing; fruit juices and as antioxidants in oils and fats. Detection and measurement of vitamin C with all its difficulties is then considered. As the author points out, it is surprising that a satisfactory procedure, whatever the type of sample, is not available, despite the enormous amount of work (fully referenced) which has been carried out. Indeed the literature has continued to provide contributions on this topic since the Warwick symposium.

The influence of ascorbic acid on the formation of N-nitrosamines in food, drugs and tobacco, together with the influence of vitamin C on the *in vivo* formation of nitrosamines are discussed in two fascinating chapters which provide a good transition to the third major theme of the book, the role of the vitamin in health and disease.

Questions have often been raised about the safety of high intakes of vitamin C by man. Formation of oxalate stones, effects on electrolytes and metals, interaction with vitamin B_{12}, mutagenicity and foetotoxicity are among the adverse side effects which have been reportedly associated with large intakes of the vitamin. However, after the critical review of the literature which forms Chapter 13, the authors conclude that ingestion of up to 10 g/day does not constitute a health risk to man. This is certainly a valuable position paper. Nonetheless reports of new studies continue to appear and in view of the popular enthusiasm for vitamin C which makes it especially liable to abuse, there is still room for some caution.

Further chapters consider ascorbic acid and immune functions, the influence of drugs on the bioavailability of vitamin C, vitamin C in surgical patients, the influence of vitamin C on lipid metabolism and vitamin C and cancer. The concluding chapter of the book considers marginal vitamin C deficiency and human health.

One of my few criticisms about this book would be that in some instances the chapters might have been more logically ordered. Perhaps vitamin C and cancer could have followed on from the discussions of nitrosamine formation. While the effect of vitamin C on the bioavailability of iron sat a little awkwardly between 'Vitamin C status in population groups' and 'Vitamin C—man's requirement'. But perhaps this is quibbling over minor details of a book which on the whole represents a valuable addition to the library of anyone interested in the current state of knowledge and areas of debate surrounding vitamin C.

Jane E. Thomas

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References. Only papers closely related to the author's work should be included; exhaustive lists should be avoided. References should be made by giving the author's surname, with the year of publication in parentheses. When reference is made to a work by three authors all names should be given when cited for the first time, and thereafter only the first name, adding *et al.*, e.g. Smith *et al.* (1958). The '*et al.*' form should always be used for works by four or more authors. If several papers by the same author and from the same year are cited, a, b, c, etc. should be put after the year of publication, e.g. Smith *et al.* (1958a). All references should be brought together at the end of the paper in alphabetical order. References to articles and papers should mention (a) name(s) of the author(s) (b) year of publication in parentheses; (c) title of journal, underlined, abbreviated according to the *World List of Scientific Publications*, 4th edn and supplements; (d) volume numbers; nnmber of first page of article. Referencrs to books and monographs should include (a) name(s) and initials of author(s) or editor(s); year of publication in parentheses; (b) title, underlined; (c) edition; (d) page referred to; (e) publisher; (f) place.

Standard usage. The *Concise Oxford English Dictionary* is used as a reference for all spelling and hyphenation. Statistics and measurements should always be given in figures, i.e. 10 min, 20 hr, 5 ml, except where the number begins the sentence. When the number does *not* refer to a unit of measurement, it is spelt out except where the number is one hundred or greater.

Abbreviations. Abbreviations for some commoner units are given below. The abbreviation for the plural of a unit is the same as that for the singular. Wherever possible the metric SI units should be used unless they conflict with generally accepted current practice. Conversion factors to SI units are shown where appropriate.

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

Figures. In the text these should be given Arabic numbers, e.g. Fig 3. They should be marked on the backs with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of a figure the top should be marked with an arrow. Each figure must bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Line diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper and should be about four times the area of the final reproduction. Lines and lettering should be of sufficient thickness and size to stand reduction to one-half or one-third. Whenever possible, the originals of line diagrams, prepared as described above, should be submitted and not photographs, The legends of all the figures should be typed together on a single sheet of paper headed 'Legends to figures'.

Tables. There should be as few tables as possible and these should include only essential data; the data should not be crowded together. The main heading should be in bold with an Arabic number, e.g. **Table 2.** Each table must have a caption in small letters. Vertical lines should not be used.

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