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# Proximate analysis of five varieties of winged beans, *Psophocarpus tetragonolobus* (L.) DC *

V. V. GARCIA† AND J. K. PALMER

## Summary

The proximate composition was determined for the mature seeds of five varieties of winged beans, *Psophocarpus tetragonolobus* (L.) DC. The results showed that the varieties analyzed had a high protein and fat content, which was similar to soybeans. The protein content (mean ± standard deviation) of the five varieties as received was 36.0 ± 1.8%, the fat content, 15.2 ± 1.2% and the moisture content 10.4 ± 1.5%. The endosperm in winged bean variety TPT-2 was found to constitute 84% of the dry weight of the seeds, the hulls about 16%. Whole beans as received contain about 14% 'dietary fibre' measured as neutral detergent fibre. The dietary fibre is concentrated in the hulls.

## Introduction

The winged bean, *Psophocarpus tetragonolobus* (L.) DC is a crop grown in Asia and Papua New Guinea with Papua New Guinea considered as the most likely centre of its geographical origin (Hymowitz & Boyd, 1977). The winged bean is the cultivated species of the genus Psophocarpus Necker while the other eight species are wild and native to Africa (Newell & Hymowitz, 1979). The green pods, leaves and seeds are edible and rich in protein. The seeds are also a source of edible oil. The potential of winged beans as a food resource has been recognized by the scientific community (Levy, 1977a,b, 1978) ever since the U.S. National Academy of Science published a descriptive booklet about it in 1975.

The first analysis of the composition of the winged bean was probably that of

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Greshoff *et al.* in the early 1950s (as quoted by Masefield, 1973) who found that the immature seeds contained 12.3% moisture, 29.8% protein and 15.0% oil, indicating exceptional promise as a food resource. Since then, additional analyses have confirmed the promising properties of the plant (National Academy of Sciences, 1975). Proximate analyses have been reported on the flowers (Claydon, 1975), immature green pods (Cerny, 1978), leaves (Cerny, 1978; Ekpenyong & Borchers, 1978), the tubers (Claydon, 1975) and the 'beans' (mature seeds) (National Academy of Sciences, 1975; Jaffe & Korte, 1976; Claydon, 1975; Ekpenyong & Borchers, 1978) all of which have been reported as being edible.

Mature winged beans have never been produced as a marketable commodity, except for the small quantities required for planting the small acreage grown. Pospisil, Karikari & Mensah (1971) were the first to record seed yields. They obtained 1254 lbs/acre (1.4 tons/ha) in their cultivation of winged beans in Ghana. This result created attention because the yield was higher than the seed yields that could be obtained for soybeans in the humid tropics. At Ibadan, multiple harvests have yielded 2.2 tons and 1.8 tons per ha of dry, mature beans. These figures are still below the seed yield potential of 2.7 tons/ha attainable for soybeans (National Academy of Sciences, 1975).

Table 1 summarizes earlier data on the proximate composition of winged beans. Although the varieties analyzed were specified in only one case (Ekpenyong & Borchers, 1978) all the results confirm the high amount of protein and lipid present in winged beans. The protein and fat content compares well with that of soybean but is higher than most other legumes (cowpea, mung bean, chickpea, etc.).

Other information on winged beans indicates they contain approximately

**Table 1.** Proximate composition of winged beans

| Analysis | 1* | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Moisture | 8.54 | 9.7 | 14 | 6.7–24.6 | 8.7 | 11.6 | 10.4 | 9.5–10.4 | 9.7 |
| Crude protein | 41.86 | 32.8 | 33 | 29.8–37.4 | 36.6 | 30.6 | 35.9 | 34.4 | 37.3 |
| Fat | 13.11 | 17.0 | 16 | 15.0–20.4 | 15.3 | 18.3 | 15.8 | 16.9 | 18.1 |
| Ash | — | 4.0 | — | 3.6–4.0 | 3.8 | 3.7 | 4.9 | 4.2 | 4.3 |
| Carbohydrates | 31.22 | 36.5 | — | 31.6–28.0 | 35.6 | 35.8 | 33.0 | 34.1 | 30.6 |
| Crude fibre | 5.27 | 5.2 | 5 | 5.0–12.5 | 3.7 | 9.4 | 9.2 | 10.7 | 5.4 |

*1. Padilla & Soliven (1933)
2. Institute of Nutrition (Philippines) (1957)
3. Tindall (1968) as cited by Pospisil *et al.* (1978)
4. National Academy of Sciences, U.S. (1975).
5. Claydon (1975)
6. Jaffe & Korte (1976)
7. Ekpenyong & Borchers (1978).
8. Gandjar (1978).
9. Kordylas (1978).

three to four times the protein found in cereals and sufficient quantities of all amino acids except methionine, cysteine and tryptophan. The deficiency in sulphur-containing amino acids could be partly corrected by combining winged beans with appropriate cereal grains containing complementary amino acids. A 19% increase in net protein utilization (NPU) was observed when winged beans and corn were combined (Cerny *et al.*, 1971).

The varieties used in many of the earlier studies on winged beans were not identified. There is a pressing need to analyze for the proximate composition of the available varieties of winged beans in order to identify the varieties with a high protein and high fat content.

The results of these studies will help plant breeders in the selective breeding of winged beans as a valuable source of food protein. This study was undertaken to investigate the proximate composition of five established varieties of winged beans.

## Materials and methods

### *Sample preparation*

Dried seeds of winged bean *(Psophocarpus tetragonolobus* [L.] DC) varieties* TPT-2, CHIMBU, WB-19, Selections 10 and 12 were used. These varieties were used because they were promising varieties grown in various experiment stations. TPT-2 was obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Dr W. Steele); CHIMBU was obtained from the Asia Foundation, San Francisco, California (Mr Louis Lazaroff); WB-19 was obtained from the University of Puerto Rico, Mayaguez, Puerto Rico (Dr F. W. Martin); Selections 10 and 12 were procured from 27 Farms, Homestead, Florida.

The dried winged bean seeds were ground in a Wiley Laboratory Mill (Arthur H. Thomas, Philadelphia, Pennsylvania) to pass through a 40 mesh sieve. For the purpose of analyzing the seed coat and endosperm separately, winged beans (TPT-2) were soaked in water for 16 h, cracked, and dried in an oven at 100°C for 30 min. The seed coat was separated from the cotyledon with the aid of a stainless steel scalpel. The seed coat and the cotyledon were then ground separately in the Wiley Laboratory Mill to pass through a 40 mesh sieve.

### *Proximate analysis of winged bean flour and seed fractions*

Crude protein, crude fibre, ash, moisture, and crude fat analyses were performed on the whole flour, dehulled flour and seed coat fractions of winged

*WB-19 is the accession number assigned for TPT-12 (IITA); Selections 10 and 12 are the numbers assigned to UPS 76 and UPS 105, respectively and originally came from Papua New Guinea.

**Table 2.** Proximate chemical composition of five varieties of winged beans (percentage as received)

| Analysis | Variety | | | | |
|---|---|---|---|---|---|
| | TPT-2 | WB-19 | Sel. No.10 | Sel. No.12 | CHIMBU |
| Moisture | 9.41 ± 0.01* | 11.6 ± 0.1 | 11.2 ± 0 | 11.4 ± 0.1 | 8.2 ± 0.1 |
| Protein | 37.4 ± 0.2 | 33.7 ± 0.2 | 36.6 ± 0.2 | 34.5 ± 0.4 | 37.8 ± 0.1 |
| Fat | 14.0 ± 0.1 | 15.9 ± 0.1 | 14.2 ± 0.1 | 14.9 ± 0.1 | 17.0 ± 0.1 |
| Ash | 3.77 ± 0.8 | 4.12 ± 0.01 | 4.23 ± 0.04 | 4.16 ± 0.02 | 3.72 ± 0.01 |
| Carbohydrates† | 35.4 | 34.7 | 33.8 | 35.0 | 33.3 |
| Crude fibre | 6.98 ± 0.14 | 6.40 ± 0.09 | 6.27 ± 0.13 | 6.45 ± 0.05 | 5.96 ± 0.05 |
| Acid detergent fibre | 12.6 ± 0.2 | 11.7 ± 0.1 | 11.5 ± 0.2 | 11.3 ± 0.2 | 11.3 ± 0.1 |
| Neutral detergent fibre | 15.6 ± 0.4 | 15.0 ± 0.1 | 14.2 ± 0.4 | 14.0 ± 0.3 | 13.1 ± 0.1 |

*Mean ± standard deviation; $n = 3$.
†Calculated by difference.

beans. Methods used were those given in the Official Methods of Analysis of the AOAC (1975) for analysis of soy flour (pg. 234). Total nitrogen was determined by micro-Kjeldahl (pg. 937) as described also in AOAC (1975) except that 100 ml digestion flasks were used. Both full fat and fat-free samples were analyzed for crude protein. Protein was calculated as N × 6.25 for winged beans. Carbohydrate was calculated by difference.

Acid detergent fibre and neutral detergent fibre were determined according to the methods of Van Soest (1963a,b) as described in the AOAC (1975) and by Southgate (1976) and by Goering & Van Soest (1970).

## Results and discussion

The results of the proximate analyses (Table 2) of the five varieties of winged beans showed that the protein content ranged from 33.7% for WB-19 to 37.8% for CHIMBU while the fat content ranged from 14.0% for TPT-2 to 17.0% for CHIMBU all on a 'as received' basis (average 10% moisture content). These results compare favourably with the previous work shown in Table 1. The corresponding values calculated on a dry weight basis are 38.1 to 41.2% protein and 15.4 to 18.5% fat content. These values are similar to those reported in the U.S. for commercial varieties of soybeans, which contain an average of 40.5% protein and 20.0% fat on a dry weight basis.

In more recent studies not included in Table 1, Harding, Martin & Kleiman (1978) analyzed the protein and oil content of thirty-two winged bean accessions that were grown in Puerto Rico. Based on one planting, they found the protein content of the winged bean to vary from 29.2 to 40.9% and the oil

**Table 3.** Proximate analysis of winged beans (TPT-2) and seed fractions (percentage as received)

| Analysis | Whole beans | Dehulled beans* | Hulls* |
|---|---|---|---|
| Moisture | 9.41 ± 0.01* | 5.51 ± 0.04 | 4.70 ± 0.21 |
| Protein | 37.1 ± 0.2 | 47.4 ± 0.6 | 9.69 ± 0.19 |
| Fat | 14.0 ± 0.1 | 16.7 ± 0.1 | 0.94 ± 0.01 |
| Ash | 3.77 ± 0.08 | 3.76 ± 0.03 | 2.05 ± 0.06 |
| Carbohydrates† | 35.4 | 26.6 | 82.6 |
| Crude fibre | 6.98 ± 0.14 | 2.56 ± 0.05 | 40.5 ± 0.1 |
| Acid detergent fibre | 12.6 ± 0.2 | 10.2 ± 0.3 | 54.8 ± 1.2 |
| Neutral detergent fibre | 15.6 ± 0.4 | 12.2 ± 0.0 | 68.1 ± 0.2 |

*Dehulled beans constitute 84.1% of the bean and the hulls constitute 15.9% of the bean.
*Mean ± standard deviation; $n = 3$.
†By difference.

content to vary from 14 to 19% on a dry weight basis. The varieties TPT-2, CHIMBU, and WB-19 (originally TPT-12) were included in their studies. Ekpenyong & Borchers (1978) found that TPT-2 beans contained 40.1% protein and 17.7% fat (dry weight basis), similar to the values obtained in this study.

The earlier data plus the results in Table 2 show that the protein content of winged beans is higher than other food legumes (dry beans, pigeon peas, cowpeas, chickpeas, broad beans and peas) recommended by the Protein Advisory Group of the United Nations (PAG) in 1973 for intensified research in relation to human nutrition and food technology. These legumes contain 22–25% protein (Esh & Som, 1952; Lal, Prakash & Verma, 1963; Singh, Singh & Sikka, 1968; Oyenuga, 1966). Three of these legumes (cowpeas, chickpeas and pigeon peas) and mung beans were also identified by Araullo (1974) as possessing the potential for increased food utilization in Asia because they are well accepted in the diet.

The distribution of nutrients in the hulls (seed coat) and the cotyledon (endosperm) was studied in variety TPT-2. The cotyledon was found to constitute 84.1% and the hulls 15.9% of the dry weight of the seed. Table 3 shows the proximate composition of the isolated seed coat and the cotyledon. The cotyledon contained the greater portion of the protein and fat of winged beans. The hulls contained most of the fibre. Kordylas (1978) separated winged beans into seed coat (hulls) and cotyledon. The cotyledon was milled to produce flour and grits after defatting in hexane. Neither the variety nor the relative contribution of the seed coat and cotyledon was reported. He found the flour to contain 60.2% protein and 2.3% fat; the grits 44.3% protein and 8.8% fat while the seed coat contained 13.0% protein and 1.3% fat (all calculated on a dry weight basis). The protein content of the milled flour was similar to the results in Table 3 for TPT-2.

The ash content of winged beans was similar to the previous values reported

(Table 1). The carbohydrate content (obtained by difference) of the winged beans was within the ranges of values previously reported (Table 1).

The crude fibre content of the five varieties of winged beans varied from 5.96% (CHIMBU) to 6.98% (TPT-2). The average value of the crude fibre (6.41%) is higher when compared to previous data obtained through 1975 but lower when compared to the three values reported since 1976 (Table 1). Ekpenyong & Borchers (1978) also analyzed winged beans (TPT-2) for crude fibre. Their results (11.89%) do not agree with the crude fibre (6.98%) obtained in the present analysis.

The neutral and acid detergent fibres were analyzed in the five varieties of winged beans because they provide a more reliable estimate of the digestibility than crude fibre (Goering & Van Soest, 1970). Acid detergent fibre (ADF) estimates cellulose and lignin. Neutral detergent fibre (NDF) estimates the total cell wall material. The difference between NDF and ADF estimates hemicelluloses. The NDF values of winged beans 'as received' (Table 2) ranged from 13.1 to 15.6% (mean, 14.4%) while the ADF varied from 11.3 to 12.67% (mean, 11.7%). The NDF is thought to give a reliable estimate of the total fibre in a vegetable foodstuff which is available for microbial fermentation in the gut. There are no detergent fibre data on soybeans or other legumes.

Some of the variation in the chemical composition of winged bean seeds probably results from agronomic factors (soil, climate, moisture, etc.) and some from varietal differences. Cartter & Hopper (1942) studied the influence of variety, environment, and fertility level on the chemical composition of soybeans. They concluded from their results that the chemical composition of soybeans (in particular the protein content) was affected by both agronomic factors and variety, but that varietal differences exerted a greater effect on protein content. Additional data will be required to establish the extent and source of the variability in winged beans.

## Conclusions

The results of the proximate composition confirmed that winged beans have a high protein and fat content (Table 2) similar to soybeans. The protein content of the five varieties analyzed was 36.0 ± 1.8%, while the fat content was 15.2 ± 12.2%, on an 'as received' basis (moisture content 10.4 ± 1.5%). The ash content (4.00 ± 0.24%) and the carbohydrate content (34.4 ± 0.9%) of the five varieties were similar to those previously reported (Table 1).

The cotyledon was found to constitute 84.1% and the hulls 15.9% of the dry weight of the seeds in TPT-2 bean (Table 3). The cotyledon contained the greater portion of the protein and fat of winged beans. The hulls contained most of the fibre.

The crude fibre content of the five varieties was 6.41 ± 0.37%. The neutral detergent fibre was 14.4 ± 1.0%, while the acid detergent fibre was 11.7 ± 0.5%.

Further studies on the carbohydrates, fatty acids and proteins of winged beans will be reported in future publications.

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# Carbohydrates of winged beans, *Psophocarpus tetragonolobus* (L.) DC*

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## Summary

Five varieties of winged beans were analyzed and found to contain 9.8 to 13.8% soluble sugars. Verbascose was present at a level of 0.2 to 0.9%; stachyose 2.2 to 3.6%; raffinose 1.1 to 2.0%; and sucrose 5.6 to 8.2%. Starch could not be detected in the mature beans of the five varieties analyzed.

## Introduction

In the preceeding paper (Garcia & Palmer, 1980), the proximate composition of five promising varieties of winged beans, *Psophocarpus tetragonolobus* (L.) DC, was reported. These varieties contained about 34% total carbohydrates (calculated by difference). Additional data on these carbohydrates are needed, as part of the effort to assess the potential of winged beans as a food crop. Since winged beans resemble soybeans in so many other respects, it is possible that the carbohydrates of winged beans are similar to those of soybeans. Dehulled, defatted soy flour was found to contain 15–18% high molecular weight polysaccharides by Aspinall, Begbie & McKay (1967) and about 13% soluble oligosaccharides (Kawamura, 1967). The oligosaccharides (verbascose, stachyose and raffinose) are important in human nutrition in that they have been reported to cause flatulence in man and animals. These three sugars escape digestion and absorption in the upper gastrointestinal tract, but are microbially degraded and fermented to yield $H_2$ and $CO_2$ when they reach the colon.

Hardinge, Swarner & Crooks (1965) and Cristofaro, Mottu & Wuhrmann (1974) have reported the oligosaccharide content of various legumes. Soybeans

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contain the highest levels of raffinose and stachyose whereas other legumes have the highest levels of verbascose. Hymowitz *et al.* (1972) determined the oligosaccharide content of varieties and strains of soybean to determine whether these carbohydrates can be eliminated genetically. They found that elimination of flatulence causing carbohydrates by breeding holds little promise since there is a high degree of genetic stability of oligosaccharides in the varieties of soybeans tested.

Kawamura, Nagao & Kasai (1977) determined the free monosaccharides and sugar alcohols present in mature soybeans using paper partition and gas liquid chromatography. The cotyledon contained glucose and fructose while the hulls contained galactose, glucose, fructose, arabinose and xylose. Sorbitol, arabinitol, xylitol and mannitol were also present. The free monosaccharides and sugar alcohols were present in minute amounts (about 0.001–0.4% of the whole seeds). Schweizer *et al.* (1978) found a new disaccharide, galactopinitol, present in soybeans, chickpeas, lentils and green beans.

High pressure liquid chromatography (HPLC) has been successfully employed in the carbohydrate analysis of oligosaccharides by Conrad & Palmer (1976), Rabel, Caputo & Butts (1976), Brobst, Scobell & Steele (1974) and Linden & Lawhead (1975). Black & Bagley (1978) determined quantitatively the oligosaccharides in soybeans by HPLC using a μ-Bondapack carbohydrate column. Cegla & Bell (1977) analyzed the oligosaccharides of oilseed flours using a combination of TLC and HPLC. TLC was employed to remove organic matter prior to HPLC to increase the life of the column.

This study was undertaken to determine the soluble carbohydrates present in winged beans. If they are to become a major food source, it is important to know how much flatulence producing oligosaccharides they contain.

## Materials and methods

### *Carbohydrate analysis (soluble carbohydrates)*

Sample preparation. Winged bean flour was prepared according to the method of Garcia & Palmer (1980). Winged bean, varieties CHIMBU (Asia Foundation, San Francisco, California), TPT-2 (International Institute of Agriculture, Ibadan, Nigeria), WB-19 (originally TPT-12, USDA, Puerto Rico) and Selections 10 and 12 (originally UPS 76 and 105 respectively, 27 Farms, Homestead, Florida) were used in this study. Five grams of ground, ether defatted winged beans were refluxed in 50 ml 85% ethanol (AOAC, 1975) for 30 min in a boiling water bath. The ethanol extract was filtered through Whatman 541 V-folded filter paper. The residue was washed 5 times with 10 ml volumes of 85% ethanol at 65°C, the mixture filtered after each wash and the washings combined with the filtrate. The ethanol was allowed to evaporate in the hood until the sample was dry. The dry sample, which contained the soluble carbohydrate, was made up to 10 ml with water, mixed and

filtered through a 0.45 μm pore diameter membrane filter to remove any insoluble material. The filtered sample was deionized on a 10 × 0.7 cm i.d. column containing a mixed bed ion exchange resin (AG 501 X-8(D), 20–50 mesh, BioRad Laboratories, Richmond, California), with a bed height of 5 cm. The top was layered with a 1 cm bed height of 1:1 mixture of AG 50W -X8, $H^+$ form, 200–400 mesh and AG 3–X4A, $OH^-$ form, 200–400 mesh (BioRad Laboratories) to give a slow flow rate. The sample was deionized in 1 ml increments, the first 3 ml discarded and the rest collected in a scintillation vial and frozen when not in use.

*HPLC standards.* Verbascose, stachyose, raffinose and sucrose were dried at 60°C in 26 inches (99 mm) Hg of vacuum for 16 h. Standard solutions of these carbohydrates (except verbascose) in water were prepared at concentrations ranging from 20 mg/ml to 50 mg/ml. Dr D. K. Salunkhe (Utah State University) kindly supplied 20 mg of verbascose (68% purity) while the other sugars were procured from Sigma Chemical Co., St Louis, Missouri. A mixed standard solution was prepared, containing verbascose at a concentration of 5 mg/ml and the other sugars at 10 mg/ml. Standard solutions of individual sugars (except verbascose) were also prepared at a concentration of 10 mg/ml. The standard solutions were kept frozen when not in use.

*Equipment.* The oligosaccharides and sucrose were examined by high performance liquid chromatography (HPLC). A Model ALC 201 chromatograph was equipped with a Model 6000 A solvent delivery system, a model R- 401 Refractive Index Detector, a Model 440 UV absorbance detector and a Model U6K Injection System (Waters Associates, Milford, Massachusetts). All data were recorded with a dual channel Omni-Scribe recorder (Houston Instruments, Austin, Texas).

*Packing of HPLC column.* Aminex 50 W X–4, $H^+$, 20–30 μm (BioRad Laboratories, Richmond, California) was converted into the $Ca^{++}$ form as described by Scobell *et al.* (1977) and the BioRad Catalog (1978). The $Ca^{++}$ form resin was packed in 30.0 × 0.5 cm i.d. stainless steel precision bore columns with a 10 μm pore-size fritted disc end fitting. An aqueous slurry of the resin was continuously stirred in a Micromeritics column packer Model 705 (Micromeritics Instrument Corp., Norcross, Georgia) and pumped into the column at 1.5–2.0 ml/min. with a Milton Roy pump, at a maximum pressure of about 2000 p.s.i.

*Separation and quantitation.* The samples and the standards were injected in 10 μl volume. The samples and standards were eluted with deionized water which had been filtered in a 0.45 μm membrane filter. The column was 60 cm in length (two 30 cm columns) and was operated at a flow rate of 0.50 ml/min. The column was equipped with an aluminum water jacket and operated at 85°C, employing a Haake FS circulating water bath (Haake, Saddlebrook, New Jersey).

The soluble sugars in winged beans were tentatively identified from the elution times relative to the individual standard sugars. Confirmation of identities was obtained by thin layer chromatography on phosphate treated plates

(Supelco, 1977) of individual sugars collected during HPLC. A periodate-p-anisidine hydrochloride solution was used for detection of the sugars (Hough & Jones, 1962). Quantification of the sample components was achieved from HPLC peak area measurements [height (H) × width at half height ($W_{½\ H}$)] relative to the peak areas of the standard.

*Carbohydrates (starch determination)*

Staining in $I_2$–KI solution was used as a qualitative test for the presence of starch. The qualitative tests were confirmed by employing the method of Palmer (personal communication, 1978) which was used for starch determinations in plantains. To 100 mg of the air-dried residue of winged beans from the soluble carbohydrates determination, was added 3 ml of 0.05 M NaAc buffer, pH 5 in a 15 ml centrifuge tube. The mixture was stirred with a magnetic bar to suspend the sample uniformly. The mixture was then heated in a boiling water bath for 5 min and cooled. Two ml of glucoamylase solution (prepared by stirring Sigma Type II glucoamylase in 50 ml cold NaAc buffer for 10 min, centrifuging at 15,000 ***g*** for 15 min at 4°C and recovering the supernatant enzyme solution) was added to the mixture. The solution was mixed and incubated at 25°C for 1 hr. The solution was then made to volume with distilled water, filtered in a 0.45 μm pore size membrane filter and deionized as described in the oligosaccharide analysis. Analysis of glucose was performed as described in oligosaccharide analysis except that a glucose standard (10 mg/ml) was used. Mg glucose × 0.90 = mg starch.

## Results and discussion

*Carbohydrate analyses*

*Starch content determination.* Based on iodine staining and on enzymatic determination, there was no starch in the five varieties of mature winged beans. Thus, winged beans are similar to mature soybeans, which do not contain starch. In other food legumes, starch is the predominant carbohydrate. It is possible that during the early stage of the development of the winged beans (i.e., green immature pods), the seed contains starch. This is the case with soybeans. An amylase inhibitor was found by Jaffe & Korte (1976) to be present in winged beans. The activity, however, was found to be low.

*Oligosaccharide content.* An analysis of the oligosaccharides in winged bean was performed because legumes commonly contain relatively high amounts. The oligosaccharides are important in nutrition because they cause flatulence in man and animals. Claydon (1975) observed that raw mature winged beans cause abdominal pains indicating the presence of flatulence factors.

The typical legume oligosaccharides (verbascose, stachyose and raffinose), as well as sucrose, were found to be present in all five winged bean varieties. These

**Table 1.** Oligosaccharide content of winged beans (g/100 g sample)

| Variety | Verbascose | Stachyose | Raffinose | Total oligo-saccharides | Sucrose | Total soluble sugars |
|---|---|---|---|---|---|---|
| CHIMBU | 0.19 ± 0.01* | 3.40 ± 0.02 | 1.98 ± 0.01 | 5.57 | 6.36 ± 0.01 | 11.9 |
| Selection 10 | 0.83 ± 0.02 | 3.32 ± 0.04 | 1.34 ± 0.00 | 5.49 | 6.03 ± 0.03 | 11.5 |
| Selection 12 | 0.68 ± 0.01 | 3.53 ± 0.03 | 1.39 ± 0.01 | 5.60 | 8.17 ± 0.01 | 13.8 |
| TPT-2 | 0.77 ± 0.03 | 2.21 ± 0.04 | 1.15 ± 0.03 | 4.13 | 5.64 ± 0.02 | 9.77 |
| WB-19 | 0.91 ± 0.03 | 3.56 ± 0.03 | 1.71 ± 0.06 | 6.18 | 6.79 ± 0.08 | 13.0 |

*Mean ± standard deviation ($n$ = 4)

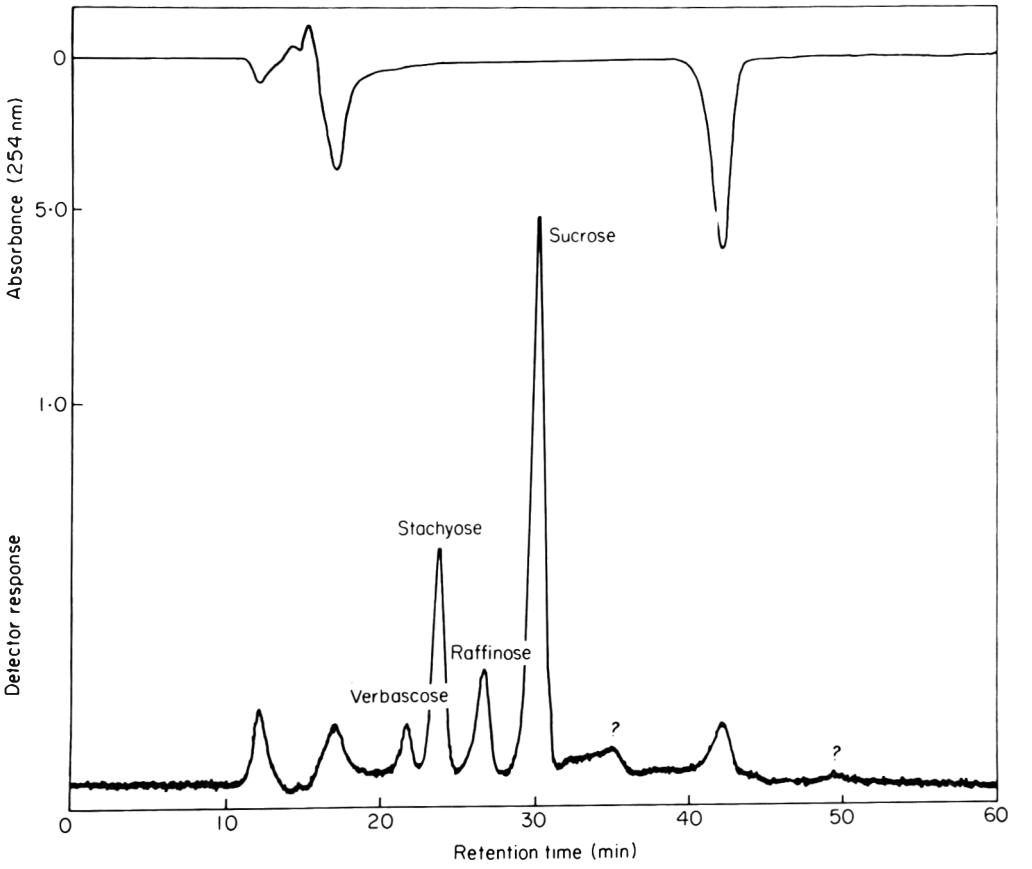


**Figure 1.** Chromatogram of the ethanol extract from defatted winged bean flour on a 60 cm column with Aminex 50 W X-4, $Ca^{++}$, eluted with water at 85°C (0 min. – sample injection). Bottom trace: RI detector. Top trace: UV absorption shows non-carbohydrate components during elution. The three unidentified peaks appearing in the bottom trace were presumed not be carbohydrates because they corresponded in retention time to the UV peaks. Small amounts of unidentified carbohydrates are marked with a question mark.

analyses are shown in Table 1 and a representative elution profile is shown in Fig. 1.

Verbascose was present at levels of 0.19% in the CHIMBU to 0.91% in the WB-19. Stachyose was present at 2.18% in the TPT-2 to 3.56% in the WB-19. Raffinose was present from 1.34% in Selection 10 to 1.98% in the CHIMBU. The total oligosaccharides were present in the range of 4.10–6.18%. Sucrose was found to be present in large amounts in Selection 12 (8.18%). Sucrose was lowest with TPT-2 (5.64%).

The total oligosaccharide content was about the same level as found in soybeans. Raffinose in winged beans is present at about the same concentration as that present in soybeans (1.3%). Stachyose is present in winged beans (3.2%) at a lower level with that found in soybeans (5.3%) by Kawamura, 1967. Verbascose was found to be present in the range of 0.19–0.91% in winged beans whereas in soybeans it was found to be present in trace amounts. The oligosaccharide content of winged bean is low when compared to other food legumes (chickpea, cowpea, field beans, lentils, etc.), which usually contain 5.7 to 7.9% oligosaccharides (Hardinge, Swarner & Crooks, 1965; Cristofaro, Motta & Wuhrmann, 1974).

Sucrose levels are about the same as found in soybeans. However, one variety (Selection 12) contained 8.18% sucrose, a level which is 30% higher than the average of the other four varieties of winged beans.

The elimination of flatulence factors by breeding in winged beans holds little promise if winged beans are like soybeans. Hymowitz *et al.* (1972) have shown that there exists a high degree of stability in the oligosaccharide content of soybeans which cannot be eliminated by breeding. The only recourse would be to find varieties which contain low concentrations of the flatulence factors.

The total soluble sugar content (oligosaccharide and sucrose) found in each variety when added to their corresponding neutral detergent fibre values as reported by Garcia & Palmer (1980) amounted to a total carbohydrate content of 25.3% in TPT-2, 25.0% in CHIMBU, 27.8% in Selection 12, 25.7% in Selection 10 and 28.0% in WB-19. These values account for 73% in TPT-2, 75% in CHIMBU, 79% in Selection 12, 76% in Selection 10 and 81% in WB-19 (mean, 77 ± 3%) of the total carbohydrates obtained by difference in the proximate analyses of the winged beans as reported by Garcia & Palmer (1980). The carbohydrate data suggest that winged beans contain a considerable proportion of carbohydrate which is solubilized during the neutral detergent fibre determination, but not solubilized in the hot 85% ethanol use and to extract the soluble sugars. Pectins are an example of a class of carbohydrates which could fall in this category.

## Conclusions

Oligosaccharides (verbascose, stachyose and raffinose) were found to be present in the five varieties of winged beans (Table 1). Raffinose is present at about

the same concentration found in soybeans (1.3%). Stachyose is present at a lower level (3.2%) than that found in soybeans (5.3%). Verbascose is present in the range 0.19–0.91% in winged beans whereas in soybeans it is present in trace amounts. The oligosaccharide content of winged beans is low when compared to chickpea, cowpea, field beans, mung beans and lentils, which usually contain 5.7% to 6.9% total oligosaccharides (Hardinge *et al.*, 1965; Cristofaro *et al.*, 1974). Sucrose levels are about the same as found in soybeans. One variety, Selection 12, contained 8.18% sucrose, a level which was 30% higher than the average of the other four varieties.

The total soluble sugars found in each variety plus their corresponding neutral detergent fibre (Garcia & Palmer, 1980) account for 77% of the total carbohydrates obtained by difference in the proximate analyses of winged beans (Garcia & Palmer, 1980). Further study will be required to identify and quantify the undetermined carbohydrates. Winged beans were found to be free of starch in the present study and it is possible that the undertermined carbohydrate is an alternate storage carbohydrate in winged beans.

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# Volatiles of apples (var. 'Schone van Boskoop'): isolation and identification

M. FEYS, P. TOBBACK AND E. MAES

## Summary

A simple method for the analysis of apple volatiles has been developed. By means of a modified vacuum-cold trap distillation an aqueous solution of volatiles, ready for direct GLC-analysis, is obtained.

Formation of artefacts (e.g. volatile aldehydes) during homogenization of the apple tissue was prevented by addition of trichloroacetic acid. About fifteen volatiles of the apple variety 'Schone van Boskoop' have been identified and quantified. More than 85% of the total volatile fraction are alcohols. The recovery for the different volatiles ranged from 60 to 95% depending on the nature of the compounds.

In a dichloromethane extract of the distillate a few minor compounds, mostly esters, were identified by mass spectrometric analysis.

## Introduction

The methods used to isolate and concentrate volatiles can affect the results of subsequent gas chromatografic analysis. The pre-treatment procedures usually cause qualitative and quantitative changes in the sample. Depending on the composition of the sample and the objectives of the study one or another procedure may be superior (Jennings & Filsoof, 1977). Headspace analysis, distillation, extraction and adsorption, or a combination of these are current methods for the isolation and concentration of volatile compounds (Weurman, 1969; Paillard, Pitoulis & Mattei, 1970; Römer & Renner, 1974).

Headspace analysis has been widely used in aroma research. Although relatively simple, the method is not very useful for quantitative studies. Headspace analysis gives an idea of the composition of the volatile fraction emitted by the

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fruit but it is difficult to relate this with the composition of the volatiles inside the fruit.

Extraction and adsorption are to a certain extent selective, depending on the nature of the solvent or of the adsorbent. Concentrating the solvent often causes the loss of lower boiling compounds and the solvent peak interferes with other compounds on the chromatogram. In addition the isolation and concentration of the volatiles mostly occurs in different steps so that the whole process becomes more complex and less suitable for routine analysis.

Finally distillation is perhaps the most commonly used method in aroma research. Since the first steam-distillations, this method has been used in a number of different forms, depending on the product and on the aim of the work. Distillation is in itself not selective for it is based on the use of a common property of all compounds of interest, namely their volatility. However the distillate is mostly too diluted for direct gas chromatographic analysis. Distillation is therefore used as a first step for isolating the volatiles and is followed by a concentration of the compounds using other methods.

It has been the objective of this study to devise a distillation procedure during which water is selectively separated from the volatiles so that a solution which is ready for direct gas chromatographic analysis is obtained.

## Experimental

Apples (1.2 kg) were homogenized in 800 ml of water in a Waring 4-litre Commercial Blendor. The pulp was transferred to a 3-litre jar and 2 ml of an anti-foaming agent (UCB 1804A) were added. The distillation set-up is shown in Fig. 1. A stream of nitrogen (15 ml/min) was passed through the apple pulp. The jar was heated with a heating mantle in order to prevent extreme cooling as a result of solvent evaporation under vacuum. Between the jar with the apple pulp and the cold trap with liquid nitrogen a cooling spiral was placed, cooled to $-17°C$ by means of a cryostat (Haake, KT 33) with methanol as cooling liquid. The whole system was connected to a vacuum pump (Cenco-Hyvac 7) with a resulting underpressure of 35 mmHg in the distillation apparatus. A second cold trap can be used to protect the vacuum pump. The distillation time was 1.5 hr.

The effectiveness of ascorbic acid (0.2% w/w fresh apples), trichloroacetic acid (4% w/w), sodium bisulphite (0.2% w/w) and different $CO_2$ concentrations (2 and 8% w/w) on the formation of artefact volatiles during blending was checked.

The recovery and the reproducibility of the distillation process was determined during a series of ten distillations with a mixture of known compounds, under identical experimental conditions as described above. For quantitative gas chromatographic analysis the mean peak area/$\mu$g for the different volatiles was used.

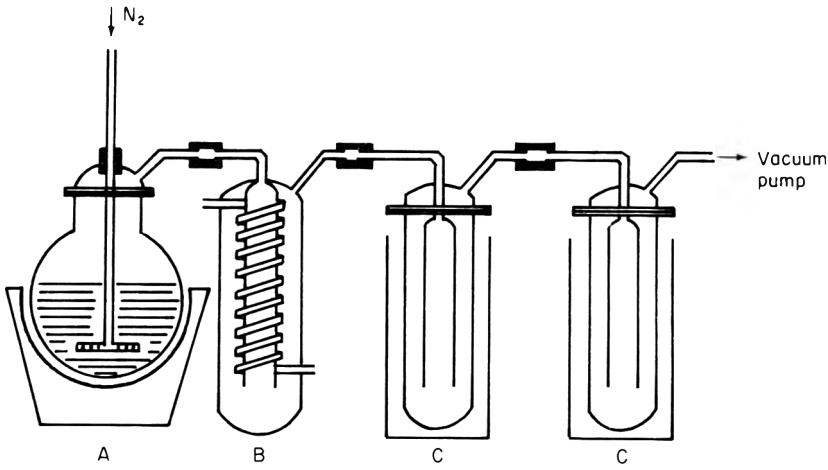


**Figure 1.** Schematic of the distillation set up for the isolation and concentration of volatiles. A, distillation jar (10°C); B, cooling spiral (−17°C); C, traps cooled with liquid nitrogen.

GLC analysis was carried out using a Hewlett-Packard 5720A instrument with flame ionization detector, connected to a Hewlett-Packard 3380A integrator. The following columns were used for the identification of the volatiles:

(i) 20% DEGS on chromosorb P–AW, 60–80 mesh, 20′ × ⅛″, SS. Column temperature 90 and 110°C, $N_2$–flow: 9 ml/min.
(ii) 15% Carbowax 20 M on chromosorb W–AW, 60–80 mesh, 16′ × ⅛″, SS. Column temperature 80°C, $N_2$–flow: 9 ml/min.
(iii) Silicon-oil SF 96 (50) + 5% Igepal Co–880, 500′ × 0.02″, SS. Temperature programmation: 60 min at 50°C, 2°C/min to 120°C. $N_2$–flow: 1 ml/min.

The 20% DEGS column was used for quantitative analysis. A dichloromethane extract of the distillate was analyzed on a Varian 2700 gas chromatograph with a 250m × 0.5mm i.d. column coated with SE–30, linear temperature programming from 20 to 220°C at 2°C/min with a carrier gas flow of 6 ml He/min. The gas chromatograph was linked to a Varian Mat 112 mass spectrometer.

## Results and discussion

### *Distillation*

The temperature of the cooling spiral (−17°C) was determined experimentally so that the most concentrated distillate was obtained without condensation of volatiles on the spiral. The mean volume of the distillate was 21.4 ml ($S_{\bar{y}}$ =

4.1). With this simple distillation method it was possible to isolate and concentrate the volatiles in one step, so that the most important volatiles could be analyzed quantitatively by gas chromatography. It is important to note that the isolation and the concentration of the compounds are based on their respective volatility so that no compounds are lost e.g. through their solubility in organic solvents. Also low boiling compounds could be analysed quantitatively. There was no interference of a solvent peak on the chromatogram since water is not detected with the columns used.

Two important disadvantages of the normal distillation method, the thermal alterations by heating of the apple pulp and the contact with $O_2$ during distillation (Drawert, 1969), could be avoided. The temperature in the distillation jar was $10 \pm 1°C$ and the remaining $O_2$ was expelled by blowing a stream of nitrogen through the pulp. This also had a favourable effect on the continuous stirring of the apple pulp during distillation.

*Formation of volatiles on disrupture of the cell structure*

The short time (10 min) between homogenization and distillation and the low temperature in the distillation jar could not prevent the formation of significant amounts of n-hexanal and trans-2-hexenal, typical aldehydes formed by enzymatic oxidation of unsaturated fatty acids. Drawert *et al.* (1965, 1966, 1973) drew attention to the possible enzymatic hydrolization and oxidation reactions and their influence on the composition of the natural aroma. The authors showed that n-hexanal and trans-2-hexenal are formed by enzymatic oxidation of linoleic and linolenic acid and they proposed the addition of inhibitors of these reactions.

In our experiments the formation of n-hexanal and trans-2-hexenal from linoleic and linolenic acid has been examined by addition of these fatty acids prior to homogenization of the apples. The effect of several inhibitors has also been compared. The results (Table 1) clearly indicate that the formation of n-hexanal and trans-2-hexenal strongly increases with the addition of linoleic and linolenic acid respectively. The inhibiting effect seemed to be the best with 0.2% (w/w) sodium bisulphite. However this is not a useful compound since it reacts with natural occurring aldehydes such as acetaldehyde so that these compounds are not found on the chromatogram. For this reason trichloroacetic acid has been used for the inhibition of the typical aldehyde formation during homogenization. Methanol as suggested by Drawert *et al.* (1966) could not be used since it would give problems during distillation and gas chromatographic analysis owing to the high volatility of the compound and its interference on the aromagrams.

When not using inhibitors the formation of acetaldehyde and the breakdown of ethyl butyrate can give some problems also, but they can be avoided by working at a low temperature and keeping the time between homogenization and distillation as short as possible. The addition of inhibitors did not influence the concentration of the other volatiles present in the distillate.

**Table 1.** Influence of the addition of some compounds on the formation of n-hexanal and trans-2-hexenal during homogenization of the apples (blank = 100%)

| Compounds % w/w fresh apples | n-hexanal % *vs* blank | trans-2-hexenal % *vs* blank |
|---|---|---|
| 0.1% linoleic acid | 302.0 | 104.5 |
| 0.1% linolenic acid | 102.0 | 435.2 |
| 2% $CO_2$ | 99.8 | 95.0 |
| 8% $CO_2$ | 65.7 | 72.3 |
| 0.2% ascorbic acid | 35.1 | 52.4 |
| 4% trichloroacetic acid | 2.6 | 6.6 |
| 0.2% sodium bisulphite | 0.2 | 1.7 |

*Distillation efficiency*

The mean distillation efficiency for the different volatiles is listed in Table 2. The percentage error with regard to the mean distillation efficiency varies mostly between 6 and 10. A greater accuracy was possible by expressing the efficiency in function of the volume of the distillate. As illustrated in Fig. 2, for a few compounds, a good linearity between the final volume after 1.5 hr distillation and the distillation efficiency has been established. The regression function and the standard deviation around the fitted regression line for each compound are also given in Table 2. By the use of these equations it was possible to determine with great accuracy the distillation efficiency for the different volatiles in the apple samples.

**Table 2.** Mean distillation efficiency with standard deviation and regression equation with standard deviation about the fitted regression line of the efficiency in function of the volume of the distillate (number of observations: 10; range of distillate volumes: 12.2–26.0)

| Volatiles | Mean dist. efficiency | $S_{\bar{y}}$ | Regression equation | $S_{\hat{y}}$ |
|---|---|---|---|---|
| Ethanol | 57.03 | 3.96 | $y = 38.78 + 0.85\,x$ | 2.01 |
| n-propanol | 66.02 | 5.51 | $y = 41.38 + 1.15\,x$ | 3.07 |
| n-butanol | 65.93 | 5.72 | $y = 38.01 + 1.31\,x$ | 2.26 |
| n-pentanol | 66.24 | 6.23 | $y = 33.72 + 1.42\,x$ | 1.77 |
| n-hexanol | 69.02 | 6.53 | $y = 37.52 + 1.47\,x$ | 2.74 |
| i-propanol | 69.71 | 4.81 | $y = 47.01 + 1.06\,x$ | 2.23 |
| i-butanol | 70.15 | 4.50 | $y = 45.35 + 1.12\,x$ | 2.03 |
| i-pentanol | 72.26 | 5.35 | $y = 47.35 + 1.20\,x$ | 1.95 |
| Acetaldehyde | 81.03 | 6.61 | $y = 47.98 + 1.55\,x$ | 2.16 |
| n-propanal | 87.29 | 8.19 | $y = 45.21 + 1.97\,x$ | 1.81 |
| n-butanal | 87.81 | 7.83 | $y = 46.93 + 1.99\,x$ | 1.60 |
| Acetone | 93.13 | 5.99 | $y = 62.29 + 1.44\,x$ | 1.29 |
| Diethylether | 73.16 | 12.05 | $y = 12.05 + 2.86\,x$ | 3.32 |
| Ethyl butyrate | 82.83 | 9.71 | $y = 35.13 + 2.23\,x$ | 3.66 |

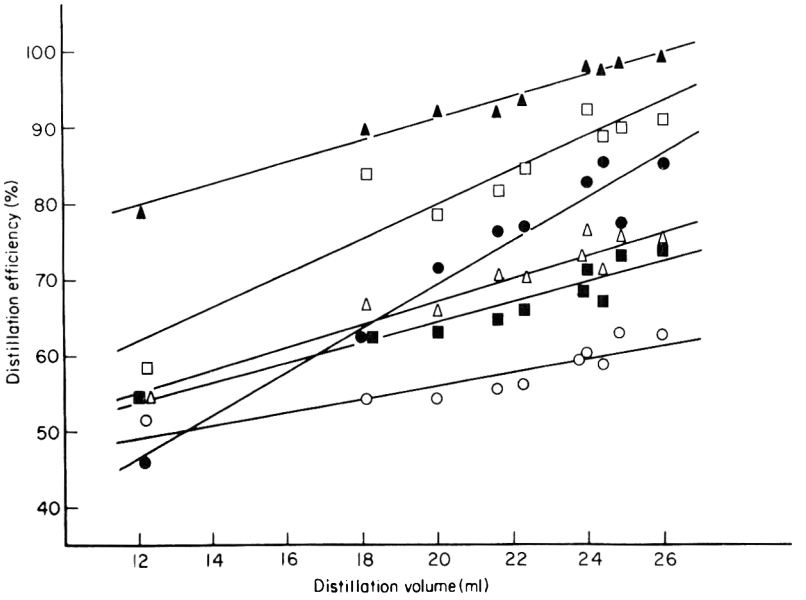


**Figure 2.** Relation between distillation efficiency and volume of the distillate after 1.5 hr distillation for a few volatiles. ▲ Acetone; □, ethylbutyrate; ●, diethylether; △ n-hexanol; ■. n-butanol; ○, ethanol.

The results indicate that the recovery of the different compounds depends on their air/water partition coefficient. Buttery, Ling & Guadagni (1969) and Buttery *et al.* (1971) showed that with each homologous series of compounds the air/water partition coefficient and hence the volatility increases with increasing carbon chain length. The methylesters and aliphatic aldehydes are the most volatile series, the methylketones have intermediary values and the alcohols are the least volatile because these are very soluble in water. The distillation efficiency is considerably higher for the aldehydes than for the n-alcohols and i-alcohols. Also within these homologous series of compounds, there is an increase from the shorter to the longer carbon chains, although the sequence within each group is not always logical. The relative concentration of the different compounds in the solution is certainly also a determining factor. The dependence of the distillation efficiency on the volume of the distillate and as a result on the distillation conditions, as expressed by the slope of the regression line, increases also with the volatility of the compounds in the aqueous solution. This value is very high for diethylether. This explains the high standard deviation for this compound when determining the mean distillation efficiency.

*Identification and concentration of the volatiles in 'Boskoop' apples*

The volatiles from the apple variety 'Schone van Boskoop' were identified on different columns by comparison with known compounds. The identification

**Table 3.** Volatiles from the apple variety 'Schone van Boskoop' and concentration of the most important compounds after 10 weeks storage at 3°C under normal atmospheric conditions

| Volatiles | p.p.m. (dry wt) |
|---|---|
| Diethylether | 0.7 |
| Acetaldehyde | 3.5 |
| n-propanal | 0.3 |
| Acetone | 0.6 |
| n-butanal | 2.7 |
| i-propanol | 0.6 |
| Ethanol | 32.4 |
| n-propanol | 12.1 |
| Ethyl butyrate | 2.0 |
| i-butanol | 5.5 |
| n-butanol | 263.8 |
| i-pentanol | 3.5 |
| n-pentanol | 3.7 |
| n-hexanol | 29.9 |

† traces of: ethyl propionate, methyl butyrate, ethyl-2-methyl butyrate, propyl butyrate, propyl-2-methyl butyrate, butyl butyrate, ethyl hexanoate, butyl-2-methyl butyrate.

was confirmed by a mass spectrometric analysis. A few quantitatively minor compounds could be identified in this way. The results are given in Table 3. The concentration was determined based on the distillation efficiency and the peak area/μg for each compound. The results clearly show that 'Boskoop' is a typical 'alcohol type' variety. More than 90% of the total amount of volatiles are alcohols. At the time of analysis n-butanol represents about 70% of the volatile fraction. Ethyl butyrate was the only ester which could be detected in a relatively high concentration. The other compounds identified by mass spectrometry were mostly butyrate esters. The concentration could not be determined but must be less than 0.05 ppm. This does not mean that these compounds are not important for the typical aroma of the apple variety 'Schone van Boskoop'. Paillard (1967) also found ethanol, ethyl butyrate, i-butanol, n-butanol and i-pentanol in the volatile fraction of 'Boskoop' apples. She also indicated the presence of ethyl acetate, butyl acetate and i-pentyl acetate. However it could be clearly shown that no acetate esters are present in our aroma distillate. Grevers & Doesburg (1965) and Paillard (1967) also mention that the amount of volatiles produced by 'Schone van Boskoop' apples is extremely small so that the study of the aroma of this variety is difficult.

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# Methods of measuring green colour and chlorophyll content of apple fruit

M. KNEE

## Summary

The change in colour from green to yellow during ripening of apples depends essentially upon disappearance of chlorophyll. Chlorophyll concentration in the fruit peel can be estimated by extraction and spectrophotometry or in terms of the reflectance of the apple surface at 675 nm. The reflectance and chlorophyll concentration data do not conform to the Kubelka-Munk equation. Reflectance at 675 nm is highly correlated with colour descriptions derived from reflectance spectra. Although a reflectance spectrophotometer gave the most precise reflectance measurements, a simple colorimetric device was modified to give nearly equivalent results.

## Introduction

The surface colour of fruit has been measured in three ways (1) by extraction and estimation of pigments, (2) by use of instruments which measure surface reflectance or (3) by visual matching against colour cards. The method chosen has varied with the nature of the experiment; physiological studies have tended to employ method (1) while studies related more directly to commercial application have employed methods (2) or (3). There has been little effort devoted to relating one type of measurement to another so that evidence is lacking about the extent to which differences in pigment composition would be apparent as colour differences or *vice versa*. Where colours have been measured by matching against cards whose colours cannot be specified on an established system of description, or by use of arbitrarily calibrated instruments, comparisons are impossible and such methods are to be deprecated. The present paper reports attempts to correlate measurement on apple fruit of extracted pigments, surface reflectance, either at specific wavelengths or integrated as CIE coordinates, and visual comparison with Munsell colour cards. The basic instru-

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ment employed for reflectance studies was a double beam reflectance spectrophotometer but the work led to the modification of an inexpensive reflectance colorimeter for similar measurements.

## Materials and methods

### *Fruit*

Most of the work was carried out with apple fruits (*Malus domestica* Borkh. of the variety Cox's Orange Pippin) grown at East Malling Research Station, either during development on the tree or during storage under conditions specified below.

### *Pigment analysis*

Chlorophyll was estimated in acetone extracts of fruit peel as described by Knee (1973).

### *Reflectance*

All reflectance measurements were made on the surface of intact fruit. A Beckman DBGT spectrophotometer with an integrating sphere reflectance accessory (DB-RF) was used for measurement of reflectance both at individual wavelengths and of spectra between 400 and 750 nm, relative to a magnesium oxide coated reference disc. Spectral reflectance data were transformed to CIE Co-ordinates under illuminant C, using weighting factors for readings at 5 nm intervals (Wright, 1958), by a BASIC computer program. A reflectance colorimeter (formerly manufactured by Evans Electroselenium Ltd. now available as Model 99 from Diffusion Systems Ltd., London, England) was used to obtain CIE readings using a 'filter wheel' with XYZ filters, again using the magnesium oxide reference disc. This instrument was later modified by fixing an acetate polarizing filter (Gallenkamp Ltd.) over the photocell with adhesive tape; this was oriented to reject light polarized in the plane of incidence. A 680 nm interference filter was obtained from Altec Laboratory Supplies and Systems Ltd., Alton, Hampshire.

### *Colour matching*

Visual matching of apples with Munsell colour cards (Tintometer Ltd, Salisbury, England) was carried out under white fluorescent lights. The colour vision of the observers was normal according to a standard series of test cards (Ishihara, 1973).

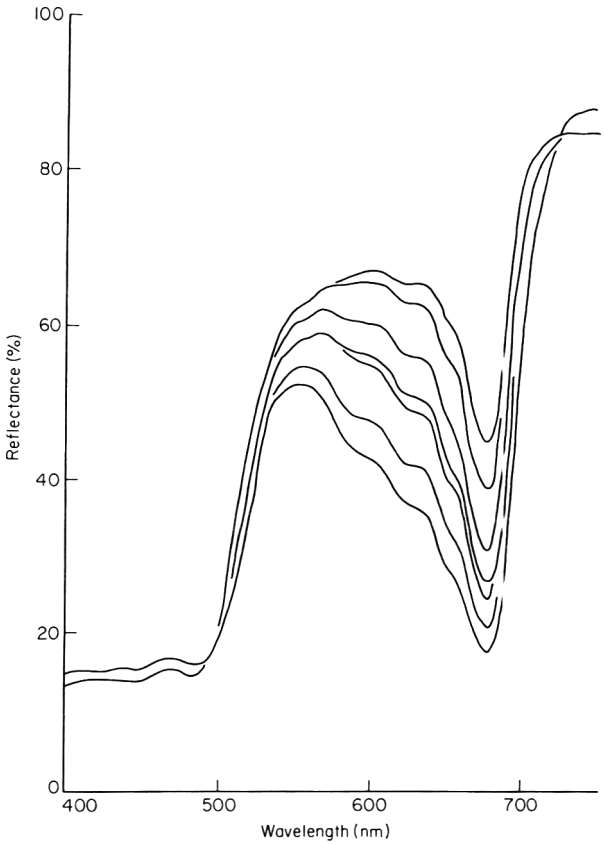


**Figure 1.** Reflectance spectra of a single Cox's Orange Pippin apple during ripening in air at 12°C. The lowest curve was recorded at harvest (12 September 1972) and the succeeding curves upwards were recorded after storage for 22, 31, 37, 45, 55 and 65 days.

## Results and discussion

### *Reflectance changes during ripening*

As apples ripen they lose chlorophyll and certain varieties including Cox, accumulate carotenoids (Knee, 1972). Examination of reflectance spectra from individual Cox apples ripening in store at 12°C showed that the greatest change was an increase in reflectance at 675 nm which was attributed to loss of chlorophyll. Progressively smaller increases in reflectance occurred at lower wavelengths down to about 520 nm und these were also ascribed to chlorophyll loss. Reflectance between 400–500 nm was about 20% and did not change; it was concluded that the increase in carotenoids, which absorb in this region, did not affect the reflectance spectrum (Fig. 1).

Red colour does not increase on apples in store but comparison of red and

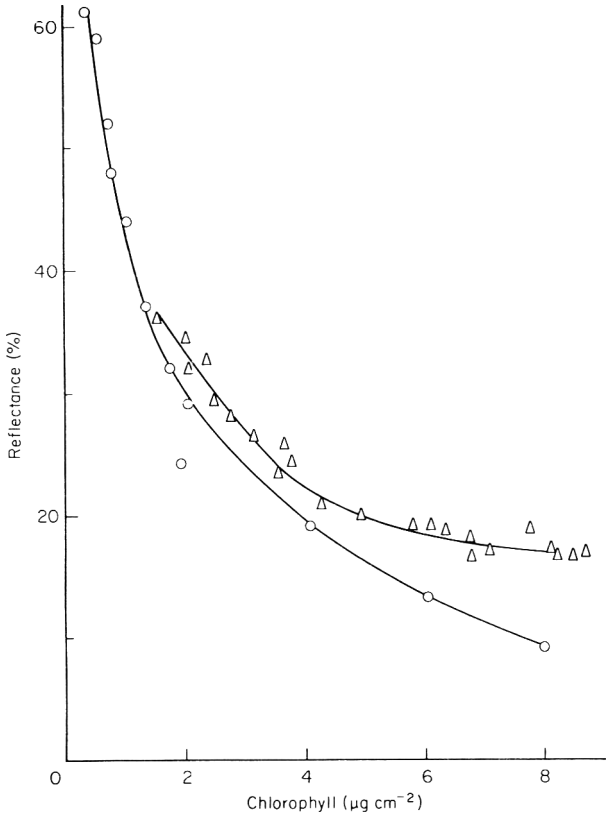


**Figure 2.** Relationship between reflectance at 675 nm and peel chlorophyll content of Cox's Orange Pippin apples. △, each point represents a sample of ten apples from weekly harvests of fruit 21 August–26 October 1972. Reflectance measured with spectrophotometer. ○, each point represents five fruit selected to have the same reflectance from samples stored at various temperatures for 2 months after harvest. Reflectance measured with colorimeter.

green surfaces showed that the biggest difference occurred at 550–570 nm. These observations are all in keeping with previously published work (Lott, 1943, 1944: Brearly & Breeze, 1966).

*Instrumental comparisons*

The most direct comparison that could be made between the spectrophotometer and colorimeter was in monochromatic measurements. The reflectance of a series of apples from green to yellow was compared at 675 nm on the spectrophotometer and using the filters, EEL 607 and 608 on the colorimeter. The apples showed a spread of reflectance values at 675 nm of from 20 to 50% but with the 607 filter the range was 80–90% and with the 608 filter 75–90%. Possibly, the colorimeter values were high because the angle of illumination in the instrument was acute; this would be expected to lead to low

penetration of the incident beam and the reflection of a large proportion as 'glare' from the glossy surface of the apple. Light reflected in this way is polarized in the plane of the incident beam and to eliminate this component a polarizing filter was placed over the photo-cell. This lowered and widened the range of readings obtained to 40–65% with the 607 filter and 35–55% with the 608 filter, corresponding to a range of 15–30% reflectance at 675 nm.

Regression analysis of 25 colorimeter readings ($y$) against reflectances at 675 nm ($x$) gave equations for the 607 filter,

$$y = 1.51x + 17.5 \ (r = 0.935);$$

for the 608 filter,

$$y = 1.47x + 10.9 \ (r = 0.940).$$

The deviations from a 45° slope and from the origin were ascribed to the broad wave band transmitted by the filters. As an improvement an interference filter with maximum transmission at 680 nm and 50% maximum at ±5 nm was fitted in the colorimeter in place of the usual filters.

Readings obtained with a range of apples from green to yellow were compared on the modified colorimeter and spectrophotometer. The readings obtained on the two instruments were almost identical over the range of 20–60% reflectance and the regression equation was

$$y = 1.01x - 1.4 \ (r = 0.993)$$

Below 20% the instruments deviated increasingly, the colorimeter giving lower values than the spectrophotometer. Possible explanations for this are suggested by comparison of pigment concentrations and reflectance values (see below).

*Reflectance and pigment content*

Reflectance at 675 nm and chlorophyll content showed an inverse curvilinear relationship (Fig. 2) so that reflectance measurements were most sensitive at low chlorophyll concentrations. This calibration of reflectance measurements cannot be regarded as absolute since it varied from one apple variety to another and even to some extent for different batches of the same variety; presumably surface features of the apple, such as russetting, influence the results. Above 4–5 μg $cm^{-2}$ the spectrophotometer recorded little change in reflectance with increasing pigment concentration, but the colorimeter was more responsive. Although the exact basis is not clear, the implication is that the modified colorimeter could be used to detect a wider range of concentration changes than the spectrophotometer.

Theoretically a linear relationship of reflectance ($R$) and pigment concentration in molar terms ($C$) can be obtained according to the Kubelka-Munk equation (Frei & MacNeill, 1973):-

$$\frac{(1-R)^2}{2R} = \frac{C}{K}$$

where K is a constant determined by the extinction coefficient of the pigment and the light scattering properties of the surface. A rigorous interpretation would require that the pigment should be dispersed homogeneously in a non absorbing diluent material. This is not the case for chlorophyll in the surface cells of an apple, and the relationship of concentration to $(1-R)^2/2R$ proved to be curvilinear, the slope being convex for the spectrophotometer and concave for the colorimeter.

*Colour measurement*

Reflectance spectra obtained with the spectrophotometer can be transformed to colour descriptions on the CIE system using tables derived from the chromaticity co-ordinates of $X$, $Y$ and $Z$. When weighted by the energy values of illuminant $C$ and converted to co-ordinates $x$ and $y$ comparison can be made with the Munsell colour system (Newhall, Nickerson & Judd, 1943). This procedure was tested on nineteen apples ranging in reflectance at 675 nm from 20 to 60%. The colour description generated corresponded to the nearest hue judged by visual matching of cards in fourteen cases. The calculated hue showed a strong inverse correlation (coefficient –0.931) with reflectance at 675 nm (Fig. 3).

In its unmodified state the colorimeter gave brightness ($Y$) values 20 to 30% higher and the hues were consistently displaced towards the red relative to results obtained with the spectrophotometer. After modification by inclusion of the polarizing filter the $Y$ values measured agreed well with those obtained on the spectrophotometer (correlation coefficient for 9 observations was 0.92, slope of line 1.05, passing within 1% reflectance of the origin). However the colour descriptions obtained were still displaced towards the red end of the spectrum.

Attempts were made to determine how the bias arose from the spectral properties of the filters and the lamp in the colorimeter but this approach met with no success. Colorimeter and spectrophotometer data for reflectance of eight Munsell colour cards covering the range of colours most usually found on apples (Hue 5GY to 5Y, value 5–7, chroma 6–10) were compared. The only significant difference between the readings obtained with the two instruments was that $x$ values were higher when measured with the colorimeter. This seemed to be a proportional difference and an empirical correction factor of 0.934 was derived from the data. Reflectance values for apples with the $x$ filter were

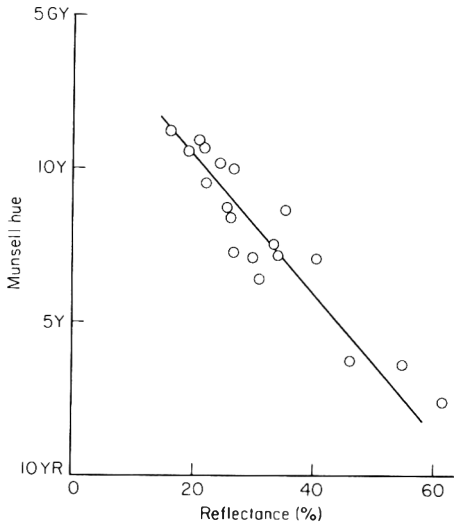


**Figure 3.** Relationship between colour description calculated from reflectance spectrum and reflectance at 675 nm for Cox's Orange Pippin apples stored in air, 2% $O_2$ and 1.25% $O_2$ for 6 months.

multiplied by this factor and CIE co-ordinates recalculated. This resulted in most colour descriptions being displaced towards green relative to the spectrophotometer values. Colour descriptions produced with the colorimeter were compared with visual assessments for twenty-five apples. Before adjustment with the $x$ correction factor the colorimeter values were displaced by an average of 1.3 Munsell hue units towards the red; after adjustment they were displaced 1.9 units towards the green. It was concluded that there was no point in adjusting $x$ values and that the remaining errors of the colorimeter would have to be accepted as a consequence of the compromises in design inherent in such a simple apparatus.

## Conclusions

Colour descriptions, reflectance values and chlorophyll concentrations in apple fruit peel can be related on scales as shown in Fig. 4. These relationships are confined to Cox's Orange Pippin and would vary for other varieties with different surface features.

Reflectance at 675 nm gives the best relative measurement of the progress of colour change within a batch of apples of a given variety in the course of storage and ripening.

Absolute reflectance data which can be converted into precise colour descriptions can only be obtained with a variable wavelength spectrophotometer. However a simple colorimeter can be used for more rapid and economical

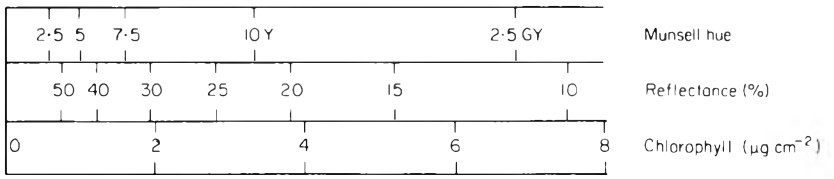


**Figure 4.** Relationship between scales of peel chlorophyll content, reflectance at 675 nm and hue on Munsell scale for Cox's Orange Pippin apples.

measurements when modified and calibrated as described above. This instrument could be applicable with other pigments in other materials, but would require empirical validation for each application.

## Acknowledgments

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# Stability and use of natural colours in foods
## Red beet powder, copper chlorophyll powder and cochineal

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## Summary

Three natural pigments – red beet powder, chlorophyll powder (in the form of a copper complex) and aqueous cochineal – were examined in order to understand better their behaviour or colouring ability in different model systems. All were heat labile but red beet colour in particular was rapidly degraded above about 14°C. Cochineal proved the most stable of the three colours. All were adversely affected by changes in pH and a reduction in water activity gave a corresponding increase in stability for each colour. Light was similarly shown to cause degradation of the natural colours. The colours were used in food products and shown to have certain applications with regard to the colour of jam and restoring the colour of peas after blanching.


## Introduction

Although the nutritional value of food is of great importance, its appeal is determined by its taste, appearance and smell rather than its food value and it has been reported that appearance and smell stimulate the flow of digestive juice and thus aid digestion (Brian & Cameron, 1977). It is thus obvious that colour is one of the major factors that determines the acceptability of a food. The natural colours of foods (chlorophylls, carotenoids etc.) are easily degraded by process and storage conditions and in order to make foods more attractive and produce a standard product artificial colours are often added during food manufacture.

Unfortunately, it has recently been shown that not all the synthetic food dyes are harmless when ingested and future trends may be towards the use of natural food colourants as more and more artificial colours are removed from the

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**Table 1.** Occurrence of natural food colours

| Pigment | Colour | Source | Solubility |
|---|---|---|---|
| Chlorophyll | Green/blue | Green vegetables, grass, stems, leaves | Oil soluble |
| Carotenoids<br>lycopene<br>α carotene<br>β carotene<br>xanthophylls | Red/yellow/orange | Carrots, tomatoes, red/yellow fruits, dairy products, green vegetables, rose hips | Oil soluble |
| Anthocyanins<br>flavonoids<br>betanins (beet colour)<br>anthoxanthins | Red/blue/yellow | Red cabbage, grapes, fruit | Water soluble |
| Haemoglobin | Red | Blood, myoglobin | Water soluble |

permitted list. This does not necessarily indicate that natural colours are harmless however, since little work has been carried out on their toxicity although the use of some natural colours in foods has been studied extensively (Buckmire & Francis, 1978; Clydesdale *et al.*, 1978; Jackson, 1976; Pasch & von Elbe, 1977). Similarly, methods of preservation of the natural colours in foods has received attention (Timberlake & Bridle, 1977; Shewfelt & Ahmed, 1978; Calvi & Francis, 1978).

The more important groups of naturally occurring plant pigments which appear to be of commercial interest as food colourants are given in Table 1. It has been determined that nature produces 100 million tons of carotenoids annually and the potential sources of certain pigments are not only very abundant but renewable.

The stability of these natural colours to changes in temperature and pH for example varies, although they are generally more labile than synthetic dyes. Although work has been carried out on the use of natural colours in foods and many problems overcome there are still certain facets of their use which require attention.

It was thus the object of this investigation to evaluate three naturally occurring colours for use in certain foods by assessing their stability under different environmental conditions.

## Materials

The three natural colours used in the study were: (1) water soluble copper chlorophyll powder – green colour; (2) water soluble beet powder – red colour; (3) concentrated, aqueous cochineal solution – red colour. These were obtained from Bush Boake Allen Ltd, London and we gratefully acknowledge the gift of these colours.

Two artificial colours were used in certain parts of the study for comparison purposes: (1) Edicol green S (green CL4); (2) Edicol amaranth (red CL9). These were obtained from I.C.I. Ltd, Manchester and we gratefully acknowledge the gift of these colours.

All other reagents were obtained from BDH Ltd, Dorset.

## Methods and results

(1) *Absorption spectra*

The wavelength of maximum absorption (λ max) was determined for each colour using a Unicam SP 800 spectrophotometer. All subsequent measurements were then made either using this instrument or a Unicam SP 500 spectrophotometer.

(2) *Consumer panel measurements*

The colour of jam and frozen pea samples was evaluated using a 12 and 10 membered panel respectively. The samples were scored on a 9 point scale for colour acceptability with 1 point corresponding to a highly unacceptable colour and 9 points a highly desirable colour. t-tests were then carried out on the results to establish any significant differences between samples using different colours in their composition.

(3) *Effect of temperature on colour stability*

Four temperatures were selected for this study, 20, 50, 75 and 100°C, and metal heating blocks were used to produce these temperatures. A known concentration of each colour was prepared in distilled water as shown in Table 2 and a known volume introduced into a sample test tube. A condenser was placed in the top of the tube and the contents heated for up to 4 hr at each

**Table 2.** Concentrations and $\lambda_{max}$ of colours used for temperature sensitivity studies

| Colour | Concentration (g/100 ml) | $\lambda_{max}$ (nm) |
|---|---|---|
| Copper chlorophyll | 0.01 | 530 |
| Red beet | 0.08 | 535 |
| Cochineal | 0.7* | 518 |
| Green S | 0.0015 | 534 |
| Amaranth | 0.0015 | 522 |

*ml/100 ml

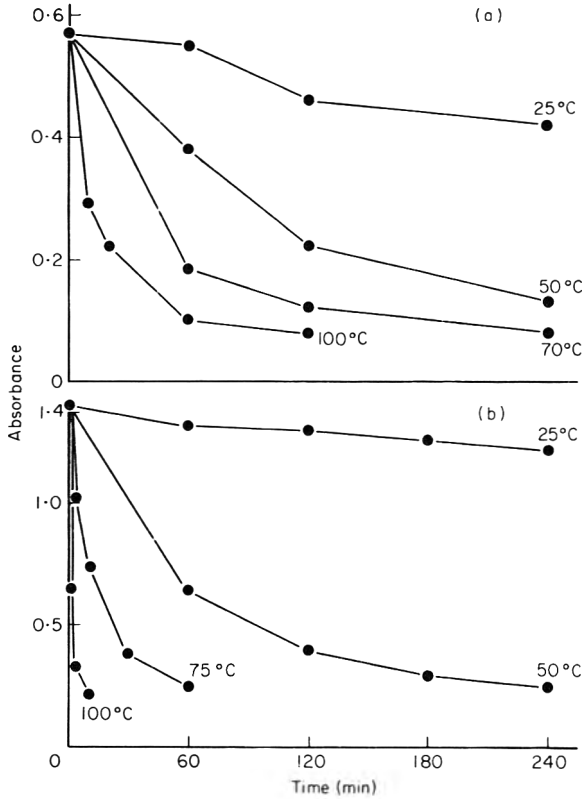


**Figure 1.** Effect of temperature on colour stability. (a). Copper chlorophyll; (b). red beet.

temperature. The absorbance of each solution was measured at the wavelength of maximum absorption (Table 2) at time zero and then at predetermined intervals throughout the test.

The results for all the colours are given in Figs. 1 and 2.

(4) *Effect of pH on colour stability*

The stability of the colours was examined at different pH values by adding a known volume of an aqueous solution of each colour to citrate-phosphate buffers in the range pH 2.2–8.0. Sodium benzoate (0.05%) was incorporated into each buffered solution to prevent microbial growth and the solutions introduced into separate heat sealable 10 ml ampoules. After sealing the ampoules were stored at 7°C in the dark and absorbance readings were taken initially and for up to 96 h. To investigate the effect of temperature at each pH the colours were also stored at elevated temperatures in tubes fitted with condensers. The concentrations of the colours and storage data are included in the results in Tables 3 and 4.

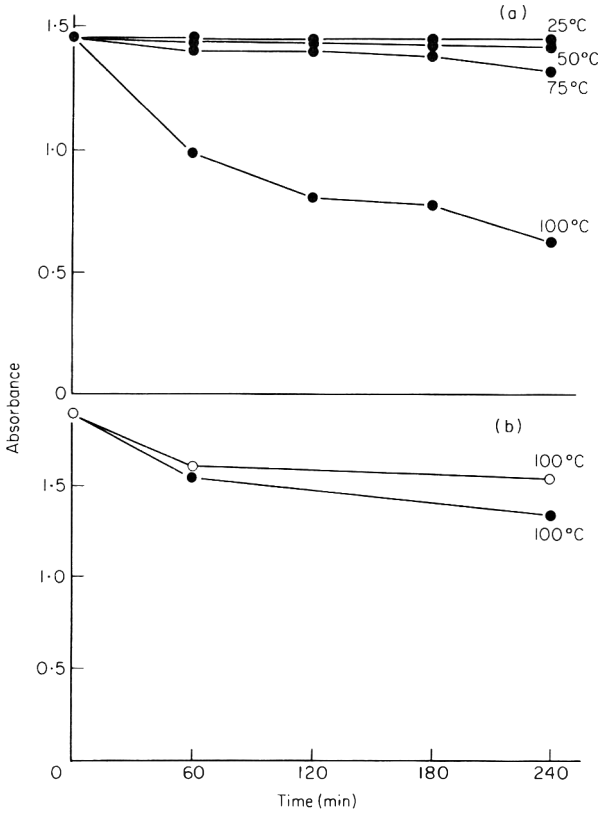


**Figure 2.** Effect of temperature on colour stability. (a), Cochineal; (b), O, amaranth; ●, green S.

(5) *Effect of water activity on colour stability*

Model systems with a water activity from 1.00 to 0.37 were prepared using water and glycerol (Pasch & von Elbe, 1975). Solutions of each colour were prepared at each water activity and incubated at elevated temperature in test tubes fitted with condensers. Absorbance readings were taken initially and for up to 2.5 hr thereafter. The results are shown in Table 5.

(6) *Effect of light on colour stability*

This study was carried out at 25°C and pH 4.0 for red beet powder and 25°C and pH 5.0 for copper chlorophyll and cochineal. Sodium benzoate (0.05%) was incorporated to prevent microbial growth and the solutions placed in heat sealable ampoules. Half the ampoules were exposed to light and the other half kept in dark storage. Absorbance readings were taken for up to 144 hr and the results are shown in Fig. 3. The concentrations of the colours were as in the pH determination experiment.

**Table 3.** Effect of pH on colour stability (absorbance) at 7°C

| pH | Colour and time of exposure | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Red beet powder | | | Copper chlorophyll | | | | Cochineal | | |
| | 0 hr | 24 hr | 48 hr | 0 hr | 24 hr | 48 hr | 96 hr | 0 hr | 24 hr | 48 hr |
| 2.2 | 0.86 | 0.61 | 0.52 | — | — | — | — | 1.08 | 0.98 | 0.96 |
| 3.0 | 0.91 | 0.73 | 0.63 | — | — | — | — | 1.30 | 0.98 | 0.94 |
| 4.0 | 0.93 | 0.77 | 0.65 | 0.32 | 0.25 | 0.24 | 0.23 | 1.44 | 1.11 | 0.98 |
| 5.0 | 0.80 | 0.55 | 0.46 | 0.36 | 0.29 | 0.25 | 0.23 | 1.53 | 1.26 | 1.13 |
| 6.0 | 0.72 | 0.44 | 0.35 | 0.39 | 0.22 | 0.17 | 0.14 | 1.60 | 1.43 | 1.35 |
| 7.0 | 0.69 | 0.39 | 0.30 | 0.45 | 0.24 | 0.17 | 0.14 | 1.65 | 1.58 | 1.56 |
| 8.0 | 0.48 | 0.33 | 0.24 | 0.51 | 0.32 | 0.24 | 0.19 | 1.62 | 1.61 | 1.58 |

Red beet concentration = 0.16 g/100 ml.
Copper chlorophyll concentration = 0.005 g/100 ml.
Cochineal concentration = 0.8 ml/100 ml.

## (7) *Effect of storage temperature on the stability of red beet colour*

The stability of the colour over the range 7–37°C was examined since this was to represent likely environmental temperatures. Sodium benzoate (0.05%) was added to each ampoule to prevent microbial growth along with a known concentration of the red beet colour. All solutions were maintained at pH 4.0 in the dark for 96 hr at each temperature. Absorbance readings were taken initially and then after 24 and 96 hr and the results are given in Table 6.

**Table 4.** Effect of pH on colour stability (absorbance) at elevated temperature*

| pH | Colour and time of exposure | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Red beet powder | | | | Copper chlorophyll | | | | Cochineal | | |
| | 0 hr | 0.7 hr | 2.0 hr | 3.1 hr | 0 hr | 0.8 hr | 2.0 hr | 5.0 hr | 0 hr | 2.0 hr | 4.0 hr |
| 2.2 | 1.00 | 0.70 | 0.48 | 0.42 | — | — | — | — | 1.12 | 1.00 | 0.94 |
| 3.0 | 1.08 | 0.95 | 0.75 | 0.64 | — | — | — | — | 1.17 | 1.04 | 0.92 |
| 4.0 | 1.05 | 0.94 | 0.71 | 0.66 | 0.36 | 0.33 | 0.18 | 0.12 | 1.38 | 1.02 | 1.02 |
| 5.0 | 0.93 | 0.68 | 0.51 | 0.46 | 0.39 | 0.37 | 0.33 | 0.26 | 1.48 | 0.92 | 0.85 |
| 6.0 | 0.82 | 0.55 | 0.45 | 0.40 | 0.43 | 0.38 | 0.29 | 0.16 | 1.52 | 0.88 | 0.76 |
| 7.0 | 0.64 | 0.45 | 0.38 | 0.30 | 0.53 | 0.46 | 0.36 | 0.18 | 1.63 | 0.76 | 0.68 |
| 8.0 | 0.50 | 0.37 | 0.30 | 0.23 | 0.61 | 0.51 | 0.43 | 0.26 | 1.56 | 0.65 | 0.46 |

Red beet concentration = 0.18 g/100 ml.
Copper chlorophyll concentration = 0.006 g/100 ml.
Cochineal concentration = 0.8 ml/100 ml.

*40°C for red beet and copper chlorophyll, 100°C for cochineal.

**Table 5.** Effect of water activity ($a_w$) on colour stability (absorbance) at elevated temperature*

| $a_w$ | Colour and time of exposure | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Red beet powder | | | | Copper chlorophyll | | | | Cochineal | | |
| | 0 hr | 1.0 hr | 2.0 hr | 2.5 hr | 0 hr | 0.7 hr | 1.2 hr | 2.5 hr | 0 hr | 1.0 hr | 2.0 hr |
| 1.00 | 1.64 | 1.35 | 1.09 | 0.99 | 0.49 | 0.31 | 0.20 | 0.10 | 1.60 | 0.76 | 0.71 |
| 0.95 | 1.56 | 1.33 | 1.09 | 0.98 | 0.49 | 0.33 | 0.22 | 0.11 | 1.20 | 0.78 | 0.73 |
| 0.87 | 1.60 | 1.36 | 1.13 | 1.05 | 0.53 | 0.30 | 0.20 | 0.12 | 1.10 | 1.08 | 1.03 |
| 0.74 | 1.58 | 1.37 | 1.14 | 1.07 | 0.61 | 0.25 | 0.20 | 0.15 | 1.10 | 1.08 | 1.03 |
| 0.63 | 1.61 | 1.38 | 1.17 | 1.10 | 0.64 | 0.26 | 0.23 | 0.19 | 1.15 | 1.13 | 1.06 |
| 0.47 | 1.64 | 1.42 | 1.25 | 1.20 | 0.69 | 0.28 | 0.25 | 0.22 | 1.12 | 1.09 | 1.04 |
| 0.37 | 1.72 | 1.51 | 1.35 | 1.32 | 0.69 | 0.30 | 0.27 | 0.23 | 1.12 | 1.09 | 1.04 |

Red beet concentration = 0.29 g/100 ml.
Copper chlorophyll concentration = 0.008 g/100 ml.
Cochineal concentration = 0.8 ml/100 ml.

*40°C for red beet, 60°C for copper chlorophyll, 100°C for cochineal.

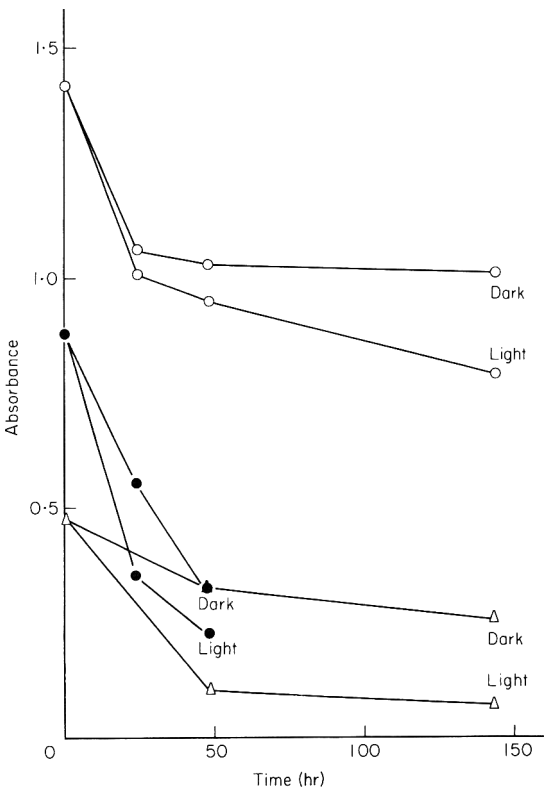


**Figure 3.** Effect of light on colour stability. ○, Cochineal; ●, red beet; △, chlorophyll.

**Table 6.** Effect of storage temperature on the stability (absorbance) of red beet powder

| | Time (hr) and absorbance | | |
|---|---|---|---|
| Temperature (°C) | 0 | 24 | 96 |
| 7 | 1.0 | 0.93 | 0.86 |
| 14 | 1.0 | 0.87 | 0.80 |
| 25 | 1.0 | 0.60 | 0.42 |
| 30 | 1.0 | 0.50 | 0.30 |
| 37 | 1.0 | 0.42 | 0.23 |

Red beet concentration = 0.18 g/100 ml.

**Table 7.** Stability of colours (absorbance) in model food systems

| System code | Sucrose | Fructose | Glucose | Citric acid | Malic acid | Glycine | Tryptophan | Ascorbic acid | Sodium benzoate | Sodium metabisulphite | BHA* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| a | — | — | — | — | — | — | — | — | — | — | — |
| b | 16 | — | — | — | — | — | — | — | — | — | — |
| c | 8 | 4 | 4 | — | — | — | — | — | — | — | — |
| d | 8 | 4 | 4 | 0.4 | — | — | — | — | — | — | — |
| e | 8 | 4 | 4 | 0.2 | 0.2 | — | — | — | — | — | — |
| f | 8 | 4 | 4 | — | — | 0.08 | 0.08 | — | — | — | — |
| g | 8 | 4 | 4 | — | — | — | — | 0.04 | — | — | — |
| h | 8 | 4 | 4 | — | — | — | — | — | 0.05 | — | — |
| j | 8 | 4 | 4 | — | — | — | — | — | — | 0.05 | — |
| k | 8 | 4 | 4 | — | — | — | — | — | — | — | 0.05 |
| l | 8 | 4 | 4 | — | — | — | — | 0.01 | — | — | — |
| m | 8 | 4 | 4 | — | — | — | — | 0.02 | — | — | — |
| n | 8 | 4 | 4 | — | — | — | — | 0.03 | — | — | — |

*BHA = butylated hydroxy anisole.
Concentration of all system components is in g/100 ml.

## (8) *Stability of colours in model systems*

Model food systems containing carbohydrate, organic acids, amino acids, ascorbic acid, preservatives and anti-oxidant were made up as in Table 7. Red beet and copper chlorophyll were used in this study, the former incubated at 50°C and the latter at 55°C and absorbance readings taken for up to 3.2 hr. The results are given in Table 8.

**Table 8.** Stability (absorbance) of colours in model systems

| System | Colour and time of exposure | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Red beet | | | | Copper chlorophyll | | | |
| | 0 hr | 0.7 hr | 1.9 hr | 3.2 hr | 0 hr | 1.1 hr | 1.8 hr | 2.7 hr |
| a | 0.86 | 0.56 | 0.35 | 0.26 | 0.80 | 0.33 | 0.25 | 0.17 |
| b | 0.86 | 0.65 | 0.38 | 0.27 | 0.86 | 0.49 | 0.39 | 0.28 |
| c | 0.86 | 0.58 | 0.35 | 0.27 | 0.85 | 0.58 | 0.52 | 0.43 |
| d | 0.82 | 0.52 | 0.32 | 0.13 | 0.36 | 0.19 | 0.18 | 0.17 |
| e | 0.82 | 0.54 | 0.31 | 0.13 | 0.38 | 0.19 | 0.18 | 0.18 |
| f | 0.84 | 0.54 | 0.35 | 0.27 | 0.72 | 0.49 | 0.44 | 0.38 |
| g | 0.84 | 0.75 | 0.64 | 0.58 | 0.49 | 0.29 | 0.29 | 0.27 |
| h | 0.86 | 0.53 | 0.35 | 0.30 | 0.75 | 0.50 | 0.47 | 0.41 |
| j | 0.85 | 0.65 | 0.43 | 0.28 | 0.50 | 0.47 | 0.45 | 0.41 |
| k | 0.86 | 0.55 | 0.35 | 0.28 | 0.76 | 0.56 | 0.52 | 0.45 |
| l | — | — | — | — | 0.46 | 0.42 | 0.41 | 0.40 |
| m | — | — | — | — | 0.46 | 0.40 | 0.39 | 0.39 |
| n | — | — | — | — | 0.46 | 0.40 | 0.39 | 0.39 |

(9) *Evaluation of red beet and cochineal as food colourants*

Red beet and cochineal were investigated as possible colourants in jam using amaranth for reference purposes. Apples were chosen as a neutral base for the jam to show up any colour differences more clearly. The apples were peeled and placed in a 1% sulphite solution to prevent browning before use and then boiled together with sucrose and pectin to a soluble solids content of about 70% w/v. The two natural and one synthetic colours were then added to separate portions of the jam last of all to prevent unnecessary heat damage to the colours and the whole mixed thoroughly and cooled. The colour concentrations were: red beet, 0.08 g/100 ml; cochineal, 0.7 ml/100 ml and amaranth, 0.0015 g/100 ml.

Ascorbic acid was also added after the boiling, with the colour, at a concentration of 0.05% in the final product. Eight jars of jam were prepared containing each colour, half were stored at 14°C and half at 25°C, both in the dark. The temperature was selected to show that red beet degrades more rapidly above 14°C. Colour measurements were made initially and at monthly intervals for 3 months as follows.

Twenty-five grams of each sample were placed in a 100 ml volumetric flask, diluted to volume with distilled water and filtered. Absorbance readings were taken on the filtrate. Colour was also evaluated using a consumer panel to judge the colour acceptability of each jam sample. A chemical analysis was carried out on each sample initially and after the 3 months' storage and no significant changes were found to have occurred. Tables 9 and 10 give the results of the colour measurements and consumer panel measurements (with statistical analysis) respectively.

**Table 9.** Stability (absorbance) of red beet and cochineal in jam

| Time (days) | Colour and storage temperature | | | | | |
|---|---|---|---|---|---|---|
| | Red beet | | Cochineal | | Amaranth | |
| | 14°C | 25°C | 14°C | 25°C | 14°C | 25°C |
| 0 | 0.57 | 0.57 | 1.00 | 1.00 | 1.00 | 1.00 |
| 30 | 0.55 | 0.48 | 0.98 | 0.98 | 0.99 | 0.98 |
| 60 | 0.51 | 0.39 | 0.94 | 0.94 | 0.99 | 0.94 |
| 90 | 0.45 | 0.34 | 0.94 | 0.94 | 0.98 | 0.86 |

**Table 10.** Consumer panel assessment of colours in jam samples at 25°C and at 14°C

| Temperature | Colour | Mean scores (± s.d.) | | | |
|---|---|---|---|---|---|
| | | 0 days | 30 days | 60 days | 90 days |
| 25°C | Amaranth | 7.6 (± 1.32) | 7.6 (± 1.41) | 7.7 (± 1.06) | 7.8 (± 0.99) |
| | Red beet | 6.3 (± 0.25) | 6.2 (± 0.36) | 6.3 (± 1.07) | 4.6 (± 1.00) |
| | Cochineal | 6.5 (± 1.04) | 6.4 (± 1.45) | 6.0 (± 1.12) | 6.6 (± 1.27) |
| 14°C | Amaranth | As at 25°C | 7.6 (± 1.25) | 8.0 (± 0.57) | 7.6 (± 1.25) |
| | Red beet | As at 25°C | 6.3 (± 0.90) | 6.6 (± 1.13) | 6.6 (± 1.20) |
| | Cochineal | As at 25°C | 6.4 (± 0.89) | 6.2 (± 0.48) | 6.5 (± 1.29) |

n.s. = not significant

(10) *Evaluation of copper chlorophyll as a food colourant*

Copper chlorophyll was investigated as a possible colourant in frozen peas to replace the natural chlorophyll converted to phaeophytin during blanching. The use of copper chlorophyll is very limited because it degrades rapidly on heating and the experiment was thus designed to use copper chlorophyll to restore the green colour of blanched peas before freezing. Frozen peas were used as a source of the vegetable since fresh produce was not available but since the object of the study was to determine colour restoration this was not considered detrimental to the investigation. A 10 lb sample of peas was thawed and divided into three parts as follows: (i) one part immersed in boiling water for 1 min, (ii) one part immersed in boiling water for 1 min and then immersed in a copper chlorophyll solution (0.05 g/100 ml) for 2 min and (iii) one part immersed in boiling water for 1 min and then immersed in a copper chlorophyll solution (0.05 g/100 ml) with added ascorbic acid (0.1 g/100 ml) for 2 min.

Each part was then divided into four smaller samples which were placed in polythene bags and rapidly frozen to −20°C. The samples were stored at this temperature until required for evaluation.

Colour measurements (reflectance) were made initially and at monthly intervals for 3 months at a wavelength of 630 nm.

The colour was also evaluated at the same time intervals using a trained consumer panel as previously described. Tables 11 and 12 give the colour measurements and consumer panel measurements for each sample.

## Discussion

It has generally been recognized that temperature during processing is one of the most important factors which affects the stability of natural food colours whilst artificial colours are little affected by heat. Red beet colour proved to be very heat-sensitive and prolonged heating produced pale yellow solutions (betaxanthines). Increased temperature produced this effect more rapidly as previously reported (von Elbe & Maing, 1973). Copper chlorophyll also proved heat labile whilst cochineal was the most resistant to elevated temperatures. The synthetic dyes amaranth and green S proved more stable as expected.

Many natural colours are ionic in nature and may thus be affected by changes in pH of the environment. The effect of pH on the colour of beets (betanins) during processing has been reported (Lusas, Rice & Weckel, 1960; Habib & Brown, 1956) and the results showed that any deviation from the natural beet pH (5.5) caused dramatic loss of colour. The results obtained in the present study using red beet powder agree with these in part although even as high as pH 5.0 some colour loss was apparent with a maximum retention about pH 4.0. Copper chlorophyll dissolves in water giving an alkaline solution and acidification yields a precipitate of the pigment with subsequent colour reduction as shown in Table 3. As low as pH 4.0 agitation of the solution reproduced the

**Table 11.** Reflectance (transmittance) measurements of colour in frozen peas

| Treatment | Transmittance (%) | | | |
|---|---|---|---|---|
| | 0 days | 30 days | 60 days | 90 days |
| Boiling water | 19.0 | 18.5 | 18.0 | 18.0 |
| Boiling water then chlorophyll solution | 7.2 | 7.1 | 7.0 | 7.1 |
| Boiling water then chlorophyll/ascorbic acid solution | 7.3 | 7.2 | 7.1 | 7.2 |

**Table 12.** Consumer panel assessment of colour of frozen peas

| Treatment | Mean scores (± s.d.) | | | |
|---|---|---|---|---|
| | 0 days | 30 days | 60 days | 90 days |
| Boiling water | 5.3 (± 0.90) | 5.5 (± 0.50) | 5.4 (± 0.49) | 5.0 (± 0.78) |
| | $P \leq 0.001$ | $P \leq 0.001$ | $P \leq 0.001$ | $P \leq 0.001$ |
| Boiling water then chlorophyll solution | 7.5 (± 0.50) | 7.7 (± 0.64) | 7.6 (± 0.49) | 7.7 (± 0.64) |
| | n.s. | n.s. | n.s. | n.s. |
| Boiling water then chlorophyll/ascorbic acid solution | 7.7 (± 0.78) | 8.0 (± 0.63) | 7.9 (± 0.54) | 7.6 (± 0.49) |

$P \leq 0.001$ (0 days) | $P \leq 0.001$ (30 days) | $P \leq 0.001$ (60 days) | $P \leq 0.001$ (90 days)

n.s. = not significant

green colour but this again precipitated on standing. Similarly cochineal proved most stable at alkaline pH and precipitation was evident at low pH values.

Water activity is very important in biological systems and it has been reported that betanin stability increases with a decrease in water activity (Pasch & von Elbe, 1975). Our results for red beet powder agree with these findings and a similar trend was found in this present study for copper chlorophyll solutions. Cochineal, on the other hand, was little affected by changes in water activity in the environment.

It is known that many natural colours are sensitive to exposure to light and that a bleaching effect is often observed. Von Elbe *et al.* (1974) reported that betanin solutions are light labile and must be protected from over-exposure. Our results indicate the same trend for red beet powder, copper chlorophyll and cochineal with copper chlorophyll being particularly susceptible.

The temperature range of 7–37°C was meant to represent a likely environmental range to which the red beet colour might be exposed and the pH of 4.0 selected as being the pH at which red beet was the most stable. The results indicate as expected that increased temperature caused decrease in colour intensity with the greatest change above about 14°C.

Many food systems appear to provide a protective stabilizing effect on natural food colours, presumably as a direct consequence of their chemical composition. In the systems studied ascorbic acid appeared to have a protective effect on red beet colour but little protective effect on copper chlorophyll colour whilst no particular advantage appeared to be gained from the other systems. Malic acid in particular had a detrimental effect on red beet colour. A mixture of glucose, fructose and sucrose appeared to have a protective effect towards copper chlorophyll as did the amino acids/sugar system and sodium benzoate, metabisulphite and BHA.

Since red beet was shown to be unstable in high $a_w$ systems its use as a food colourant is obviously limited to those foods with a low $a_w$, of the correct pH and which are not subject to extreme heat processing. Red beet has been used successfully in gelatin desserts, meat substitutes, sausage, dairy products and confectionery (von Elbe & Maing, 1973; von Elbe *et al.*, 1974b; Pasch, von Elbe & Sell, 1975; von Elbe, 1977). This present study was designed to investigate the use of red beet (and also cochineal) in jam. Amaranth was used for control purposes. The results indicate that jam coloured with amaranth had a more acceptable colour than either cochineal or red beet and that no significant difference was shown between cochineal or red beet except after 90 days at elevated temperature, when the red beet colour was reduced in intensity. The panellists did not indicate that the naturally coloured jam was distasteful but they preferred the synthetic colour, as might have been predicted.

A slightly different application was tried using the copper chlorophyll in that the colour was intended to restore lost colour rather than to provide colour in the food. The results of the study suggest that copper chlorophyll will indeed restore successfully lost colour in blanched peas. The addition of ascorbic acid to the copper chlorophyll solutions had no greater effect than copper chlorophyll alone. Whilst the increase in green colour was more or less uniform throughout the samples a few peas had a two-tone colour.

## Conclusions

The results indicate that whilst natural food colours are heat labile a certain protection can be afforded by selection of a suitable pH for the system, selection of suitable water activity and the exclusion of light. Cochineal proved most stable of the colours examined and red beet the most labile especially at temperatures over 14°C. There are indications however that these colours could replace the synthetic dyes in certain applications.

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# Methods for the production of acceptable bread from storage-deteriorated flours

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## Summary

Three practical means of overcoming the problem of the production of acceptable bread from storage-deteriorated flour have been devised. These comprise (1) the use of extra fat in breadmaking, (2) the use of extra yeast to increase proof volume at constant time, and (3) extending proof-time. The first two of these methods are acceptable for use commercially and have been tested successfully in commercial as well as laboratory scale trials. The third method may be difficult to apply in automatic plant bakeries. Additives which were ineffective included gluten, soya, skimmed milk powder, and glyceryl monostearate. Other changes proving ineffectual included increased dough temperature, increased oxidant level and variations in dough water levels.

## Introduction

Prolonged storage of flour results in a deterioration in its baking performance, yielding loaves of poor volume and crumb structure which are commercially unacceptable (see Bell *et al.*, 1979). In the course of a collaborative study of changes in the composition and baking quality of flours during storage, carried out with the Food Laboratory (Norwich) of the Ministry of Agriculture Fisheries & Food (MAFF), means of restoring the quality of loaves made from badly-deteriorated flours to a commercially acceptable standard were devised, and submitted to laboratory, (Bell & Fisher, 1977; Bell *et al.*, 1979), and, in selected cases, commercial scale production trials.

Trials of both types were carried out by the Chorleywood Bread Process (CBP) (see, e.g. Chamberlain, 1969), since this is quantitatively the most important of the breadmaking processes in use in the U.K. The applicability of the restorative procedures to other breadmaking processes such as the traditional straight dough, long fermentation process (LFP; *loc. cit.*) and the Activated Dough Development Process (ADD; *loc. cit.*) is also discussed.

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## Materials and methods

Flours deteriorated as a result of prolonged storage at ambient temperature were obtained either from the stocks laid down specially for the collaborative trials or through the MAFF. For the former flours, designated weak, medium and strong, according to their original baking quality, controls stored at −20°C in an inert atmosphere were available for comparison. No 'controls' of this nature were available for the other flours. The 'medium' flour was made from a grist obtained by mixing equal weights of the wheats giving rise to the 'weak' and 'strong' flours. Full details of flours and storage conditions are given in Bell *et al.*, 1979.

The fat used in breadmaking was 'Covo' (slip point 42.5°C, van den Berghs and Jurgens Ltd., Burgess Hill, W. Sussex).

Recipe (parts): Flour 100; yeast 2.1; salt 1.8; fat 0.7 or 0; water as required by the water absorption of the flour (Bell *et al.*, 1979); ascorbic acid 30 parts per million (p.p.m); potassium bromate 45 p.p.m. for weak flours, 50 p.p.m. for medium and strong flours.

Procedure: Doughs from 1400 g flour were mixed to 40 kj/kg (5 wh/lb) in a Morton variable speed double Z-blade mixer operating at 300 rev/min at atmospheric pressure. Final dough temperatures were in the range 29.5 – 31.5°C. Doughs were hand-divided to give four 454 g pieces which were moulded into cylinders, rested 10 mins, remoulded, proved at 43°C in baking tins (base 140 mm × 75 mm, top 160 mm × 100 mm; height 80 mm), to a height of 10 cm (unless otherwise stated) then baked in an electrically-heated oven for 25 min at 220°C. Loaf volumes were measured by seed displacement.

'Acceptable' loaves had no obvious defects of crumb structure (large holes, cores of hard crumb etc) and loaf volumes of about 1500 ml or more per 14 oz loaf. Deteriorated flours gave loaf volumes in the range 1100–1400 ml. 'Acceptable' 28 oz loaves made in commercial bakeries have volumes of about 2750 ml or more.

## Results

### (1) *Use of extra fat*

Results of a typical experiment on the effect of fat in dough made from a long-stored commercial flour are shown in Table 1.

A commercial trial involving production of 28 oz loaves from another commercially-milled stored flour gave loaf volumes for 0.7% and 2.1% fat addition of 2706 ml and 2968 ml respectively ($P<0.05$; preliminary 14 oz-scale trial (not statistically designed) with this flour gave loaf volumes of 1308 and 1508 ml respectively).

Table 2 summarizes the results obtained with flours specially laid down and

**Table 1.** Volumes of loaves made from stored flours with different levels of fat

| Concentration of fat (% w/w flour). | Loaf volume (ml) |
|---|---|
| 0.7 | 1239 |
| 2.1 | 1530 |
| | Increase 291 ($P<0.01$) |

stored for 4½ years (Bell *et al.*, 1979), using fat at 0–6 lbs/280 lb sack of flour (0–2.1% w/w of flour). Each value is the mean of four replicates from a single mixing. The significance of the linear regression of loaf volume on fat concentration was computed for each type of flour, giving values of 5%, 1% and 1% for the weak, medium and strong control flours respectively, and 0.1%, 0.1% and 1% for the same flours stored at ambient temperatures.

Other commercial fats designed for use in the CBP gave similar results, as did lard and a number of commercial cake and biscuit shortenings. In a test in which ten such fats were each used at concentrations of 0.7, 1.4 and 2.1% by weight of flour, each regression of loaf volume on fat concentration was found to be significant at the 1% level of probability.

The efficacy of the use of extra fat in making acceptable bread from storage-deteriorated flour has thus been confirmed with a number of such flours in laboratory trials and in industrial trials in commercial bakeries.

(2) *Use of other additives*

Many other additives were tested, each at a range of concentrations, as 'restoratives' for deteriorated flour. These included emulsifiers (glyceryl mono-

**Table 2.** Volumes of loaves made using varying levels of added fat, from flours stored at −20°C in an inert atmosphere, or stored in air at ambient temperatures

| Fat level | CONTROLS | | | AMBIENT-STORED | | |
|---|---|---|---|---|---|---|
| (lb/sack) | Weak | Medium | Strong | Weak | Medium | Strong |
| 0 | 1201 | 1199 | 1306 | 1191 | 1122 | 1139 |
| 0.5 | 1296 | 1209 | 1313 | – | – | – |
| 1.0 | 1374 | 1387 | 1399 | – | – | – |
| 1.5 | 1391 | 1531 | 1531 | – | – | – |
| 2.0 | 1396 | 1531 | 1616 | 1255 | 1290 | 1348 |
| 2.5 | 1391 | 1539 | 1631 | 1341 | 1331 | 1378 |
| 3.0 | | | | 1389 | 1369 | 1445 |
| 3.5 | | | | 1435 | 1428 | 1498 |
| 4.0 | | | | 1451 | 1492 | 1471 |
| 5.0 | | | | 1475 | 1504 | 1490 |
| 6.0 | | | | 1466 | 1540 | 1521 |

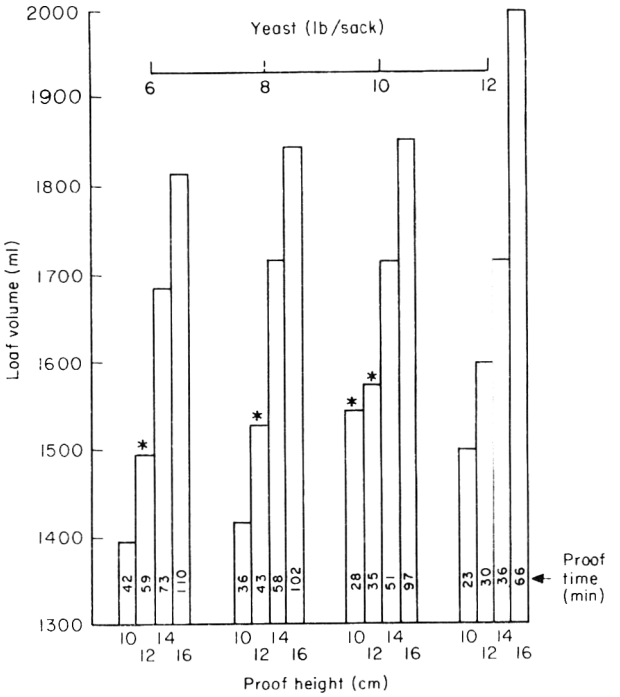


**Figure 1.** Histograms showing the loaf volumes achieved by using four different levels of yeast in CBP doughs made from stored flour. Each variant was proved to four different heights. *. Acceptable bread.

stearate, stearyl tartrate, cetyl alcohol); antioxidants (hydroquinone, butylated hydroxytoluene, nordihydroguaiaretic acid); fungal amylase; malt flour; soya flour; various gums; skimmed milk powder, sugar, gliadin and gluten: none was effective. Flour phospholipids and flour glycolipids did exert a restorative effect, as did some commercial lecithin preparations.

(3) *Variations in recipe*

Changing the levels of oxidant, salt or water used in the recipe was unavailing.

(4) *Variations in doughmaking procedure*

(a) *Effect of dough temperature.* Variation of dough temperature in the range 25–36°C gave loaves with volumes of 1065–1108 ml (with fat) and 1186–1219 ml (without fat).

(b) *Use of modified dough fermentation ('proof') procedures.* It seemed likely that increased loaf volume might result from increasing the volume of the proved dough pieces, as measured by proof height, either by extending proof time, increasing yeast level, or both. Identically mixed doughs were therefore proved to four predetermined heights at each of four yeast levels.

Loaf volumes, proof heights, proof times and yeast levels are shown in Fig. 1. Of the sixteen variants shown, the first and fifth, reading from left to right, were rejected because the bread volume was too small. In all cases where proof height was 16 cm, the dough condition after proof was very delicate, and the bread produced contained very large holes at the top and a coarse open cell structure. None of the bread proved to 14 cm was acceptable, as the external crust was generally rough and torn on the sides, and the crumb was too open.

Of the conditions which produced acceptable bread (marked with an asterisk in Fig. 1) the first would be difficult to apply in an automatic plant bakery because lengthening of proof time would reduce plant throughput, the second would be acceptable, and the third and fourth would create problems because of the very short proof time. Thus, of all the test variations, only one (yeast 8lb/sack, proof height 12 cm) combined the right conditions with the right bread quality to be considered as a possible commercial solution in plant bakeries to the problems of using this particular stored flour. Manual and semi-automatic bakeries could adapt more readily to any of the proof and yeast changes which gave acceptable bread.

A further 1400 g scale trial was carried out to a randomized block design in which doughs made by CBP with various levels of yeast were proved to constant time (50 min) and proof heights, loaf heights and loaf volumes were recorded. Results are shown in Table 3.

Statistical analysis showed significant differences in loaf volume between all the variants ($P<0.01$ for all comparisons except 3 versus 4 for which $P<0.05$).

A commercial trial using the same flour for the production of 28 oz loaves was carried out, with the results shown in Table 4.

The difference in loaf volume between variants 2 and 3 was significant at the 5% level; all other differences were significant at the 1% level.

The quality of the bread produced using 5lb/sack yeast was poor, with dense crumb structure firm to the touch and poor in colour. Crumb structure became progressively more open and softer as the yeast level was increased. At 7.25 lb/sack loaf volume became commercially acceptable, and the crumb structure, though slightly more open than usual, was likewise acceptable.

**Table 3.** Baking test of stored flour using various levels of yeast

| Variant | Yeast level (lbs/sack) of 280 lb | Mean proof height (cm) | Mean loaf height (cm) | Mean loaf volume (ml) |
|---|---|---|---|---|
| 1 | 5 | 10.4 | 11.0 | 1191 |
| 2 | 5.75 | 11.4 | 11.4 | 1316 |
| 3 | 6.5 | 12.1 | 12.5 | 1431 |
| 4 | 7.25 | 12.7 | 12.9 | 1494 |
| 5 | 8.0 | 13.2 | 13.4 | 1571 |

**Table 4.** The effect of additional yeast on the volume of commercially-made bread prepared from stored flour

| Variant | Yeast level (1b per sack) | Proof height (cm) | Loaf volume (ml) |
|---|---|---|---|
| 1 | 5 | 10 | 2351 |
| 2 | 5.75 | 11 | 2418 |
| 3 | 6.5 | 11 | 2496 |
| 4 | 7.25 | 12 | 2756 |
| 5 | 8 | 15 | 2867 |

## Discussion

The data in Table 2 show the full restoration of the baking quality of the weak and medium flours by the use of extra fat, and the partial restoration of the quality of the strong flour. In all these cases the bread produced was of commercially-acceptable volume and crumb structure. Few flours of the many tested have been found to be incapable of restoration in this way, and these had been stored for up to nine years.

Although the trials described have been carried out using the CBP, tests made by the LFP and ADD processes have also yielded similar results. Indeed the deterioration in baking quality manifests itself later with doughs made with fat using LFP or ADD than with those made using the CBP (Bell *et al.* 1979).

Flour phospholipids and glycolipids were effective but prohibitively expensive.

Of the three practicable methods for the restoration of the baking performance of storage-deteriorated flours in the CBP which we have found, namely the use of extra fat, the use of extra yeast, and prolongation of proof, the first two are applicable with minimal change in normal commercial bakery procedures and would therefore be more acceptable to the plant baking industry than the third.

The use of extra yeast and prolongation of proof are equally applicable to the ADD process, where dough maturity is achieved rapidly by means of a suitable mixture of oxidising and reducing agents (Chamberlain, *loc. cit.*), but the position is less simple with LFP. In this case dough maturity is achieved by prolonged fermentation of the dough in bulk, the duration being strongly influenced by the level of yeast and the temperature. An arbitrary alteration of yeast level would not therefore be acceptable in this process, though a prolongation of final proof would.

Increasing the yeast level, where acceptable, is a very useful means of overcoming the main problem with stored flour of poor gas retention in the oven. The extra volume gained in proof is maintained during baking. In practice, therefore, the baker would simply adjust the height of the dough piece at the end of proof, by increasing the yeast level in the recipe, until he was satisfied

with the volume of the resultant bread. To avoid producing an undue amount of bread of small volume, an immediate increase in yeast level of 1 lb per sack of flour (0.36% w/w) could be recommended when using stored flour. No other change would be necessary: the procedure is thus practical and easy to carry out.

The cost of applying this ameliorative action would be much less than the cost of adding extra fat. Furthermore, a bakery always stocks yeast, but as many bakers add fat only via their composite improver, fat as such might not always be suitable.

A successful trial of this procedure on the commercial scale has been carried out.

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# Diffusional analysis of air drying of grain sorghum

C. SUAREZ, P. VIOLLAZ AND J. CHIRIFE

## Summary

Fick's law of diffusion (moisture flux proportional to the moisture gradient) for diffusion out of spheres was successfully applied to describe the drying of grain sorghum. It was found that the diffusion coefficient of water was independent of moisture content in the approximate range of 21–6% (dry basis) moisture content. An Arrhenius type temperature dependency of moisture diffusivity was found, the energy of activation being 7.5 kcal/g mol.

## Introduction

The drying of cereal grains is a problem of great practical importance and has been studied extensively. A number of workers have developed empirical correlations for the prediction of drying rates or have studied the relation among the variables which control the drying of single exposed grains (Hukill, 1947; Becker & Sallans, 1955; Hukill & Schmidt, 1960; Thompson, Peart & Foster, 1968). Foster (1973) reviewed most of the research in the area of grain drying theory and calculations. Drying of grain sorghum has become increasingly important as the grain is harvested earlier and at a higher moisture content. However, relatively little basic research has been performed on drying of grain sorghum (Paulsen & Thompson 1973) as compared to other grains such as wheat and maize.

The purpose of this study was to investigate the fundamental aspects of the drying of grain sorghum when fully exposed to air of constant temperature and humidity. For this purpose the standard solution of the transient diffusion equation is applied to describe the drying phenomena.

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## Experimental

### *Laboratory drier*

This consisted of a centrifugal fan which blows air over twelve 0.75 kW electric bar elements into a chamber at the base and then upwards through a vertical duct. The vertical duct has a flow-smoothing section of small glass spheres. A metal cup with screened bottom and lid served as drying chamber and was mounted on the outlet of the duct. A high constant air velocity (8–10 m/sec) was used in all experiments in order to minimize external resistances to the moisture loss. This through-flow of air produced a vigorous fluidizing action in the grain sample. The inlet air dry bulb temperature was regulated (± 0.1°C) by an electronic proportional controller; wet and dry bulb thermometers were fitted in the drying chamber. Relative humidity of the drying air was calculated from the wet bulb depression. A single layer of grain (about 20 g) was used for each drying experiment, the progress of which was followed by weighing the sample periodically on a precision balance (± 0.001 g). For this purpose, the drying cup was removed, rapidly weighed and replaced in the drier at regular intervals.

### *Materials*

Red grain sorghum was used in all experiments; it was received with an average moisture content of about 10% (dry basis). It was necessary to add water to the sorghum for the drying experiments. This was done by humidification over a saturated solution of barium chloride which provided a constant relative humidity of about 90%. The grain was placed in evacuated vacuum desiccators containing the saturated salt solution and kept at 7°C until equilibrium was reached. The grains were irradiated with u.v. light in order to prevent (or delay) the growth of microorganisms during the equilibration period (about 2 weeks). This method of humidification gave more satisfactory results than the usual procedure of adding liquid water (Becker & Sallans, 1955; Steffe & Singh, 1979) to the grains. Moisture content of grains at equilibrium was found to be 21% (dry basis) and this value was used as initial and uniform moisture content of the grains in the drying runs.

The grains were screened prior to humidification and drying in order to obtain samples of uniform size. Size distribution of the samples utilized in the drying experiments ranged between 0.24 cm to 0.28 cm. Volumetric tests were conducted to obtain radius estimates of the sorghum grains. The total volume of a large quantity of grains was measured using a pycnometer (with toluene as the fluid). The average grain volume was calculated by dividing the total sample volume by the total number of grains. The equivalent spherical radius was then computed from the formula for the volume of a sphere ($V = 4\pi r^3/3$) using the average grain volume.

Moisture content of the grains was determined gravimetrically using a vacuum oven method at 70°C over magnesium perchlorate.

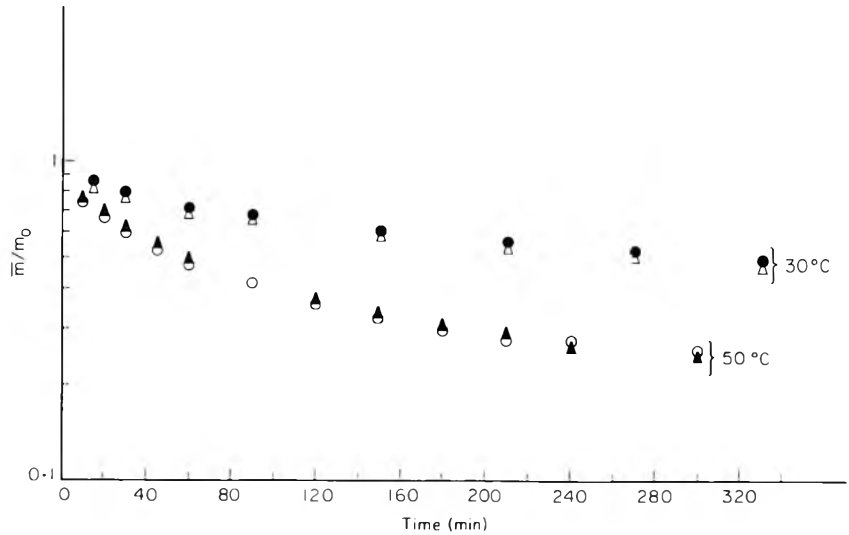


**Figure 1.** Effect of air flow rate on drying curves. Reproducibility of drying experiments. △, v = 10 m/sec; ●, v = 8 m/sec; ▲, ○, v = 8.5 m/sec.

*Determination of equilibrium moisture content*

Selected values of equilibrium moisture content at the various drying temperatures were determined by equilibration against saturated salt solutions which provided known constant relative humidities. The measurements corresponded to the desorption branch of the isotherm.

The details of the experimental technique have been published elsewhere (Iglesias, Chirife & Lombardi, 1975).

## Results and discussion

By studying the effect of different velocities of air on the moisture content of material during drying, it is possible to determine which resistance (internal or external) controls the rate of drying (Vaccarezza, Lombardi & Chirife, 1974a). From the results shown in Fig. 1 it can be seen that in the range of air velocities used in this study, internal moisture movement is the main resistance to the moisture loss rate because the drying rate is not affected by increasing the air velocity from 8 m/sec to 10 m/sec. Reproducibility of drying runs is also observed in Fig. 1 which shows a very good agreement between different pairs of drying runs performed under identical conditions.

As drying is a simultaneous heat and mass transfer process, correlations of moisture (and temperature) changes should involve solution of coupled differential equations (Harmathy, 1969; Husain, Chen & Clayton, 1973). As a consequence of heat transfer the temperature of a food undergoing dehydration

increases rapidly at the beginning of drying towards the air dry bulb temperature (Vaccarezza *et al.*, 1974b) and theoretically, internal temperature gradients may exist.

However, in the case of sorghum drying the heat transfer effects may be neglected and the process treated as a purely mass transfer one. This is due to the following reasons. Due to its relatively low initial moisture content (about 21% d.b.) the difference between the grain and air dry bulb temperature may be considered negligible with little error from the beginning of drying (Vaccarezza *et al.*, 1974; Alzamora, Chirife & Viollaz, 1979). Further, the internal grain temperature during drying may also be considered uniform due to the low Biot number, $Bi = h\,r/k$, usually found for normal air drying of foods (Alzamora *et al.*, 1979). In these conditions Fick's second law for diffusion out of spheres may be used to fit the experimental drying data of grain sorghum

$$m^* = \frac{\overline{m} - m_e}{m_0 - m_e} = \frac{6}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{n^2} \exp\left(-n^2\,\pi^2\,D_{eff}\,\theta/r^2\right) \qquad (1)$$

where $\overline{m}$ is the average moisture content (dry basis), $m_0$ is the uniform initial moisture content, $m_e$ is the equilibrium moisture content (at the air dry bulb temperature), $r$ is the radius of grain, $\theta$ is the drying time, and $D_{eff}$ is the moisture diffusivity. Diffusion coefficients for water in grain sorghum were found by comparing actual and predicted (eqn 1) drying curves. For this purpose, eqn (1) was programmed on a digital computer and used to simulate drying. The values of equilibrium moisture content for each drying condition (temperature, relative humidity) were experimentally determined as described

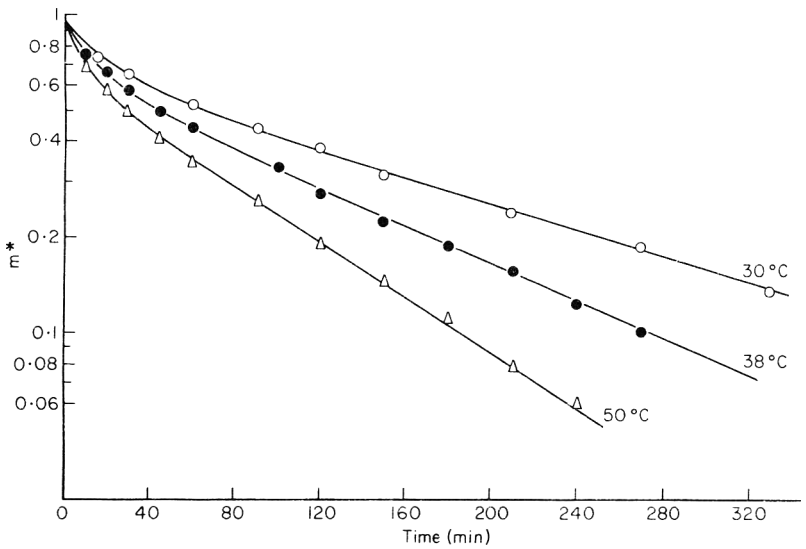


**Figure 2.** Comparison of measured and predicted drying curves for grain sorghum at various air dry bulb temperatures. ○, ●, △, experimental; ——, calculated.

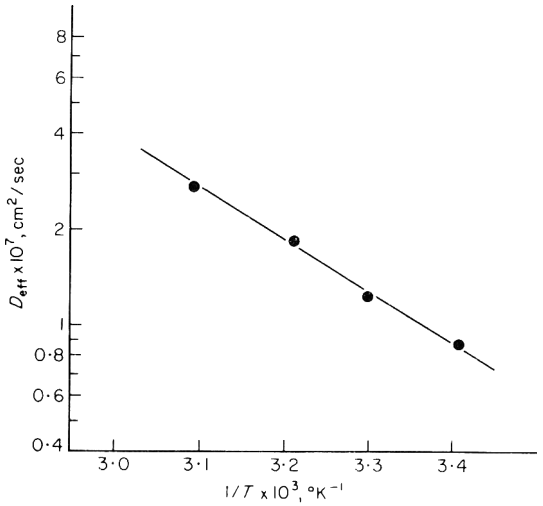


**Figure 3.** Effect of temperature on the diffusion coefficient of water in grain sorghum.

in the 'Experimental' section. In each drying simulation the diffusivity was allowed to vary until the sum of the squared deviation between the actual and theoretical curve was a minimum. Typical results comparing the predicted and measured drying curves for grain sorghum at three different temperatures are given in Fig. 2. These curves indicate the accuracy with which the diffusion theory with constant diffusivity could predict the drying behaviour.

For $m^*$ approximately smaller than or equal to 0.3, Fick's law for spheres (eqn 1) reduces to a straight line (in semilogarithmic coordinates) the intercept of which is $6/\pi^2 = 0.608$. The intercept of the various experimental lines representing log $m^*$ *vs* time, shown in Fig. 2, were calculated and found to be about 0.618 in very good agreement with the theoretical value. This is further proof of the validity of Fick's law for describing the rate of moisture movement during drying of sorghum.

The diffusion coefficients found by the above procedure were plotted in Fig. 3 as ln $D_{eff}$ *vs* $1/T$. It can be seen that the plot is essentially a straight line, from which the activation energy for diffusion may be estimated using an Arrhenius type equation,

$$D_{eff} = A \exp(-E_a/RT) \tag{2}$$

The activation energy was calculated from the slope of the straight line in Fig. 3 and was found to be, $E_a = 7.5$ kcal/g mol.

Table 1 compares this value with activation energies for water diffusion in various food materials of approximately similar moisture content, as reported by several authors. It can be shown that the value for sorghum is somewhat lower than that of most others. Although data in the literature relating to

**Table 1.** Activation energy ($E_a$) for water diffusion in various food materials

| Material | $E_a$ (kcal/g mol) | Moisture content (dry basis) | Reference |
|---|---|---|---|
| Fish | 8.8 | 0.1 | Jason (1958) |
| Cellulose (cotton) | 8.3–9.0 | 0.1 | Daruwalla & Shet (1964) |
| Starch gel | 8.1 | 0.063 | Fish (1958) |
| Wheat | 12.9–14.6 | 0.12–0.30 | Becker & Sallans (1955) |
| Rice | | | |
| (starchy endosperm) | 6.8 | 0.34–0.13 | Steffe & Singh (1979) |
| (bran) | 10.7 | | |
| Sorghum | 7.5 | 0.21–0.06 | This work |

diffusivity of water in drying cereal grains were obtained using rewetted materials instead of 'fresh' (or naturally moist) ones, it may be argued that the diffusivities of rewetted materials may be different from that of the naturally moist one. This has been studied for drying rice by Steffe & Singh (1979). They performed a statistical test which indicated that the diffusivities of the fresh and rewetted starchy endosperm and bran were equal.

*The driving force behind diffusion*

It has been shown that Fick's law in terms of moisture content represents an accurate way of predicting drying times of sorghum at several different temperatures. Similarly, Becker & Sallans (1955) found that the diffusion coefficient of water in wheat was also independent of moisture content in the range 12–30%. Other authors have also used Fick's law in terms of moisture content to interpret drying rates at relatively low moisture contents. In most cases however, it has been found that the moisture diffusivity was not constant with moisture content, but diffusion coefficients were calculated based on an appropriate solution of equation

$$\frac{\delta m}{\delta \theta} = \frac{\delta}{\delta x}\left(D_{\text{eff}} \frac{\delta m}{\delta x}\right) \tag{3}$$

over each segment of the drying period considered (Fish, 1958; Bluestein & Labuza, 1972; Román, Rotstein & Urbicain, 1979).

It has been suggested that vapour phase diffusion rather than liquid diffusion is the mechanism of moisture transport in dried foods where the equilibrium relative humidity is below saturation (Van Arsdel, 1947; King, 1968). In this case, the driving force behind diffusion is the water vapour pressure rather than the moisture content. According to this view, this fact was responsible for the

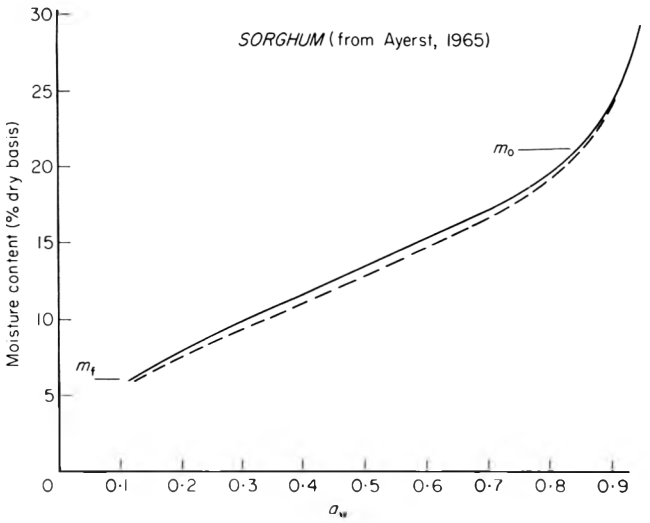


**Figure 4.** Desorption isotherms of grain sorghum at different temperatures. (from data of Ayerst, 1965). ---, 35°C; ——, 25°C.

lack of constancy of moisture diffusivity (as defined by eqn 3) with moisture content, as reported by various authors.

The concept of water vapour pressure and moisture gradients as driving force for diffusion are proportional (and may be used interchangeably) only if a linear water sorption isotherm exists and in the absence of an internal temperature gradient (Chirife, 1979). Based on certain assumptions, King (1968) derived an equation relating the effective diffusivity (Eqn 3), $D_{eff}$, to various physical properties of the food and environmental conditions. In the absence of any heat transfer control it may be written

$$D_{eff} = \frac{M_w D'}{T \rho_s R_v} \left( \frac{\delta a_w}{\delta m} \right) T P_o \frac{P}{P - P_w} \tag{4}$$

This equation clearly illustrates the link between moisture and vapour pressure gradient as driving forces behind water diffusion. As most food isotherms are not linear ($\delta a_w / \delta m$) $T$ is not a constant (Viollaz *et al.*, 1978), and $D_{eff}$ should vary with moisture content.

Ayerst (1965) measured the desorption isotherms of grain sorghum in the moisture content range of interest to the present study. His tabulated data have been plotted in Fig. 4. It can be seen that the shape of the isotherm is such that it may be considered linear for most of the moisture range of interest (about 6–21% d.b.) to our study. If one accepts the validity of eqn (4) this may explain the observed constancy of $D_{eff}$ with moisture content of grain sorghum.

The observed activation energy for grain sorghum, 7.5 kcal/g mole, does not however, satisfy King's model. According to eqn (4) the most temperature

sensitive term is $P_0$, so the expected activation energy for $D_{eff}$ should be close to the latent heat of water, 10.2 kcal/g mole, which is the 'activation energy' for the variation of $p_0$ with temperature. In fact, the expected activation energy for $D_{eff}$ should be even somewhat higher than 10.2 kcal/g mole due to the other temperature sensitive term in eqn (4), namely $D'$.

It may be concluded that the observed behaviour of grain sorghum is not in itself sufficient to prove the validity of a particular drying mechanism (liquid or vapour diffusion). Nevertheless, the data show that Fick's law in terms of moisture gradient constitutes an excellent model for describing the drying behaviour of sorghum and consequently may be safely used for all practical purposes.

## Nomenclature

$a_w$ : water activity = $p/p_0$
$A$ : constant, cm²/sec
$b$ : vapour space permeability, g-mole/atm cm sec
$D'$ : effective vapour space diffusion coefficient, cm²/sec
$h$ : heat transfer coefficient, cal/°C cm² sec
$k$ : thermal conductivity, cal/°C cm sec
$M_w$ : molecular weight of water, 18 g/g mole
$P_0$ : vapour pressure of pure water, atm
$P_w$ : partial pressure of water, atm
$P$ : total pressure, atm
$R_v$ : gas constant, 1.98 cal/°K mole
$T$ : absolute temperature

*Greek letters*

$\rho_s$ : dry solids density, g/cm³

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# Properties of concentrates containing milk protein and non-milk carbohydrates

A. W. M. SWEETSUR AND D. D. MUIR

## Summary

Various edible carbohydrates were combined with skim-milk concentrate, prepared by ultrafiltration (UF), to obtain a novel range of milk products with total solids contents greater than 40%. These products were very heat stable, their stability being inversely proportional to the reducing power of the carbohydrate in the mixture. In contrast to both unaltered UF concentrate and conventional concentrate prepared by evaporation, the heat stabilities of the UF concentrate/carbohydrate mixtures could be further improved by addition of urea. As with both conventional and UF concentrates, the heat stabilities of the mixtures could be increased by additions of simple aldehydes. Attention has been drawn to possible uses for these mixtures in the food industry.

## Introduction

Skim-milk is a wholesome and nutritious food but when production exceeds the market demand the surplus is often disposed of as animal feed. The search for new uses for skim-milk centres on processes for modification or fractionation of the milk to yield novel human food. Recent fundamental studies of the heat stability of concentrated milk have demonstrated that soluble milk salts are one important determinant of heat stability (Muir & Sweetsur, 1978a). In addition, we have also shown how reduction of soluble salts during concentration of milk by ultrafiltration results in protein-rich material able to withstand conditions equivalent to sterilization (Sweetsur & Muir, 1980). This paper deals with an extension of these studies in which we aim to define the conditions in which concentrated mixtures of milk protein and carbohydrate may be formulated to withstand sterilization.

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## Materials and methods

Milk samples were obtained from the Hannah Research Institute Farm bulk tank. The fat was removed from all samples as described by Davies & White (1966) and the skim-milk stored in the dark at 5°C until used.

Carbohydrates (fructose, glucose, lactose, maltose, sucrose, maltitol and sorbitol) were obtained from Sigma London, Ltd, (Kingston-upon-Thames, Surrey, England) as crystalline solids, with the exception of maltitol which was obtained as a 70% aqueous syrup. A commercial sample of dried hydrolysed starch with a dextrose equivalent of 28 was also used in some experiments.

Urea ('Aristar' grade) and aldehyde reagents were added to the milk as 2.5 (w/v) aqueous solutions before adjustment of pH. Formaldehyde ('Analar' grade) was obtained as a 40% solution. The other aldehydes were crystalline solids obtained from Sigma, London, Ltd.

Skim-milk was concentrated by ultrafiltration at 50°C using a laboratory-scale apparatus with a flat membrane (PM–30 membrane in a TCF–10 ultrafiltration unit from Amicon Limited, High Wycombe, England).

Total solids content of the skim-milk concentrate was estimated by the appropriate British Standards Institute (1963) method and by the rapid method of Wilson as described in Muir & Sweetsur (1978b).

Total nitrogen (TN) and non protein nitrogen (NPN) measurements were made as described by Sweetsur (1976).

Lactose was measured by the method of Grimbleby (1956) as amended by Biggs & Szijarto (1963).

Coagulation time (CT) – pH profiles for both the UF concentrates and the concentrate/carbohydrate mixtures were measured at 120°C by the method of Sweetsur & White (1974).

## Results

*The effect on concentrate stability of addition of reducing sugars*

Skim-milk was concentrated to 18.7% TS by ultrafiltration (approximately × 4.0 volume reduction) and a selection of reducing sugars were dissolved in the concentrate by gentle warming to a final concentration of 33 g per 100 ml. The CT-pH profiles for the original concentrate and that of mixtures with glucose, maltose and fructose are shown in Fig 1. The control sample was extremely heat stable at 120°C but, although the mixtures were significantly less stable, after pH adjustment they could withstand treatment for 10 min at 120°C – that is, conditions typical of in-can sterilization.

*The effect on concentrate stability of addition of non-reducing sugars*

A comparison was made of the effect of adding sucrose, sorbitol and maltitol (33 g per 100 ml) to concentrate as before. In each case, the CT–pH profiles of

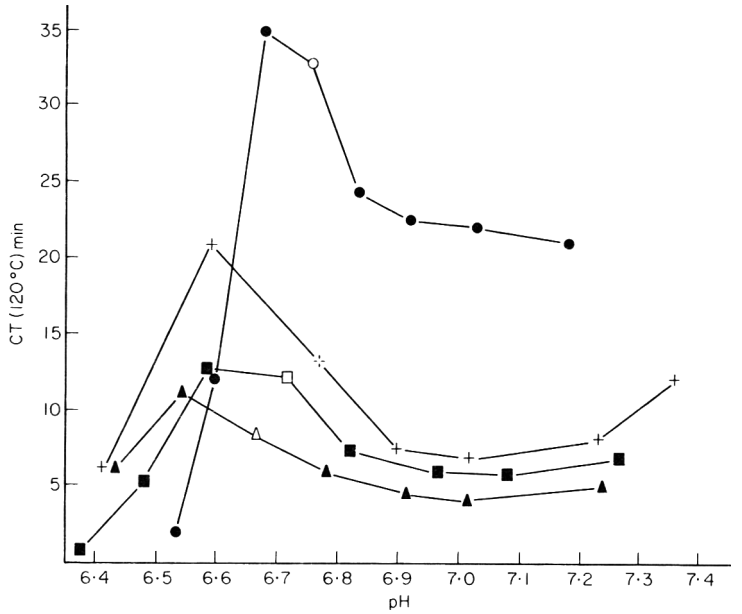


**Figure 1.** The effect of addition of non-reducing sugars (33.3 g/100 ml) on the heat stability of UF concentrated milk (18.7% TS). ●, Control; ▲, plus glucose; ■, plus maltose; +, plus fructose. Open symbols represent 'unadjusted' pH.

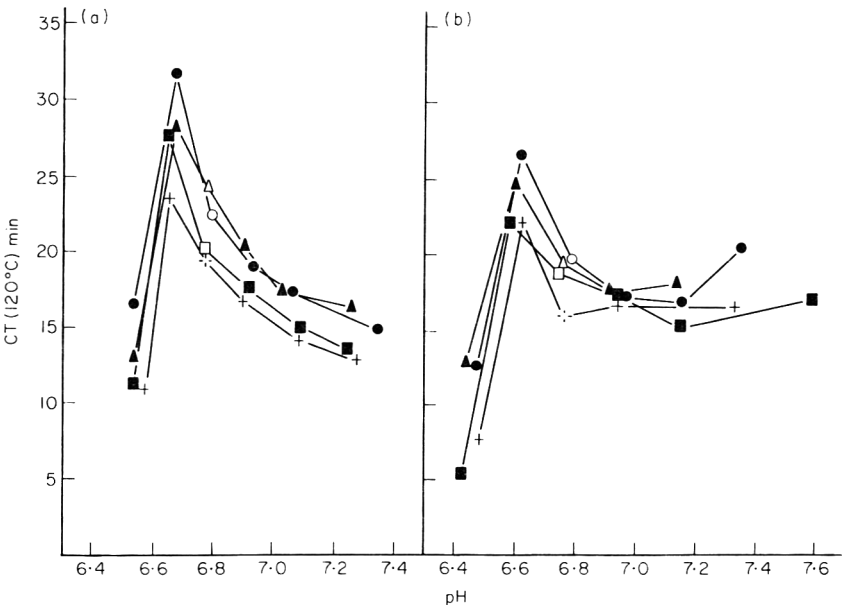


**Figure 2.** The effect of volume reduction by ultrafiltration on the heat stabilities of concentrate and concentrate/sucrose mixtures. ●, Volume reduced 4 fold; ▲, volume reduced 4.5 fold; ■, volume reduced 5 fold; +, volume reduced 5.5 fold. (a). Concentrate; (b) concentrate/sucrose mixture. Open symbols represent 'unadjusted' pH.

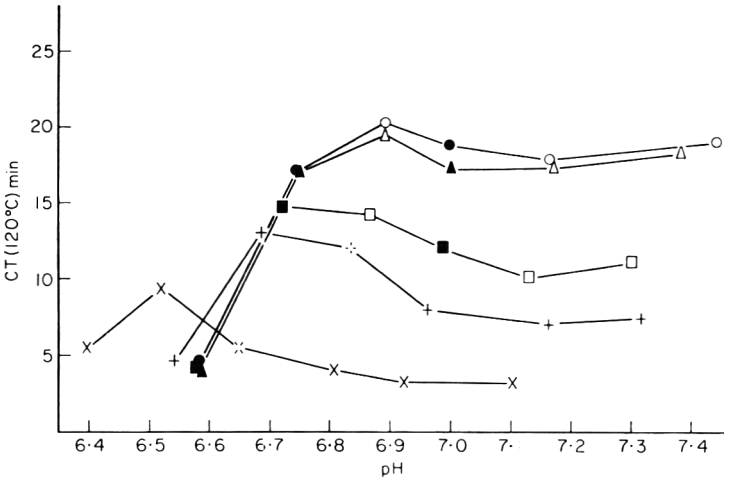


**Figure 3.** The effect of addition of glucose/sorbitol combinations (33.3 g/100 ml) on the heat stability of UF concentrated milk (24.5% TS). ●, 100% sorbitol; ▲, 1% glucose/99% sorbitol; ■, 10% glucose/90% sorbitol; +, 25% glucose/75% sorbitol; ×, 100% glucose. Open symbols represent 'unadjusted' pH.

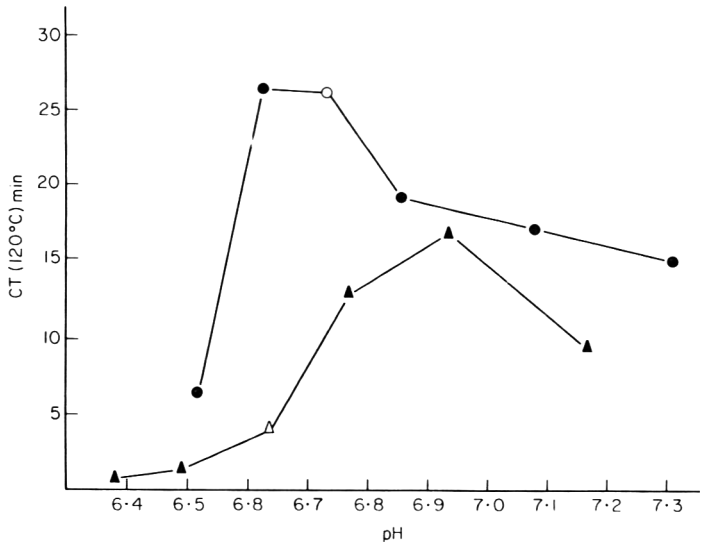


**Figure 4.** The effect of addition of a dried hydrolysed starch (dextrose equivalent = 28) on the heat stability of UF concentrated milk (21.5% TS). ●, control; ▲, plus starch (33.3 g/100 ml). Open symbols represent 'unadjusted' pH.

**Table 1.** Composition of UF concentrate and carbohydrate mixtures

| Mixture | Total solids (%) | Protein* (%) | Lactose (%) | Carbohydrate (%) | Milk salts (%) |
|---|---|---|---|---|---|
| UF concentrate | 26.70 | 20.86 | 4.93 | — | 0.91 |
| UF concentrate/ lactose permeate | 27.11 | 10.81 | 15.45 | — | 0.85 |
| UF concentrate/ Carbohydrate permeate | 27.11 | 10.81 | 4.65 | 10.80 | 0.85 |

* (TN–NPN) × 6.38

the carbohydrate containing mixtures were similar to that of the control, despite the fact that the concentration of TS was more than doubled. The effect is shown in detail for sucrose addition in Fig. 2. The amount of added sucrose was kept constant at 33 g per 100 ml but concentrates prepared by different degrees of ultrafiltration were used as the base material. Even when the volume reduction of the skim-milk was × 5.5, sucrose addition did not result in destabilization. On the basis of these results, formulations of milk-protein concentrate with non-reducing sugar will stand in-can sterilization without difficulty.

*The effect of addition of mixtures of reducing and non-reducing sugars*

The previous results suggest that the heat stabilities of concentrate/sugar mixtures are dependent on the reducing powers of the carbohydrates. To test this hypothesis a reducing sugar (glucose) was combined in various ratios with its non-reducing alditol analogue (sorbitol). These combinations were then added to UF concentrate, prior to the determination of their CT–pH profiles. As the reducing power of the mixture decreased (with increasing sorbitol concentration) the heat stability improved (Fig. 3).

The above results were corroborated by formulating a mixture of a UF concentrate and a dried hydrolysed starch (Dextrose equivalent = 28). The CT–pH profile of the mixture (Fig. 4) was comparable with that which would have been predicted for sugar combinations containing 28% reducing carbohydrate (cf. Fig. 3).

*The effect of sugar addition at constant total solids and soluble milk salt concentration*

A number of explanations may be advanced to account for the high heat stability of mixtures of non-reducing sugar with milk protein concentrate. For example, the sugars could have increased the stability of the system *per se* or alternatively acted as inert diluents. To elucidate the effect, various

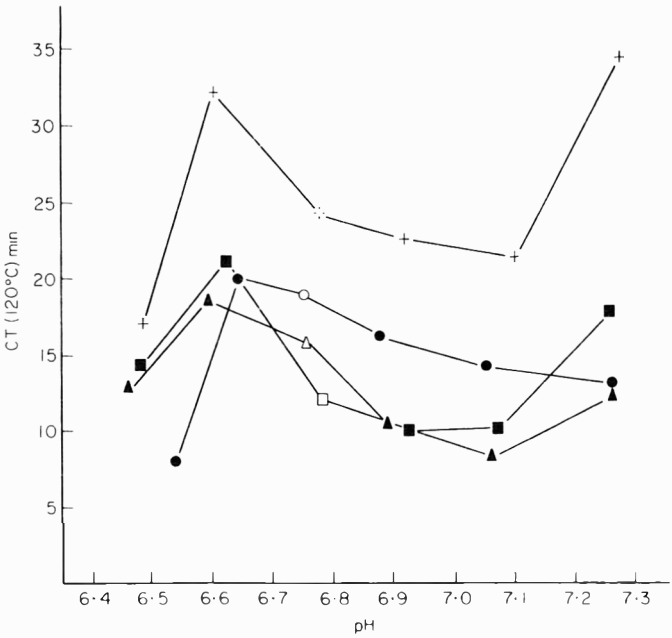


**Figure 5.** The effect of addition of carbohydrate on the heat stability of UF concentrated milk at constant levels of total solids and soluble salts. ●, Control; ▲, plus glucose; ■, plus lactose; +, plus sucrose. Open symbols represent 'unadjusted' pH.

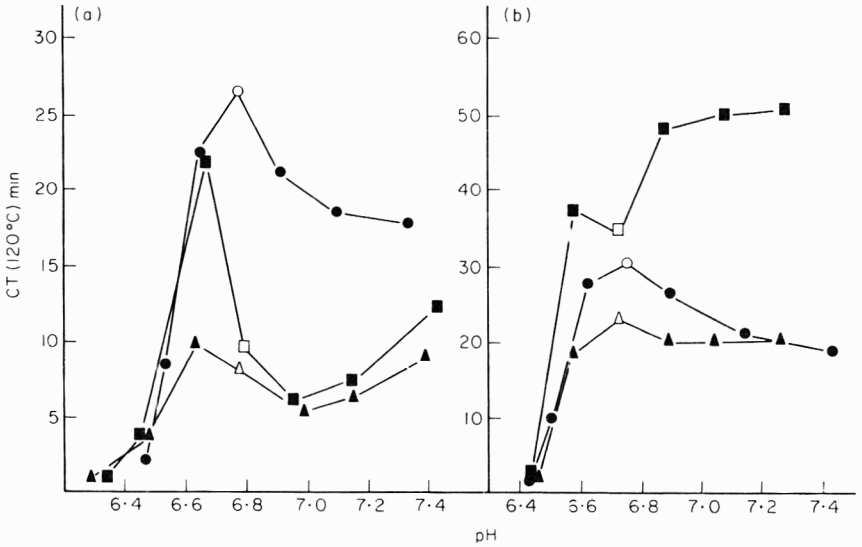


**Figure 6.** The effect of addition of urea (200 mg/100 ml) on the heat stabilities of UF concentrate/carbohydrate mixtures. ●, UF concentrate; ▲, UF concentrate/carbohydrate mixture; ■, UF concentrate/carbohydrate mixture plus urea (a) UF concentrate/fructose (44.6%); (b) UF concentrate/sucron (43.6% TS). Open symbols represent 'unadjusted' pH.

carbohydrate-milk protein mixtures were examined at constant total solids and salt concentration. This end was achieved by adding sugar to the permeate from the ultrafiltration process to a final concentration of 27% TS – that is, the same total solids level achieved for the concentrate in this experiment. The sugar/permeate mixtures and the milk protein concentrate were mixed in the proportions required to produce a similar protein: sugar ratio (1:1) to those obtained by direct sugar addition. The compositions of the mixtures are shown in Table 1.

It was found that the maximum heat stabilities of the mixtures containing lactose and glucose were similar to that of the unaltered UF concentrate (20 min at 120°C), but that the mixtures containing sucrose, sorbitol, maltose and fructose were more stable. The effectiveness in improving heat stability increased with the decreasing reducing power of the carbohydrates, i.e. sucrose ≒ sorbitol > maltose ≒ fructose > lactose ≒ glucose, until a maximum heat stability of 32 min was obtained for the mixtures containing sucrose and sorbitol. Figure 5 shows the CT–pH profiles for three of the concentrate/carbohydrate mixtures.

*The effect of addition of urea and aldehydes*

The effect of addition of urea (200 mg/100 ml) on the heat stabilities of mixtures of UF concentrate with both a reducing (fructose) and a non-reducing (sucrose) carbohydrate is shown in Fig. 6. The heat stabilities of mixtures of UF concentrates and other carbohydrates were also improved by addition of urea, but the increases in stability were greater for the combinations of concentrate and non-reducing carbohydrates.

The effect of addition of formaldehyde (125 mg/100 ml UF concentrate) on the heat stabilities of mixtures of UF concentrate with a reducing (glucose) and a non-reducing (sorbitol) carbohydrate is shown in Fig. 7. Addition of DL–glyceraldehyde and 2-deoxyribose induced similar increases in heat stability.

## Discussion and conclusions

The results of this work demonstrate that a novel range of heat stable compounds can be obtained by combining UF concentrate with edible carbohydrates to produce mixtures with a final TS level in excess of 40%.

The heat stabilities of the concentrate/carbohydrate mixtures depend on the sugar added (Figs. 1, 2, 6 and 7) but, even at the poorest responses, the mixtures were remarkably stable – skim-milk concentrated to 40% TS by conventional evaporation would coagulate instantly at 120°C. The unaltered concentrate and the mixtures containing the non-reducing carbohydrates sucrose, sorbitol and maltitol were similar in coagulation behaviour at 120°C (*cf.* Figs 2, 3 and 7) and would easily withstand sterilization (120°C for 10 min). However, when reducing carbohydrates such as glucose, maltose and fructose were added to UF concentrate a drop in heat stability occurred (Fig. 1). The results obtained with

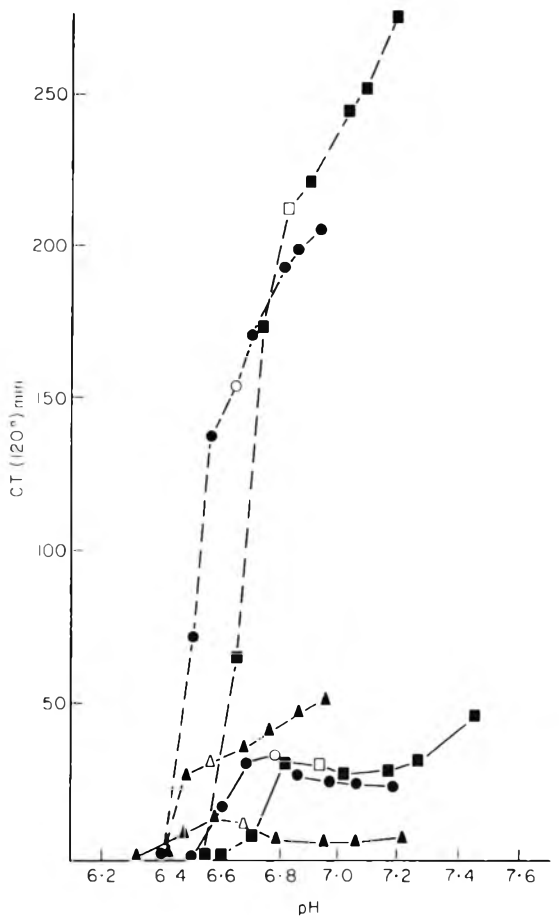


**Figure 7.** The effect of addition of formaldehyde (125 mg/100 ml) on the heat stabilities of UF concentrate/carbohydrate mixtures. ●——●, UF concentrate (16.3% TS); ▲——▲, UF concentrate plus glucose (33.3 g/100 ml); ■——■, UF concentrate plus sorbitol (33.3 g/100 ml); ●--● UF concentrate plus formaldehyde; ▲--▲, UF concentrate/glucose mixture plus formaldehyde; ■--■, UF concentrate/sorbitol mixture plus formaldehyde. Open symbols represent 'unadjusted' pH.

combinations of glucose and its non-reducing alditol analogue (sorbitol) demonstrate that the reducing power of the carbohydrate is the principal determinant of heat stability.

The CT–pH profiles obtained with mixtures of concentrates and carbohydrates at constant TS and milk salt content (Fig. 5) appear to demonstrate that the non-reducing sugars enhance heat stability *per se*. However, it is more probable that the non-reducing carbohydrates simply behave as inert diluents because their maximum heat stabilities (at a non-sugar solids content of 16.3%) were comparable with those of unaltered UF concentrate at 16.3% TS (Fig. 7). If this were the case, it could then be postulated that reducing sugars destabilize the protein caseinate system as a consequence of Maillard type condensation

reactions between carbohydrate and the micellarprotective κ – casein (Sweetsur & White, 1975).

In contrast to either conventional or UF concentrates (Muir & Sweetsur, 1978b; Sweetsur & Muir, 1980), the heat stabilities of UF concentrate/carbohydrate mixtures could be increased by additions of small quantities of urea. Muir & Sweetsur (1978b) demonstrated that as the TS content of milk was reduced, the coagulation mechanism changed from type II (2-stage) to type I (single-stage), and that urea was only effective in delaying the onset of type I protein coagulation. The present results suggest that the coagulation mechanism in concentrate/carbohydrate mixtures is closer to type I than type II, i.e. more like that occurring in unconcentrated skim-milk. This stabilizing effect of urea could have been predicted from the shapes of the CT–pH profiles for UF concentrate/carbohydrate mixtures. A partial recovery in heat stability at high pH values is observed with these mixtures (Figs 1, 2, 3, 6 and 7), resulting in their CT–pH profiles being more like those obtained with skim-milk than those obtained with concentrate (*cf.* Muir & Sweetsur, 1978b).

Additions of simple aldehydes, and certain sugars that are believed to act as aldehydes at high temperatures, have been found to markedly improve the heat stability of skim-milk, conventional evaporated skim-milk concentrate (Holt, Muir & Sweetsur, 1978) and UF skim-milk concentrate (Sweetsur and Muir, 1980). The investigations reported here demonstrate (Fig. 7) that such additions can also be used to increase the heat stabilities of UF concentrate/carbohydrate mixtures. It should be noted, however, that aldehydes and some of the other compounds added are currently not included in the United Kingdom (1977) regulations regarding permitted additives for condensed milk and dried milk products.

From the milk processor's viewpoint, the UF concentrate/edible carbohydrate mixtures appear to have two major advantages over conventional skim-milk analogues. Firstly, they are much more stable to heat, and most of the mixtures, even with their TS contents greater than 40%, would be able to withstand sterilizing conditions. Secondly, the possible substitution of most of the lactose with other sugars offers the possibility of producing both sweeter mixtures and formulations that would be more acceptable to ethnic groups with lactose intolerance.

## Acknowledgments

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# Inhibition of potential food poisoning microorganisms by sorbic acid in cooked, uncured, vacuum packaged turkey products

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## Summary

Vacuum packaged, oven-roasted turkey breasts and sliced turkey breast luncheon meat were prepared with and without potassium sorbate or sorbic acid. Control and treated products were inoculated with one of the following organisms: *Salmonella, Staphylococcus aureus,* or enteropathogenic *Escherichia coli.* The samples were vacuum packed and stored at 15°C for 10 days. The addition of 0.25% sorbate to the breasts and 0.12% sorbic acid in the slices provided excellent protection against the growth of *Salmonella, E. coli,* and *S. aureus* in uncured, cooked, vacuum packaged turkey.

## Introduction

The use of vacuum packaging has been shown to be effective in increasing the shelf life of poultry and other meats (Clark & Lentz, 1972: Pierson, Collins-Thompson & Ordal, 1970; Sander & Soo, 1978). Robach *et al.* (1980) reported that the use of vacuum packaging in combination with sorbic acid markedly extended the shelf life of cooked, uncured turkey products.

Pierson, Smoot & Stern (1979) reported that 0.13 and 0.26% potassium sorbate was effective in suppressing growth of *Staphylococcus aureus* in bacon. Tompkin *et al.* (1974) demonstrated that 0.1% sorbate markedly retarded salmonellae growth and delayed the growth of *S. aureus* and *Clostridium botulinum* in cooked uncured sausage. To & Robach (1980) reported that a 5% sorbate dip on fresh chickens reduced the growth of *S. aureus* and several species of *Salmonella* inoculated onto the broiler carcasses. There has been little work on the growth of pathogenic bacteria in vacuum packaged, cooked meat products.

The purpose of this study was to determine the effectiveness of potassium

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0022–1163/80/1000–0543 $02.00 

sorbate and sorbic acid in controlling the growth of three potential food poisoning bacteria inoculated onto cooked, uncured turkey products stored at 15°C.

## Materials and methods

### *Turkey products*

Vacuum-packaged, oven-roasted turkey breasts and turkey breast luncheon meat were prepared by Louis-Rich, Inc., West Liberty, Iowa. The products were shipped by refrigerated truck to Monsanto laboratories in St Louis, Missouri, for microbiological evaluation.

### *Sorbate treatments*

Two types of turkey products were evaluated in this experiment. Oven-roasted breasts were pumped with turkey broth, salt, and phosphate. Sorbate was added by dissolving 2.6% potassium sorbate into the pump solution. Sorbic acid was added to the emulsion of the sliced luncheon meat at a target level of 0.2%. Both products were cooked at 82°C to an internal temperature of 71°C prior to vacuum packaging and refrigerated shipment to Monsanto.

### *Cultures*

Two naladixic acid resistant strains of salmonellae (*Salmonella infantis* and *S. enteritidis*) were obtained from Professor G. Snoeyenbos of the University of Massachusettes. The two strains were grown overnight at 37°C in trypticase soy broth (TSB: BBL). The salmonellae were then pooled for inoculation. Two strains of *S. aureus* (S–6 and ATTC 12600) were prepared in a similar manner. Two strains of *Escherichia coli* (0125 B15, obtained from Professor E.H. Marth, University of Wisconsin, and ATCC 11775) were also grown overnight in TSB and pooled for inoculation.

### *Inoculation*

Pooled cultures of the same organism were diluted in 0.005M phosphate buffer (pH 7.2) to give an inoculum of $10^4$ cells/ml. The turkey breasts were inoculated by immersing twelve breasts into the 12 litre inoculum suspension of the appropriate organism for 1 min, drained for 5 min, vacuum packaged and stored at 15°C. A fresh 12 litre inoculum suspension was prepared for each batch of product. The sliced product was inoculated by spreading 0.1 ml of the inoculum suspension on the surface of each slice. Slices were vacuum packaged, three slices per package, and stored at 15°C.

*Microbiological evaluation*

On 0, 2, 4, 6, 8 and 10 days of storage, samples were visually inspected for signs of spoilage and microbial counts for the four inoculated organisms determined on the corresponding packages, using two packages per variable. Sliced luncheon meats were sampled by stomaching one slice (average weight 28 g) in 99 ml of sterile peptone water (0.1% proteose Peptone, Difco) for 1 min. Whole breasts were washed by shaking the entire sample (average surface area 600 $cm^2$) in 200 ml sterile peptone water in a plastic bag for 1 min with intermittent rubbing of the surface. Serial dilutions were made in 0.1% peptone water, spread-plated on the appropriate selective media and incubated at 37°C for 24 hr before counting. *Salmonella* spp. used in this study were resistant to naladixic acid and were thus enumerated on brilliant green agar (BBL) with 100 p.p.m. naladixic acid. Colonies were transferred to lysine iron agar slants (BBL) for confirmation. *S. aureus* was enumerated on Baird-Parker medium (Difco) and *E. coli* on violet red bile agar (BBL).

*Sorbate analysis*

Both products were analyzed for residual levels of sorbic acid by the HPLC method described by Robach *et al.* (1980).

## Results and discussion

Sorbate analysis on day of arrival indicated 0.24% sorbate in the breast product and 0.12% sorbic acid in the sliced product.

Figure 1 shows the inhibition of *Salmonella, S. aureus* and *E. coli* on vacuum-packed, sliced turkey luncheon meat by sorbic acid. Control samples inoculated with either *Salmonella* or *E. coli* gave off-odour after 6 days of

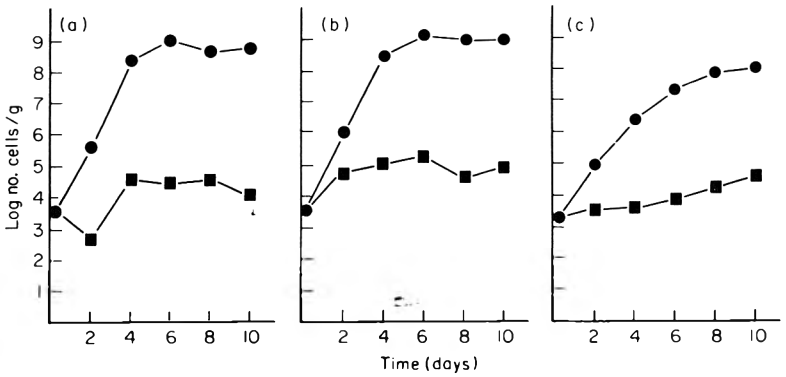


**Figure 1.** Effect of 0.2% sorbic acid on the growth of (a) *E. coli*; (b) *Salmonella*; (c) *S. aureus* in vacuum packed turkey slices stored at 15°C. ●, control; ■, sorbate.

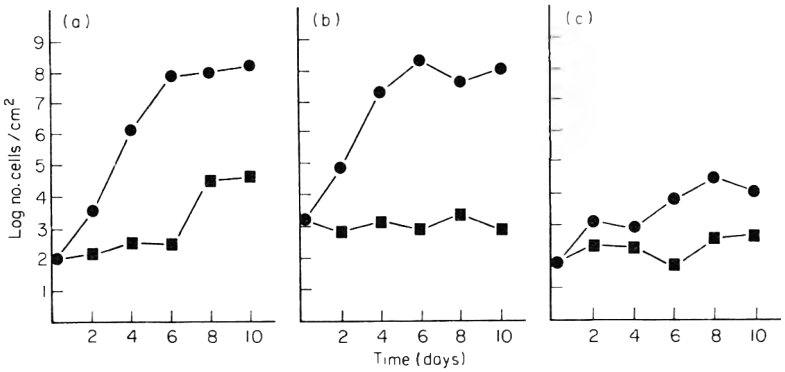


**Figure 2.** Effect of 0.26% potassium sorbate on the growth of (a) *E. coli*; (b) *Salmonella*; (c) *S. aureus* in vacuum packed turkey breasts stored at 15°C. ●, control; ■, sorbate.

storage when the counts were approaching $10^9$ cells/g. After 8 days of storage these samples had a distinct spoilage odour. The samples inoculated with *S. aureus* lost their firm texture after 8 days' storage when the counts reached $10^8$ cells/g. In contrast, the sorbate-treated product effectively inhibited the growth of all three pathogens throughout the 10-day storage at 15°C. None of the packages in the sorbate-treated lots exhibited off-odours in the 10-day test.

Figure 2 shows the effect of potassium sorbate on pathogen growth in vacuum packaged turkey breasts. As in the sliced control product, *Salmonella* and *E. coli* grew rapidly on the control breasts and reached a maximum of $10^8$ cells/cm² in 6 days. In addition to off-odour, on day 8 a pink colour developed on the meat surface not covered by skin. *S. aureus* did not grow well on the breast product, only increasing 2 log cycles in 10 days. The use of 0.26% sorbate effectively stopped growth of *Salmonella* and *S. aureus* through the 10-day storage period. There was a 2–3 log cycle increase in *E. coli* numbers after 6 days of 15°C storage. However, final levels were 3.5 log cycles below those of the controls.

This study demonstrates that sorbates are extremely effective in controlling the growth of *Salmonella* and *E. coli* in vacuum packaged, uncured turkey products. Sorbates also provide marked protection against the growth of *S. aureus* in these products.

## Acknowledgments

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# Taste properties of cocoa drinks with an added bitter/sweet sugar: intensity/time effects

G. G. BIRCH AND G. OGUNMOYELA

## Summary

Cocoa drinks treated with either sucrose or the bitter/sweet sugar, methyl α-D-mannopyranoside were evaluated for their intensity/time response determined by taste panellists using a moving chart recorder. Both additives caused an increase in persistence as well as intensity of response, sucrose for sweetness and methyl α-D-mannopyranoside for bitterness.

Tasting of the sucrose-treated drinks, after presaturating panellists' tongues with quinine sulphate solutions, caused no significant differences in perceived sweetness. However, presaturating panellists' tongues with sucrose solution did cause significant differences in bitterness perception of the drinks containing methyl α-D-mannopyranoside.

These results accord with a previous observation of the taste response to methyl α-D-mannopyranoside in pure water and suggest that the bitter/sweet molecule may exert its effect by simultaneously spanning both basic taste receptors.

## Introduction

A previous report from this laboratory (Birch & Mylvaganam, 1976) showed that the taste response to the bitter/sweet glycoside, methyl α D-mannopyranoside, could be specifically impaired by presaturating panellists' tongues with either sucrose or quinine sulphate solutions, the former treatment depressing its sweetness. These results, as well as giving a clue to the mechanism of action of bitter/sweet molecules on taste receptors, suggested practical problems which might result from the use of simple additives in food systems. In particular the effect of a single molecule with two or more basic taste-modifying

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properties cannot be fully evaluated without understanding how other food components might modify its interaction with the taste receptor.

With these difficulties in mind we have studied the effects of adding methyl α-D-mannopyranoside to cocoa drinks by a novel time/intensity recording technique which in turn allows a more thorough interpretation of total gustatory response than is possible by conventional methods.

We have further investigated the effects of added surfactant in an attempt to gain a deeper insight of the chemoreceptive functions of bitter/sweet additives.

## Materials and methods

Cocoa drinks were made up by mixing 5 g of commercial cocoa powder (Cadbury's, Bournville, containing sodium carbonate, salt and flavourings) alone or with sucrose (AR grade, May and Baker, Dagenham) or methyl α-D-mannopyranoside (MMP) (Sigma Chemical Co.) and tap water (100 ml) at 95–100°C in a 250 ml beaker. Some solutions (C and D) also contained 0.5 g surfactant-glycerol monostearate, (GMS) food grade from BDH, Poole or lecithin (L). i.e. Wylfo 'A', obtained from Wynmouth Lehr and Fatoils Ltd., London – in 100 ml of cocoa drink solution. Compositions of the four solutions tasted at any one sitting by panellists were therefore as follows:-

A = Standard, 5 g cocoa powder/100 ml, no added sugar, glycoside or surfactant
B = Cocoa powder + sucrose (1.5 g or 7.5 g) or MMP (1.3 g or 7.5 g) per 100 ml, no added surfactant
C = B + 0.5 g GMS
D = B + 0.5 g L

Four solutions (10 ml each), presented to each of ten panellists at 50°C were evaluated at one sitting using a moving chart recorder (Telsec Type X, moving at 12 cm $min^{-1}$) linked to a potentiometer 'dial box' the dial of which could be moved from 0 to 10 arbitrary units and back again according to the intensity of basic taste experienced by the panellist. The dial box was located some twenty feet from the moving chart recorder to avoid panellist distraction. Traces of intensity against time were thus obtained for each basic taste from which intensity was taken as peak height and persistence time as the time for taste duration. Panellists were instructed to rinse their mouths with tap water and pause at least one minute between drinks. Retasting was allowed. All appropriate solutions were tasted in the same way before presaturation of panellists' tongues was attempted.

Each panellist undertook each tasting twice and means of the two determinations were obtained. Overall means of 10 panellists in each case are presented in the Tables, although means for each panellist were subjected to a 4-way analysis of variance taking samples, concentrations, presaturation effect and panellists as variables.

For presaturation studies panellists were instructed to hold 10 ml of 0.005% quinine sulphate solution in their mouths for 10 sec, spit out and then immediately taste the sample and assess its sweetness. Alternatively they were instructed to hold 10 ml of 50% sucrose solution in their mouths for 10 sec, spit out and then immediately taste the sample and assess its bitterness (Birch & Ray, 1979). It was observed that those panellists who gave high scores were consistent in this and the same was true of panellists who consistently gave low scores. This in no way affected the validity of the technique, since a comparison with the conventional numerical scale procedure showed no significant differences between results obtained by both methods.

## Results and discussion

Methyl α-D-mannopyranoside is a bitter/sweet glycoside but its sweetness is so low as to be masked by the intrinsic bitterness (due to alkaloids etc.) of cocoa. Panellists tasting cocoa drinks containing methyl α-D-mannopyranoside were only therefore able to recognize the bitterness due to the presence of the glycoside. Sucrose-containing cocoa drinks were therefore separately assessed for sweetness.

Table 1a lists the mean sweetness or bitterness persistence times ($T_p$) and intensities ($I_t$) of cocoa drinks rendered sweet by addition of 1.5% sucrose or bitter by addition of 1.3% methyl α-D-mannopyranoside. Table 1b lists the same parameters after presaturation of panellists' tongues with 0.005% quinine sulphate solution or 50% sucrose respectively.

There is no marked effect of presaturation with quinine sulphate on the sweetness of the drinks, either in terms of intensity or persistence, but there is evidence of depression of the bitterness of the glycoside after presaturation with sucrose. Columns C and D in each Table list the effects on sweetness and bitterness of the drinks when surfactant – glycerol monostearate (GMS) and lecithin (L) – is included. Surfactant tends to enhance intensity and time of sweetness response (Birch & Ogunmoyela, 1980) but at the low concentrations of the two basic taste stimuli considered in Table 1 this is not very apparent.

Table 2a lists the mean sweetness or bitterness persistence times ($T_p$) and intensities ($I_t$) of cocoa drinks rendered sweet by addition of 7.5% sucrose or bitter by addition of 7.5% methyl α-D-mannopyranoside. Table 2b lists the same parameters after presaturation of panellists' tongues with 0.005% quinine sulphate solution or 50% sucrose respectively. Although many of the cocoa drinks in Table 2 have lower sweetness intensities and persistences after presaturation with quinine sulphate than before, the effect is not marked. However, the bitterness of the glycoside-containing drinks in Table 2 is markedly lower in both intensity and persistence after presaturation of panellists' tongues with 50% sucrose solution in all cases except one drink containing lecithin. Again columns C and D show the effects of added surfactant (GMS and lecithin respectively).

**Table 1a.** Mean persistence times and intensities of sweetness and bitterness of cocoa drinks containing low concentrations of sucrose or methyl α-D-mannopyranoside before presaturation (10 panellists)

| | Mean persistence times ($T_p$) (sec) | | | | Mean intensities ($I_T$) (% max of arbitrary scale) | | | |
|---|---|---|---|---|---|---|---|---|
| | $T_{PA}$ | $T_{PB}$ | $T_{PC}$ | $T_{PD}$ | $I_{TA}$ | $I_{TB}$ | $I_{TC}$ | $I_{TD}$ |
| Sweetness | 4.8 | 7.5 | 8.7 | 6.6 | 10.3 | 21.3 | 25.1 | 21.5 |
| s.d.* | (4.0) | (4.2) | (5.2) | (4.2) | (7.9) | (10.1) | (15.8) | (12.8) |
| Bitterness | 28.2 | 34.9 | 35.8 | 32.4 | 56.5 | 58.6 | 62.9 | 55.8 |
| s.d.† | (17.7) | (18.7) | (29.6) | (20.9) | (19.7) | (20.3) | (22.6) | (22.6) |

*Sweetness solutions composed as follows: A = Cocoa (5 g) + water (100 ml); B = A + 1.5 g sucrose; C = B + 0.5 g GMS; D = B + 0.5 g L.

†Bitterness solutions composed as follows: A = Cocoa (5 g) + water (100 ml); B = A + 1.3 g methyl α-D-mannopyranoside; C = B + 0.5 g GMS; D = B + 0.5 g L.

Analysis of variance tests for significance: persistence times for sweetness and bitterness significantly different between samples ($P<0.001$); sweetness and bitterness intensities significantly different between samples ($P<0.001$).

**Table 1b.** Mean persistence times and intensities of sweetness and bitterness of cocoa drinks containing high concentrations of sucrose of methyl α-D-mannopyranoside before presaturation (ten panellists)

| | Mean persistence times ($T_p$) (sec) | | | | Mean intensities ($I_T$) (% max of arbitrary scale) | | | |
|---|---|---|---|---|---|---|---|---|
| | $T_{PA}$ | $T_{PB}$ | $T_{PC}$ | $T_{PD}$ | $I_{TA}$ | $I_{TB}$ | $I_{TC}$ | $I_{TD}$ |
| Sweetness | 7.9 | 9.0 | 14.1 | 12.4 | 37.4 | 51.9 | 61.7 | 51.8 |
| s.d.* | (2.7) | (3.1) | (6.2) | (7.2) | (10.8) | (14.5) | (17.9) | (10.8) |
| Bitterness | 14.4 | 26.1 | 28.8 | 35.3 | 56.4 | 69.9 | 75.2 | 61.2 |
| s.d.† | (5.7) | (15.5) | (33.0) | (25.8) | (9.9) | (18.5) | (15.3) | (22.5) |

*†As in Table 1a, but solutions B, C, D containing sucrose or methyl α-D-mannopyranoside at 7.5 g/100 ml level.

Persistence times for sweetness and bitterness significantly different between samples ($P<0.001$)

Sweetness or bitterness intensities significantly different between samples ($P<0.001$)

Panellist responses significantly different between low and high concentrations for sweetness ($P<0.001$) but not for bitterness. (Tables 1a and 1b compared.)

The marked effect of presaturation with sucrose on the bitterness of cocoa drinks containing methyl α-D-mannopyranoside is better illustrated by the product of persistence and intensity (PI) which may be regarded as a truer representation of total gustatory response (e.g. as has been emphasized previously by Birch (1978) and utilized by Larson-Powers & Pangborn (1978)). Tables 3a and 3b list these products for sweetness and bitterness respectively, before and after presaturation of panellists tongues, as explained above. Only in the case of the bitterness of drinks containing the glycoside is there a consistent

**Table 2a.** Mean persistence times and intensities of sweetness and bitterness of cocoa drinks containing low concentrations of sucrose or methyl α-D-mannopyranoside after presaturation (ten panellists)

| | Mean persistence times ($T_P$) (sec) | | | | Mean intensities ($I_T$) (% max of arbitrary scale) | | | |
|---|---|---|---|---|---|---|---|---|
| | $T_{PA}$ | $T_{PB}$ | $T_{PC}$ | $T_{PD}$ | $I_{TA}$ | $I_{TB}$ | $I_{TC}$ | $I_{TD}$ |
| Sweetness | 3.6 | 6.2 | 8.5 | 7.4 | 11.3 | 22.1 | 26.4 | 25.7 |
| s.d.* | (3.7) | (3.3) | (5.4) | (5.5) | (11.4) | (11.0) | (16.9) | (19.0) |
| Bitterness | 13.9 | 15.8 | 25.2 | 19.0 | 40.5 | 56.1 | 55.8 | 52.3 |
| s.d.† | (7.5) | (5.2) | (22.9) | (13.2) | (13.5) | (23.7) | (23.3) | (22.6) |

*†As in Table 1a.

Persistence times for sweetness and bitterness significantly different between samples ($P<0.001$)

Sweetness or bitterness intensities significantly different between samples ($P<0.001$). Presaturation effect significant for bitterness ($P<0.001$) but not sweetness.

**Table 2b.** Mean persistence times and intensities of sweetness and bitterness of cocoa drinks containing high concentrations of sucrose or methyl α-D-mannopyranoside after presaturation (ten panellists)

| | Mean persistence times ($T_P$) (sec) | | | | Mean intensities ($I_T$) (% max of arbitrary scale) | | | |
|---|---|---|---|---|---|---|---|---|
| | $T_{PA}$ | $T_{PB}$ | $T_{PC}$ | $T_{PD}$ | $I_{TA}$ | $I_{TB}$ | $I_{TC}$ | $I_{TD}$ |
| Sweetness | 7.6 | 10.2 | 11.4 | 9.5 | 33.5 | 47.8 | 56.3 | 63.5 |
| s.d.* | (3.6) | (4.3) | (4.8) | (3.4) | (8.9) | (15.3) | (14.0) | (14.8) |
| Bitterness | 13.2 | 15.0 | 19.4 | 18.7 | 54.3 | 56.2 | 68.7 | 64.7 |
| s.d.† | (9.1) | (9.3) | (17.2) | (13.9) | (10.7) | (18.1) | (22.6) | (22.1) |

*†As in Table 1b.

**Persistence times for sweetness and bitterness significantly different between samples ($P<0.001$).

Sweetness or bitterness intensities significantly different between samples ($P<0.001$). Presaturation effect significant for bitterness between samples ($P<0.001$) but not sweetness (Tables 1b and 2b compared).

and significant drop in PI after presaturation. This result accords with a previous report from this laboratory (Birch & Mylvaganam, 1976) which noted the loss of both sweetness and bitterness of methyl α-D-mannopyranoside in pure water after presaturation of panellists' tongues with either strongly sweet or strongly bitter soiutions. It is consistent with the concept of 'polarization' of the glycoside on the taste receptor such that one 'end' of the molecule binds with a sweet receptor site and the other 'end' with a bitter receptor. Since the molecule spans both sites simultaneously, impairment of one mode of binding by presat-

**Table 3a.** Mean product values of persistence times and intensities of sweetness and bitterness of cocoa drinks containing sucrose or methyl α-D-mannopyranoside before presaturation (ten panellists)

| | Mean product of persistence time/intensity (*PI*) | | | |
|---|---|---|---|---|
| | $PI_A$ | $PI_B$ | $PI_C$ | $PI_D$ |
| Sweetness (1.5 g%) | 47 | 158 | 243 | 152 |
| s.d.* | (65) | (152) | (253) | (189) |
| Sweetness (7.5 g%) | 306 | 498 | 861 | 608 |
| s.d.* | (149) | (317) | (408) | (269) |
| Bitterness (1.3 g%) | 1727 | 2276 | 2713 | 2005 |
| s.d.† | (1440) | (1795) | (3061) | (1723) |
| Bitterness (7.5 g%) | 827 | 1929 | 2620 | 2381 |
| s.d.† | (380) | (1428) | (3355) | (2636) |

*†As in Tables 1a and 1b.

*PI* values for sweetness and bitterness highly significantly different between samples ($P<0.001$).

**Table 3b.** Mean product values of persistence times and intensities of sweetness and bitterness of cocoa drinks containing sucrose or methyl α-D-mannopyranoside after presaturation. (ten panellists)

| | Mean product of persistence time/intensity (*PI*) | | | |
|---|---|---|---|---|
| | $PI_A$ | $PI_B$ | $PI_C$ | $PI_D$ |
| Sweetness (1.5 g%) | 64 | 140 | 247 | 239 |
| s.d. | (95) | (164) | (248) | (329) |
| Sweetness (7.5 g%) | 260 | 512 | 639 | 586 |
| s.d. | (139) | (328) | (338) | (223) |
| Bitterness (1.3 g%) | 592 | 944 | 1645 | 1030 |
| s.d. | (391) | (612) | (1810) | (881) |
| Bitterness (7.5 g%) | 714 | 1062 | 1547 | 1391 |
| s.d. | (479) | (723) | (1726) | (1483) |

*†As in Tables 1a and 1b.

*PI* values for sweetness and bitterness highly significantly different between samples ($P<0.001$).

Presaturation effect significant between samples for bitterness ($P<0.001$) but not for sweetness. (Tables 3a and 3b compared).

uration, prevents it from binding with the other. It is of interest that this same behaviour is discernible in cocoa drinks despite the plethora of possible interfering substances which are present.

We have previously reported (Birch & Ogunmoyela, 1980) that surfactants added to cocoa drinks can elevate both their sweetness intensity and persistence. The effect is again noticeable in the higher concentrations investigated here but further information about their mode of interaction with the receptor can be obtained by studying their effects before and after presaturation of panellists' tongues with quinine sulphate solutions. For example, the sweetness response products (PI) for sucrose-sweetened drinks are elevated (Table 3) by GMS (column C) before and after presaturation of panellists' tongues with quinine sulphate solution, but inspection of the separate time and intensity factors (Tables 1 and 2) show that these do not behave in the same way. Thus, although both time and intensity of response are elevated by addition of GMS before presaturation, the increase in PI after presaturation (due to GMS) is almost entirely made up of a substantial increase in intensity. This can be interpreted as resulting directly from the occupation of hydrophobic bitter receptors sites (Wingard *et al.*, 1978) by quinine sulphate molecules, thus *sparing* the GMS molecules for complexing with sucrose molecules and facilitating their accession to fresh sweet receptor sites. For a given concentration of sucrose molecules a distribution pattern must exist between localized concentration (Birch, Latymer & Hollaway, 1980) at a few sites (persistence effect) and occupation of additional sites (intensity effect). Clearly after presaturation with quinine sulphate solution addition of GMS favours the latter possibility. The effects of lecithin (L) are less well-defined and possibly reflect the complex interplay of surfactant and rheological properties of this additive.

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# Influence of heat and sulphur dioxide treatments on some quality characteristics of intermediate-moisture banana

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## Summary

The effect of duration of heat treatment (blanching) on the firmness, dehydration rate, colour, and pectin esterase (PE) activity, was investigated in sulphited, unsulphited, blanched and non heat-treated intermediate-moisture banana (IMB). The product was significantly firmer if the bananas had been blanched, and in either event, firmness as measured with a texturometer, also increased with increasing $SO_2$ concentration. The average rate of water removal (dehydration) increased, with increasing blanching time, up to about 4 min, and increased also with increasing $SO_2$ concentration. There was a decrease in PE activity in both blanched and non-heat-treated IMB, with increasing $SO_2$ content. The effect of sulphiting on pectolytic enzyme activities does not appear to have been reported previously.

## Introduction

Intermediate moisture food products (IMFP) are usually developed with the intention of retaining the quality characteristics, such as colour, flavour, appearance and texture, as closely as possible to those of the raw material. On the other hand, IMFP are expected to be reasonably stable under storage and marketing conditions and should be easily rehydratable if required (Brockman, 1973; Potter, 1970). The stability is due to their lower water activity ($a_w$) as compared with that of the raw material, but this is not as low as in a fully dehydrated product. In order to prevent deterioration following post-production contamination, both in fully dehydrated and in IM fruit products, preservatives such as benzoates or sorbates are generally added. Such preserva-

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tives do not inactivate enzymes which could affect the colour (polyphenolases, etc.) texture (pectolases) or flavour (lipoxidases) of either IM or fully dehydrated fruits and vegetables (Acker, 1969).

One exception is $SO_2$, which possesses bactericidal properties and also inhibits enzymatic (Embs & Markakis, 1965) and non-enzymatic (McWeeny, Knowls & Hearne, 1974) darkening. Embs & Markakis (1965) found that the mechanism of $SO_2$ inhibition of browning caused by polyphenol oxidase (PPO), is the $SO_2$ reaction with some substrate compounds (such as the enzymatically produced O-quinones – from the existing polyphenols), as well as direct enzyme inactivation by the $SO_2$. The literature does not appear to note that $SO_2$ has any effect on pectolytic enzyme activity, or on textural properties of $SO_2$ -preserved fruit and vegetable dehydrated products.

Another method of inactivating enzymes in industrial processed fruit and vegetables, is heat treatment. Blanching is used to inactivate enzymes responsible for quality deterioration during processing and storage of food products. On the other hand, blanching is not always advisable for most commercially prepared tropical fruit products, because of its negative effects on their delicate textural properties and other quality characteristics (Czyrinciw, 1969). For example, heat treatment of banana is one of the important factors responsible for a pink discoloration in the products (Ranganna & Parpia, 1974). Damaged banana tissue (after peeling, etc.) darkens very quickly, due to enzyme oxidation of dopamine and other polyphenols (Weaver & Charley, 1974), and some non-enzymic darkening and pink discoloration are observed after heat treatment of banana pulp (Ranganna & Parpia, 1974).

When plant tissue is heat-treated during commercial processes, such as blanching, cooking, drying, etc., some physico-chemical changes in its structural components would be expected, so that the resulting texture characteristics of the processed material are different from those of the fresh plant tissue. Mathee & Appledorf (1978) found that cooking processes cause significant changes in crude-fibre content and other polysaccharide components of various vegetables. They report that cooking liberates from the cell wall cellulose and pectic substances, or can even cause their hydrolysis, with consequent changes in their water binding capacities. Another factor which could cause texture breakdown, with effects such as variations in firmness (toughness) and water liberation or binding, is pectin degradation. Luh, Ozbilgin & Liu (1978) reported changes in firmness of canned apricots during storage, due to pectin degradation catalyzed by added polygalacturonase of mould origin.

Such factors which affect the cell wall permeability also affect the drying behaviour of plant tissue during dehydration (Rotstein & Cornish, 1978). Cellulose and pectic substances are among the main water-binding components of the cell wall and of plant tissue. Changes in their chemical and physical properties could affect the drying behaviour of fruits and vegetables during dehydration, by modifying the cell wall water-binding capacity (Parrot & Thrall, 1978).

The effects of heat treatment and $SO_2$ addition on some quality characteris-

tics of intermediate moisture banana (IMB) were now studied. The effects of heat and $SO_2$ treatments on PE activity in IMB are reported.

## Materials and methods

For each experimental condition, batches of 5–10 kg of physiologically mature banana fruits, *cv*. Gross-Michel, obtained from a commercial plantation in Venezuela, were artificially ripened, under controlled conditions, up to about 24.5 – 25.5° Brix (less than 1% starch). The ripening conditions were: 20±2°C, at about 85% r.h. The ripe bananas were hand peeled and cut longitudinally into equal halves before further treatment. The soluble solids (°Brix) content of the fresh bananas was measured with an Abbe refractometer, in a blended (5 min) representative sample of about 200 g fresh banana halves.

### *Heat treatment (blanching) and sulphitation*

The banana halves were dipped in boiling water for various times up to 7 min and immediately cooled to about 35°C or less in a soft-water bath, at room temperature (2–3 min). Unblanched and blanched bananas were sulphited separately by dipping for 10 min in sodium bisulphite solutions of various concentrations of up to 20,000 p.p.m. $SO_2$ (see 'Results and discussion'). The $SO_2$ content in the IMB was determined by the modified Monier-Williams method (AOAC, 1970; Method No. 27080).

### *Dehydration and storage*

The treated banana halves were dehydrated under standard conditions on perforated stainless steel trays, with cross-through air-flow. The drying conditions were 3 hr at 70°C (air r.h. = 30%), followed by 3–6 hr at 55°C (r.h. = 50%), so that the final moisture content of the IMB was between 30% and 40% (on wet basis). The bananas were dried in a Proctor Standard 10 RAX cabinet drier (Proctor & Schwartz) with automatically controlled air temperature and humidity, and at constant air velocity. All the samples of each experiment were dried simultaneously, in order to ensure uniform drying conditions.

The IMB were packed between polyethylene sheets and stored in cartons at room temperature (21±2°C) for 1–6 weeks, before their quality characteristics were evaluated. The different batches of each experiment were stored for equal periods of time (±24 hr), before quality evaluation and analysis.

### *Texture*

The texture (firmness) was measured with an FTC (Food Technology Corporation – Lee-Kramer) Texturepress Mod. TP–1, connected to a Varian TR–1, Texturecorder. Three to five representative, 100 g, samples of IMB halves for

each experimental condition were laid carefully in the direction perpendicular to the cutting blades. The firmness measurement conditions were: hydraulic pressure, 100 lb; velocity of movable (cutting) blades, 1 cm/sec; rings, 300 or 3000 lb. The 'firmness' values given are the arithmetic means of individual values for each sample under the same experimental condition, expressed as the maximal shear-compression force applied to cut the banana halves.

*Dry matter (moisture) content*

The dry matter content (DM) was analyzed on three fresh samples of IMB for each experimental condition, by drying them in a vacuum oven (650 mmHg vacuum at 60°C), to constant weight (about 16 hr), by the method described by Hart & Fisher (1971).

*Rate of water removal*

The average rate of water removal was calculated from the weight losses during the 'dehydration' process and from the difference in the DM between the fresh and IM banana halves. The values given represent the mean values of 3–5 experiments for each experimental condition. The banana halves were dehydrated simultaneously for all the experimental conditions of heat treatment and sulphiting – in order to ensure uniform drying conditions. The total loss in weight (evaporated water) from the fresh to the IM bananas is divided by 6 (hr) and expressed as a percentage of the weight of the fresh banana halves.

*Colour (discoloration, browning)*

The reflectance of the IMB was measured directly, with a representative sample of the IMB halves covering the bottom of a standard glass vessel, with a Gardner Color Difference Meter – model XL10 CDM. The results are expressed in L b/a values (L = lightness; + b = yellowness; + a = redness), which were found to be significantly correlated with the visibly lighter or darker colour of the various tested samples.

*Pectin esterase (PE) activity*

PE was extracted from about 100 g blended representative sample (for each experimental condition), by the method described by de Swardt & Maxie (1967), and the PE activity was determined as described by Singleton & Rossi (1965).

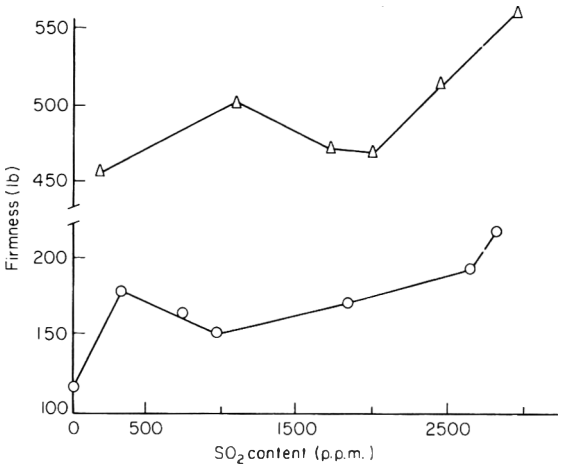


**Figure 1.** Heat treatment (3 min dipping in boiling water), sulphur dioxide content and firmness of intermediate moisture banana. △, Heat treated; ○, non heat-treated.

## Results and discussion

### *Texture*

The effects of heat treatment and $SO_2$ concentration on textural properties of IMB, were evaluated by 'firmness' measurements, as described above. The firmness of the blanched product (3 min in boiling water), compared with that of the unblanched product, as affected by the $SO_2$ content after 6 weeks' storage, is shown in Fig. 1. These results are representative of ten replicate experiments.

The blanched product was found to be firmer than the unblanched, both having about the same moisture content (38±2%). Cooking usually causes textural alteration in the tissue, which becomes softer or 'tougher' depending on its composition and physico-chemical characteristics. These alterations, as well as alterations in textural properties during the drying and storage, are probably responsible for the observed differences in firmness of heat- *vs.* non heat-treated IMB. The 'firmness' values of the IMB were found to be between 120 and 220 lb shear-force in the unblanched product, and between 450 and 550 lb in the blanched product.

The $SO_2$ content of the IMB halves also affected the 'firmness'. In the unblanched product, this increased from 120 lb when not sulphited, to 220 lb when about 2500 p.p.m. $SO_2$ was present in the IMB; in the blanched product the measured increase was from 450 (no $SO_2$) to 550 lb (2500 p.p.m. $SO_2$). One of the reasons responsible for the observed effect of $SO_2$ on firmness could be the $SO_2$ – inactivation of pectolytic enzymes (and the consequent influence on the pectic components), which were not fully inactivated up to 3 min heat treatment, as is shown below.

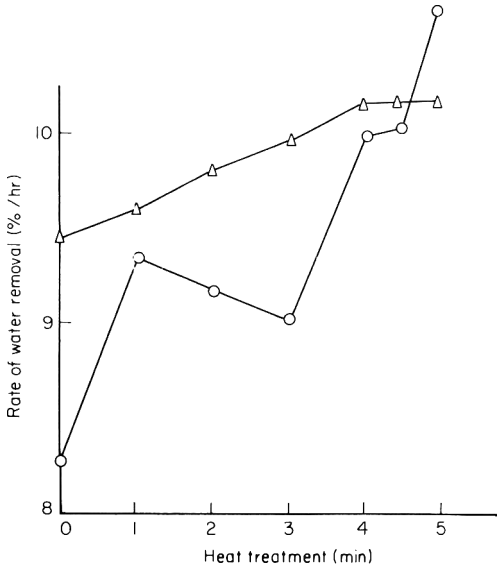


**Figure 2.** Average rates of water removal during dehydration of intermediate moisture banana. ○, Non-sulphited; △, dipped in $SO_2$ solution.

*Dehydration rate*

Heat treatment affects both the permeability of the cellular membrane (Rotstein & Cornish, 1978) and the water-binding capacity of plant fibrous materials (Mathee & Appledorf, 1978). Sulphur dioxide was also found to affect the permeability of a certain cellular membrane in banana tissue (Ramirez-Martinez *et al.*, 1977). The effect of heat treatment was therefore investigated, on the rate of water removal during dehydration of sulphited IMB (10 min in 3000 p.p.m. $SO_2$ solution); the results are shown in Fig. 2. These results represent the average rate of water removal by dehydration, as calcu-

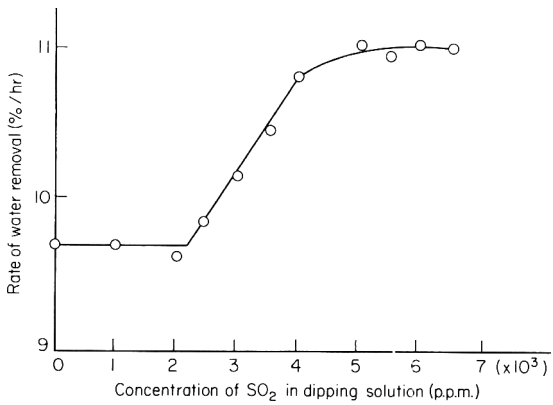


**Figure 3.** Effect of sulphur dioxide treatment on average rates of water removal during dehydration of intermediate moisture banana.

lated from differences in weight of the differently treated samples, all dried under the same conditions, as well as from their final dry matter content, at the end of the dehydration process. Even if there are differences in the 'rates' in the different experiments, due to raw material variability, the trends observed in three similar experiments were similar to the figures presented here. Drying was more rapid for the sulphited than for the unsulphited product, possibly due to the combined effects of blanching and $SO_2$ on the permeability of the cell membranes, as well as to their influence on the water-binding capacities of the fibrous matter. The differences between sulphited and unsulphited products disappear after 4 min of heat treatment, possibly due to the complete destruction of the cell membrane on blanching. The variations in the intermediate values of water removal observed were probably caused by natural differences in the composition and structure of the raw banana halves, even at a uniform degree of ripeness.

The influence of the degree of sulphiting of the bananas on the rate of water removal (dehydration) of the IMB, is presented in Fig. 3. Samples of about 5 kg each were blanched and dipped in sodium bisulphite solutions of different concentrations (including plain tap water) for 10 min, and then treated as described above. The average rate of water removal (percentage water removed per hour) was calculated as above. The results given in Fig. 3 represent the three similar experiments, carried out under the same drying conditions. No differences in the rate of water removal were observed between samples dipped in $SO_2$ solution of up to 2000 p.p.m. The rate in this case was about 9.7%/h. Further increases in the $SO_2$ concentration in the dipping solution affected the 'rate', and at 5000 p.p.m. $SO_2$ it was *ca* 11%/h. No significant differences in the rate were observed when increasing the $SO_2$ concentration from 5000 to 6500 p.p.m. Some variations in the rates of the water removal were observed in the different experiments, but the trend was similar to that in Fig. 3. The results of these experiments also indicate an influence of the $SO_2$ treatment on physico-chemical changes in the water-binding components of the IMB tissue and/or on the cellular membrane permeability, which are expressed in the observed faster dehydration rates (facilitated water removal) during the drying process.

*Colour*

Sulphur dioxide prevents brown discoloration of enzymic and of non-enzymic origin (Embs & Markakis, 1969; McWeeny, Knowles & Hearne, 1974). On the other hand, $SO_2$ causes bleaching of anthocyanin pigments (Desrosier, 1963). As bananas are known for their fast darkening of damaged tissue, the effect of $SO_2$ on the IMB colour was studied. Banana halves were dipped in a 3000 p.p.m. $SO_2$ solution for 10 min following heat treatments of up to 7 min, dehydrated to about 30% moisture, and the IMB stored for 6 weeks. The influence of the $SO_2$ and the heat treatment on the resulting colour values is given in Fig. 4.

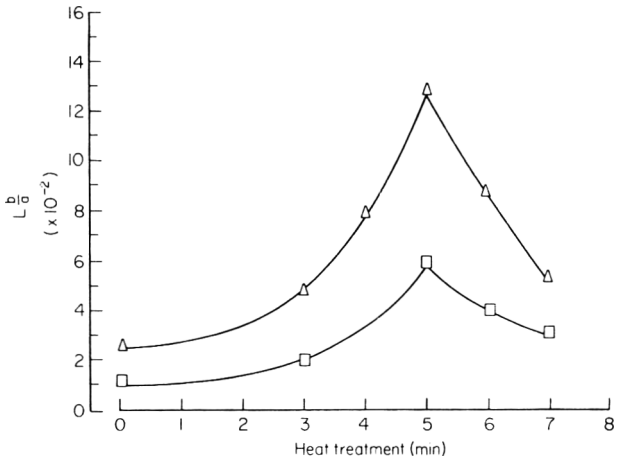


**Figure 4.** Influence of heat and sulphur dioxide treatments on colour of intermediate moisture banana. △ $SO_2$-treated; □ heat-treated only.

The colour (L b/a) values of the blanched product, were compared with the same in non-blanched. The L b/a values increased (prevention of darkening) with increasing blanching time for up to 5 min, in both sulphited and unsulphited IMB. Longer blanching (6–7 min) caused a marked decrease in the L b/a values (faster darkening). The prevention of darkening after blanching and $SO_2$ treatments may have been due to inactivation of the polyphenolic enzymes responsible for enzymic browning of bananas. On the other hand, a longer blanching time could be responsible for cooking effects, which may cause significant changes in the banana tissue's permeability. This, in turn, could be responsible for the liberation of substrate compounds into the media, causing non-enzymic browning and/or a decreased ability for retention and reaction with the $SO_2$, at low $SO_2$ concentration level (about 200 p.p.m. in the IMB at the beginning of storage).

In conclusion, the heat and $SO_2$ treatments of banana fruits prior to dehydration have a marked effect on the colour of the IMB, as well as on its texture. Some negative effects, such as toughening (increased firmness) and darkening, are observed at certain levels of these treatments.

*Pectin esterase inactivation*

Another negative effect of heat and $SO_2$ is the astringent taste observed in the IMB, heat-treated for longer times and at higher $SO_2$ concentrations, as reported by Ramirez-Martinez *et al.* (1977). Adequate blanching is generally applied for enzymic (including PE) inactivation. PE is one of the enzymes responsible for pectin degradation, so that PE could affect some structural-textural properties of plant materials relatively rich in pectins. On the other hand, $SO_2$, which inhibits polyphenolic enzymes such as PPO. is apparently not

**Table 1.** Effect of $SO_2$ content on pectin esterase (PE) activity in intermediate moisture banana halves*

| Non heat-treated | | Heat-treated† | |
|---|---|---|---|
| $SO_2$ (p.p.m.) | PE (μmol/g min) | $SO_2$ (p.p.m.) | PE (μmol/g min) |
| 0 | 26.0 | 0 | 5.0 |
| 340 | 4.2 | 180 | 2.7 |
| 710 | 0.9 | 1020 | 0.9 |
| 960 | 2.7 | 1150 | 0.0 |
| 1810 | 3.5 | 1700 | 0.0 |
| 2030 | 0.8 | | |
| 2620 | 2.0 | | |
| 2820 | 0.0 | | |

*Dehydrated to about 30% moisture content.
†For 3 min in boiling water.

known to have any effect on activity of pectolytic enzymes. When studying the effects of blanching and $SO_2$ on the IMB, a decrease of PE activity was observed in the product, with increasing $SO_2$ concentration (Table 1).

The banana halves were prepared, blanched, $SO_2$-treated, dehydrated to about 30–33% moisture, and stored for 6 weeks, before the PE activity in the resulting IMB was measured. The results given in Table 1 represent the observed PE inactivation in five such experiments. Some differences in initial PE activity, as well as different degrees of inactivation, were observed in the different experiments, possibly due to natural variations in the banana tissue and differing concentrations of $SO_2$ present in the IMB, after similar treatments. On the other hand, the trend was always similar to that presented here.

A marked decrease in PE activity was observed in both the blanched and non-blanched products, with increasing $SO_2$ concentration in the different samples. The PE activity in the unsulphited, non heat-treated IMB was 26 μmol/g min, while at about 2800 p.p.m. $SO_2$ the PE was completely inhibited. After the blanching, the PE activity in the unsulphited IMB was about 5 μmol/g min. The enzyme was completely inhibited at about 1150 p.p.m. $SO_2$ in the IMB. The variations observed in the samples with intermediate concentration in the un-blanched product may have been due to natural variations in the initial PE activity in the fresh bananas. Blanching of 4.5–5 min completely inactivated the PE.

As mentioned in the 'Introduction', no reports were found in the literature that $SO_2$ inhibits pectolytic enzymes. On the other hand, Negoro (1972) reports that both PE and polygalacturonase (PG) in apples, were inhibited by tannin-like polyphenols. Ramirez-Martinez *et al.* (1977) found that there is an increase in the availability of certain polyphenolic compounds, responsible for astringency in IMB, after heat and $SO_2$ treatments. It is therefore presumed that, heat

and $SO_2$ caused an increase in tannin-like polyphenols in the IMB, with a consequent PE inactivation, caused by the same polyphenolic compounds. A correlation between the increase in polyphenols' availability in the IMB (after heat and $SO_2$ treatments), and the degree of inactivation of both PE and PG in the IMB, was investigated and the findings will be presented separately.

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# Book reviews

**Clinical Nutrition.** By M. Bennion.
New York: Harper and Row, 1979. Pp. viii + 564. ISBN 0 06 453526 6. US $24.00.

This book is primarily written for students of dietetics, with the medical student in mind. However, for the nutritionist or medical practitioner it would be a useful adjunct to standard texts on nutrition. A considerable amount of medical terminology is used throughout, and for this reason, the text may aim too high for the average dietetics student in this country. There is no glossary of medical terms, so it would have to be used in conjunction with other texts.

Each chapter contains up to a hundred up-to-date references. Many of these are from the American literature, except where the data are unavailable from this source, but the text is nonetheless useful for that. Throughout the book is written well, with a strong practical approach and features useful, clearly labelled diagrams and figures. Six major areas are discussed: nutrition care; over- and under-nutrition; nutrition and stress; gastrointestinal disorders; metabolic abnormalities; and cardiovascular and renal disorders. As well as information primarily of use to the dietician such as food exchange lists, physiological aspects of the digestive system and absorption and the role of the liver in nutrition are discussed. There are particularly good sections on nutritional anaemias, gastrointestinal disorders, atherosclerosis, coronary heart disease and diabetes mellitus. The section on hyperlipoproteinaemias contains a description of the current classification of these disorders with sample menus for each type.

On the whole the subject has been well reviewed but as the writing of such a book is a tremendous undertaking, it is to be expected that there would be a few criticisms. Certain subjects are only briefly discussed and yet are very important in this area. Two in particular are nutrition and cancer, and food allergies, which are only allocated very small sections. In addition, keeping abreast with new hypotheses in such a wide subject area is difficult. For example, the author has not critically discussed the hypercellularity theory of obesity, which recent evidence does not support. Another example is that in the section on intestinal disorders, a table is presented giving crude fibre figures for selected foods, despite dietary fibre values being available for some years.

Despite these criticisms, the book is very useful, the approach is well balanced and it is certainly to be recommended as reading for the nutritionally-inclined scientist.

*Ann F. Walker*

**Fundamentals of Food Canning Technology.** By J. M. Jackson and B. M. Shinn.
Westport, Connecticut: Avi Publishing Co. Inc., 1979. Pp. x + 406. ISBN 0 87055 257 0. US $22.00.

The purpose of this book is to present the specialized theory and practice of canning technology in one volume. The book can broadly be divided into four sections. These deal with the theory of heat sterilization, different types of containers, different types of product and a miscellaneous section including labelling, storage, quality assurance, nutritional content and waste disposal.

The first chapter is concerned with the 'Development of the Canning Industry': the emphasis here and throughout the book is on the American industry.

The second chapter by Pflug & Esselen, entitled 'Heat Sterilization of Canned Food', is a revised and updated version of their excellent chapter, first published in *Food Processing Operations*. This is one of the clearest written accounts on the theory of heat treatment and is well worth reading.

Chapters 3, 4 and 5 deal respectively with rigid metal containers, glass containers and closures, and flexible packaging for thermoprocessed foods. The main emphasis here is on the manufacture and the quality of the containers. New materials for rigid containers and the newer manufacturing techniques are also described. Flexible packaging is the newest technique for thermoprocessed foods and the chapter deals adequately with packaging materials, sealing and associated problems. Filling and processing are also dealt with.

Chapter 6 on general canning procedures is all of four pages and could well be omitted. Chapters 7 to 12 deal with canning procedures for fruits, vegetables, speciality products, meat and poultry, marine products and dairy products. The choice of commodities described in each section reflects the major interests of the American canning industry and its consuming public, e.g. the vegetables dealt with are green beans, beets, corn, peas, spinach, tomatoes and tomato juice.

The speciality products chapter is short (six pages) and the products themselves are disappointingly 'unspecial'; nothing more ordinary than soups, baby foods and baked beans are mentioned. Perhaps this is being a little unkind as anybody who has tried to make their baked beans taste like a well known commercial variety will probably tell me: baked beans in tomato sauce do warrant their own code of practice in Great Britain.

The marine products section describes the canning of salmon and is of interest, especially after the recent case of botulism.

The dairy product section deals mainly with evaporated milk. UHT treatment (called HTST sterilized products in the text) and aseptic packaging of dairy products are briefly discussed. No mention is made of two of our favourites, namely rice pudding and custards, again reflecting the differences in eating habits.

The chapter on the factors affecting the nutrient content of canned foods is, in my opinion, the most interesting in the miscellaneous section. Changes take

place during processing and during subsequent storage: particularly important during storage is the leaching of soluble materials into the brine or syrup and the subsequent loss of these if the liquids are discarded. There is a large amount of available data in this area, much of it confusing and contradictory. Much work remains to be done to clarify the situation, particularly if nutritional labelling is to be introduced. The chapter ends by briefly describing the introduction of nutritional labelling and its impact on the canning industry. Useful examples are given.

Chapters on waste disposal, and quality assurance complete the picture.

The book ends with forty-five pages of appendices. Of particular interest are the production figures for canned fruit and vegetables, both for America and several other countries, and the consumption figures for fresh, frozen and canned pudding in America. There is an interesting State by State guide to the processing seasons for all commonly canned fish and vegetables.

Finally there are tables of nutrient values for some canned food items: it may be of interest to compare these data with the data contained in the *Composition of Foods Tables* (HMSO).

The book is edited and has six contributors. However, there is not too much repetition. As with most AVI publications the units are predominantly British. Nevertheless in some chapters masses are recorded in kilograms (kg) and pressures in Pascals (Pa). The diagrams are satisfactory but some of the photographs have not reproduced too clearly. Compared to other books of similar length, it is not overpriced, and it would provide its owner with a comprehensive account of the activities of the American canning industry.

*M. J. Lewis*

**Food-borne Micro-organisms of Public Health Significance.** (2 Vols), 3rd edn. Ed. by K. A. Buckle, G. R. Davey, M. J. Eyles. G. H. Fleet and W. G. Murrell.
N.S.W. Branch Food Microbiology Group of the Australian Institute of Food Science and Technology, CSIRO Division of Food Research, and the School of Food Technology of the University of New South Wales. Published by the University of NSW, 1979 Pp. xxii + 521. ISBN 0 9597441 4 2. Aust. $32 incl. postage.

This is the 3rd edition of a publication designed to support an intensive laboratory course on the detection in foods of micro-organisms of public health significance, with many well-known microbiologists being involved in the course and thus in the preparation of these books. Although primarily intended as the manual for the course, the previous editions have already become well known and used in the U.K. and, no doubt, in other countries to support taught

courses in food microbiology. (The 2nd edition was reviewed in *Journal of Food Technology,* **13,** 155.)

Volume 1 discusses microbiological criteria for food, the microbial ecology of foods, and the microbiological and statistical aspects of quality control and sampling. It then provides short monographs on the most important groups of organisms to have public health implications when present in foods. The coverage provided here is very variable, although reflecting the priorities commonly found in current industrial practice. For example, *Salmonella* is covered by three monographs, whereas only one short section is devoted to viruses and one section to mycotoxins and toxigenic fungi.

Volume 2 covers the procedures and techniques for sampling, for examination for specific organisms, for examination of specific products, and recipes for media and reagents.

There are a number of substantial changes in this edition. Some of these result from the publication, in the intervening period, of the 2nd edition of Volume 1 of the ICMSF book *Micro-organisms in Foods*, and from developments in *Codex Alimentarius* and in the work of the Council of Australian Food Technology Associations. Also many of the developments in methodology which have occurred since 1976 are discussed. Just two examples are the excellent 17-page chapter on serology and the fluorescent antibody technique as applied to *Salmonella,* and in the chapter on staphylococcal enterotoxin there is additional information on the characteristics of the enterotoxins and on the radioimmunoassay technique.

Some of the changes in epidemiology and in epidemiological thinking have been included. For example there is a small section on infant botulism and its possible implication in the sudden infant death syndrome, and a discussion of the possibilities of gastro-enteritis being caused by ingestion of small numbers of *Salmonella*. One notable omission is of *Campylobacter.* Until microbiologists in other countries start examining foods, and faecal specimens from victims of gastroenteritis, for the presence of these bacteria. it will not be known whether the apparent prevalence of *Campylobacter* is a phenomenon peculiar to the U.K. — one suspects that it is not.

In Volume 2, there have been added 14 pages on 'Examination of specific products' – a section which was left empty in the previous edition.

There are very few printing errors. One or two have persisted from the 2nd edition, e.g., 'the normal intestinal flora also *effects* the susceptibility to infection' (my italics). One new error captured my imagination when it was stated that pyrolysis g.c. is 'potentially an interesting method of finger painting micro-organisms'! A few idiosyncratic items appear. For example the book perpetuates the myth that to detect staphylococcal enterotoxin by the microslide technique it is necessary to prepare a template with holes 1.587 mm in diameter – a feat worthy of NASA! Also it is a pity that the MPN tables give Most Probable Numbers for sets of positive results such as 0,0,2; 0,3,0; and 1,0,3 without indicating the improbability of such combinations.

One useful change has been the insertion of the diagrams into the appropriate

position in the text (in the previous edition they were commonly placed at the end of each chapter). A disappointment is that continuous page numbering and an index have still not been provided.

To sum up, the books, and especially Volume 1, provide a valuable addition to institutional libraries, particularly those catering for the needs of degree courses in food science and food technology.

*W. F. Harrigan*

**Cold Tolerant Microbes in Spoilage and the Environment.** Ed. by A. D. Russell and R. Fuller.
(Society for Applied Bacteriology Technical Series No. 13). London: Academic Press, 1979. Pp. xii + 169. ISBN 0 12 603750 7. £9.80.

This volume, described in the preface as comprising contributions from experts on 'psychrotrophic' bacteria resulting from the demonstrations made at the Autumn 1977 meeting of the S.A.B., provides an excellent overview of this group of bacteria. The importance of these bacteria with respect to the main food commodities, i.e., vacuum-packed beef (Dainty *et al.*), poultry (Barnes *et al.*), fish (Shewan & Murray) and milk (Law *et al.*) is comprehensively covered with extensive and valuable bibliographies pertaining to each subject. However, the absence of any consideration of low temperature spoilage in the important context of chill distribution of prepared and convenience foods and in the wider areas of red meat and meat product technology detracts slightly from the excellence of this book.

Although it was probably a reflection of the desire to provide a balanced broad treatment of the subject, the inclusion of the contribution by Morris relating to the response of algae to freezing without any consideration of the important effects of freezing and thawing on psychrophilic or psychrotrophic bacteria themselves seems illogical and incomplete. In addition the inclusion of the, albeit interesting, contribution on an inflatable anaerobic glove bag by Leftley & Vance seems inappropriate to the general theme of the book.

The importance of the spoilage effects of heat-resistant exo-enzymes produced by bacteria growing at low temperatures in milk is well described by Law *et al.*, while the suppression of psychrophiles in milk by lactoperoxidase is described in detail by Reiter & Marshall.

Lee's contribution on that most important spoilage psychrotroph *Alteromonas (Pseudomonas) putrefaciens* is concisely but comprehensively presented and a classical treatment of the heat resistance of cold-tolerant spores is given by Michels. The construction and application of temperature gradient incubators by Baker & Orr is of appreciable technical interest but did not emphasize aspects relative to low temperature growth. The contribution by Wynn-Williams on Antarctic Terrestrial Microbiology is a useful reference text for this little known area of study.

While the book title carefully avoids the etymological problem of the terms 'psychrophile' and 'psychrotroph', Herbert and Bhakoo restrict their review mainly to 'true psychrophiles' while Shewan and Murray seek to distinguish the two groups so far as isolates from fish are concerned. Relevant bacteria in a dairy context are defined by Law *et al.*, as 'psychrotrophs' while Barnes *et al.*, regard the terms 'psychrophile' and 'psychrotroph' as synonymous for isolates from poultry carcasses.

Perhaps it is time that bacteriologists, or even microbiologists, in the various fields of study should finally make up their minds about these definitions in terms of the genera and species to which they apply.

*J. Scholefield*

**Aquaculture and Algae Culture: Processes and Products.** By A. Shaw Watson.
Park Ridge, New Jersey: Noyes Data Corporation, 1979. Pp. 310 US $32.00.

This book is really a list of U.S. patented inventions for use in the business of aquaculture. In a broadly philosophical introduction many of the old platitudes about the social desirability of fish farming are given yet another airing but some caution is expressed in the view that aquaculture must be cost-effective. Nevertheless, the claim that by the year 2000 'world aquaculture production may reach 50 million metric tons' seems over-optimistic.

Following the introduction, a series of function orientated chapters present a varied mixture of technology past, present and future. Under 'Types of Products', the cultivation of a whole range of fish, shellfish and plants is briefly but not authoritatively described. Some patented inventions are mentioned for salmon culture but the core of the book is the extensive list of patented processes and devices included in the next chapter called 'Steps in Aquaculture Production Processes'.

This chapter of almost 160 pages is broken down into sub-sections such as 'Seeding', which deals with hatchery problems and the placement of juvenile fish in natural waters, 'Genetics and Selective Breeding', 'Controlling Diseases and Predators' and sundry other identifiable parts of fish farming practice. Basically, each part comprises a brief statement of problems and perspectives followed by a list of patented procedures. Some topics, such as genetics, seem ill supplied with inventions whilst others, such as crustacean hatchery equipment, are over-endowed with a variety of devices or processes which seem to reflect an attitude reminiscent of the early years of the industrial revolution. The diagrams are slightly quaint and also seem to be from an earlier age. Perhaps fish farming still is in an early exploratory stage but one cannot avoid the feeling that most of the patents involve over-sophistication or undue complication and are not, in fact, part of anyone's plans for fish farming. The text is almost totally deficient in the description of practical applications of the contrivances under

patent. Some inventions completely stretch one's credulity: an 'Artificial Habitat for the Growth and Study of Deep Sea Marine Organisms' and a 'Vertical Fish Rearing Tank Design with Provision for Fish Removal at Various Levels' are good examples of fantasy.

A chapter on 'Production Techniques for Fish' dwells largely on closed circuit culture with several systems described in great detail. None of it looks convincing in a practical sense and once again there is no evidence that water recycling systems are employed anywhere. The chapter concludes with a few words on polyculture and ocean ranching. No invention here but then these are simple aspects of fish farming which are already successfully pursued.

The last major chapter is on 'Algae Production and Processes and Products'. Again, much inventiveness and imagination is apparent but little pragmatism. The feeling persists that the value of aquatic algae is not commensurate with the grand industrial design for producing it. Single cell protein systems seem to have inspired the algae system inventors but while the former seem able to thrive on organic energy sources the algae seem forever dependent on sunshine or some artificial equivalent.

Two short chapters on 'Economics of Aquaculture' and 'Legal and Regulatory Aspects of Aquaculture' complete the book. These are too general to be a source for reference. They are also based on practice in the U.S.A. and therefore have little overall value for fish farmers.

In general the book is a good insight into the ingenuity of man. It makes fascinating reading but will not generate much in the form of fish farm output.

*C. Purdom*

**Human Nutrition and Dietetics,** 7th edition. By S. Davidson, R. Passmore, J. F. Brock and A. S. Truswell.
Edinburgh: Churchill Livingstone, 1979. Pp. x + 641. ISBN 0443 01765 4 (hard covers) 0443 01764 6 (limp). £14.00, limp.

Five years ago the 6th edition of this internationally renowned textbook appeared. The review of that edition which appeared in *Journal of Food Technology* (**10,** 699) considered that the book 'maintains its justifiable reputation as a reliable source of basic information and is in a class of its own'. The preface to the 7th edition outlines the nature of the major revisions which have been made. There are stated to be three new chapters on 'Fuels of the Tissues' (Chapter 8),'Food Processing' (Chapter 22), and 'Consumer Protection' (Chapter 26), although it must be observed that the appearance of the first two of these results primarily from a rearrangement of topics. There is also a short but useful chapter added on 'Community Nutrition' (Chapter 53). Many changes and additions to the text have been made, with a large number of references to work published since the appearance of the previous edition.

The casual observer might suppose that the present edition contains less

material than the previous edition of 756 pages of approximately the same page size. However, the typeface and typesetting is more economical on space and I estimate that the text of the book has in fact been increased by approximately 10%. In spite of this, I found the typeface and presentation of the 7th edition more attractive and easier to read than its predecessor.

This review can mention only a few of the many additions and modifications to the text of the new edition. Amongst the new and updated material is information and references on available and unavailable carbohydrates, and the recent work on chemical measurement techniques for dietary fibre and on the physiological effects of dietary fibre – including the possible role of the pentosans found in wheat bran. Chapters 8 and 9 (Energy Balance) include new material on, for example, ATP generation and energetics. In Chapters 11 to 14 there is updating of the information on zinc, chromium, manganese and selenium; vitamins D, E and K; vitamins C and $B_{12}$ and folic acid. Chapter 15 on 'Dietary Standards' presents the WHO (1974) as well as the U.S. NAS–NRC (1974) RDIs, and there is a better discussion of these RDIs.

I found it rather strange that more space was devoted in Chapter 20 to descriptions of the different types of alcoholic beverages manufactured than was given in Chapter 19 to the range of fish and fish products consumed around the world. Chapter 24 on 'Bacterial Food Poisoning' has been substantially rewritten. However, the statement that *Bacillus cereus* 'infection (*sic*) occurs usually in those who eat in Chinese restaurants' is inaccurate unless related specifically to the reported incidents in the U.K. In Central and Eastern Europe *B. cereus* food poisoning is more likely to be found associated with the consumption of the goulash-type of meat dishes.

The Chapter on 'Food Toxicity' has been rearranged and much improved, although a reference or two to appropriate monographs on mycotoxins would be helpful to interested readers. The new chapter on 'Consumer Protection' gives a good short account of the food law in the U.K., E.E.C. and U.S.A., but international standards and specifications such as those of *Codex Alimentarius* get perhaps too brief a mention.

In the sections on nutritional diseases there has been addition and modification to, *inter alia*, anorexia nervosa, protein-energy malnutrition, beriberi and gastro-intestinal diseases.

These are but some examples of the changes to be found in this edition. As always, any reader is likely to find many interesting and surprising items. For example, the reviewer learnt that eating raw fermented fish (which contains thiaminase) may lead to a serious reduction in the thiamin in the diet in many Asian countries; and in another chapter that the food dye tartrazine (used in orange fruit squashes and drinks) may cause urticaria and asthma.

Suggestions for the next, 8th, edition could include an expansion of the section on fish and fish products, and also it would be most welcome if the next edition could give the same extensive treatment to the cooking and food preparation of cereal grains and staples other than wheat as is accorded wheat in this edition.

One major improvement in the layout of the book has been the insertion of the photographs in the text pages as appropriate, instead of their being presented as a block of plate inserts. There are a few typographical, spelling and grammatical errors, most of which occur in the sentences and sections new to this edition. When these are unambiguous spelling errors or simple grammatical errors little harm is done. More care should be taken however that errors in syntax do not lead to misleading statements. For example, the omission of two commas from page 70 results in the statement that 'Proteins like starch are rapidly digested in the duodenum . . . '. This would have been better expressed as 'Like starch, proteins are rapidly digested in the duodenum . . . '.

Summing up, this is a book that is attractively presented and easy to read (although the soft back version was already showing bad signs of wear by the time I had finished reading it for the purpose of this review). It remains an excellent reference work for use by food scientists and technologists as well as nutritionists, dieticians and others in medically-oriented occupations. It is so readable that it will perform excellently as a student text. A book thoroughly to be recommended for the personal library of everybody interested in the nutritional aspects of food.

*W. F. Harrigan*

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**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 | $\mu m = 10^{-6}$ 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 | = 4·5461 l |
| 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'.

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# Contents



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