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FOOD PROCESSING  
and  
PRESERVATION**

**Edited by  
T.P. LABUZA**

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# JOURNAL OF FOOD PROCESSING AND PRESERVATION

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# SHELF-LIFE OF ASEPTICALLY BOTTLED ORANGE JUICE

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## ABSTRACT

*In comparison with the regularly thermally processed food product, the aseptically filled ones which receive considerably less heat treatment, is thought to have better taste, texture and color, and exhibit less damage to nutritive properties.*

*In this work the storage stability of aseptically bottled juice stored at 4, 15, 25°C was compared to that of hot filled juice. Criteria for stability were ascorbic acid retention, browning as measured by optical density, tristimulus color reflectance and organoleptic evaluation.*

*It was found that the governing factor of establishing shelf-life was storage temperature rather than the method of filling.*

*The quality parameters for the juices stored at 4 and 15°C were similar for both methods of filling. No difference was found in sensoric evaluations between aseptically filled juices and hot-filled juices stored at the same temperatures after 60 days storage. Immediately after filling the aseptically filled juice was judged slightly better but this difference disappeared rapidly during storage even at 4°C. In all cases juices stored at the lower storage temp. were judged better than those stored at the higher temperature regardless of filling method.*

## INTRODUCTION

The term "aseptic packaging" refers to the filling of a commercially presterilized product into a presterilized container, followed by hermetic sealing with a presterilized closure under an atmosphere free of micro-organisms.

As compared with terminally heat processed products, the aseptically processed products are thought to have a better taste, texture and color, exhibit less damage to nutritive properties and show less change in heat sensitive components.

Brody (1975, 1976) gave a thorough review on the uses of aseptic packaging for various foods. Lund and Lawler (1966) described the cold filling of orange juice into bottles, and Scott (1974) emphasized the importance of storage at low temperature on organoleptic properties of the juice. Data on the kinetics of the changes of the aseptically filled juice during storage as compared with hot-filled juice is limited and the object of this study was to obtain such data.

### MATERIALS AND METHODS

Ascorbic acid was determined by a 2,6 dichlorophenol indophenol titration as described in official Methods of AOAC (1970). The extraction solution was metaphosphoric acid.

Optical density of the juice was measured after precipitating the pulp with lead acetate, and reading the optical density of the clear solution at 420nm with a Bauch & Lomb Spectronic 20 spectrophotometer while water was the blank.

The CIE tristimulus color values of the juice were measured with a Zeiss Elrepho Colorimeter, and calibration was done with a Hunter reference plate No. D-25-1331.

Organoleptic evaluations were carried out using the triangular test in order to find differences between differently treated samples. Organoleptic evaluations were done on a 1 to 5 hedonic scale (5 being best) to establish general acceptability of products. For the evaluation, samples stored at 2°C were given as a reference with a score of 5. In all evaluations there were at least 10 panel members.

### EXPERIMENTAL

Freshly extracted, screened and deaerated orange juice was aseptically filled in a commercial plant into one liter bottles (see Fig. 1). Juice from the same batch was hot filled in a different line. Both types of juices were stored at 4, 15 and 25°C and analyzed periodically.

### RESULTS AND DISCUSSION

Results of chemical analyses of the hot and aseptically filled juices are presented in Fig. 2-4.

The aseptically filled juices stored at 15 and 25°C showed less browning



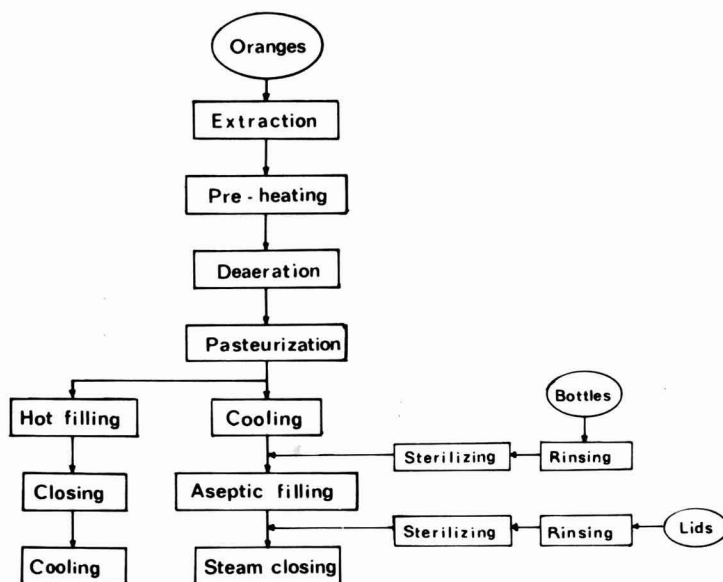


FIG. 1. SCHEMATIC FLOW DIAGRAM

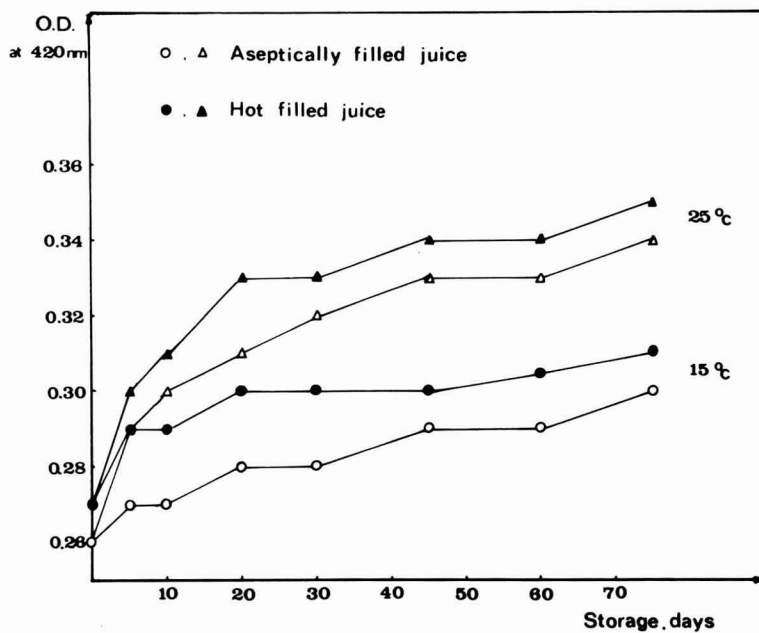


FIG. 2. BROWNING OF ASEPTICALLY AND HOT FILLED JUICES, STORED AT 15°C AND 25°C

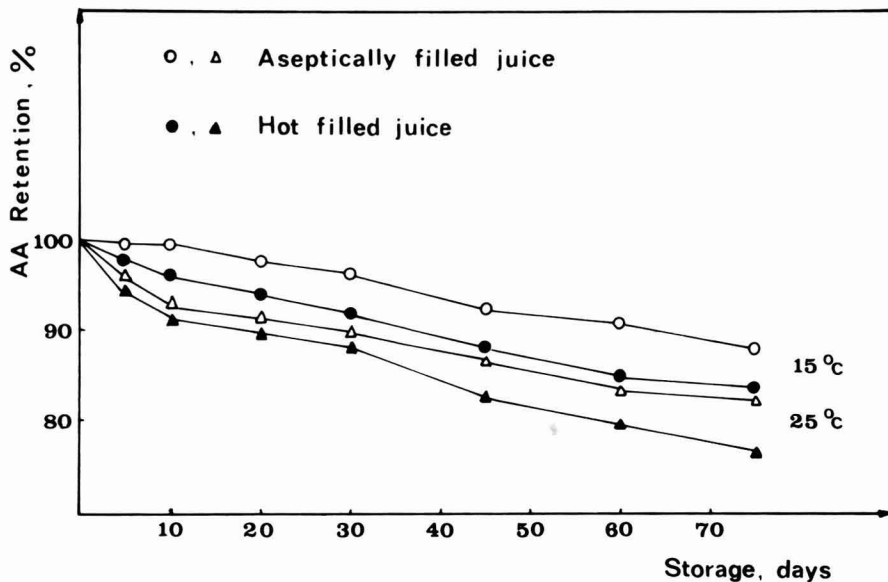


FIG. 3. AA RETENTION OF ASEPTICALLY AND HOT FILLED JUICE, STORED AT 15°C AND 25°C

as compared to the hot filled juices stored at the same temp (Fig. 2). This seems to indicate that the longer thermal treatment occurring during hot filling had a detrimental effect on browning.

No change in browning could be observed in both types of juices stored at 4°C for over 4 months.

Ascorbic acid (AA) retention is shown in Fig. 3. The initial content of vitamin C for both types of juices was the same (55 mg%). The aseptically filled juices stored at 15 and 25°C had better AA retention (about 10%) than the hot filled juices stored at the same temperature. Since browning in citrus juices is considered to be mainly due to AA degradation it is therefore quite evident that the trend in AA retention (Fig. 3) corresponds to the trend shown for browning (Fig. 2).

There was no difference in AA losses in both types of juices stored at 4°C. During 70 days the AA content decreased in both juices by only about 5%.

The CIE tristimulus color values of the juices are shown in Fig. 3. A decrease in brightness (Y) values of both juices was observed during storage combined with an increase in amount of the red color (x) (Fig. 4).

The changes in CIE values are also in accordance with the browning readings as shown previously.

No differences in appearance could be observed by a panel, between

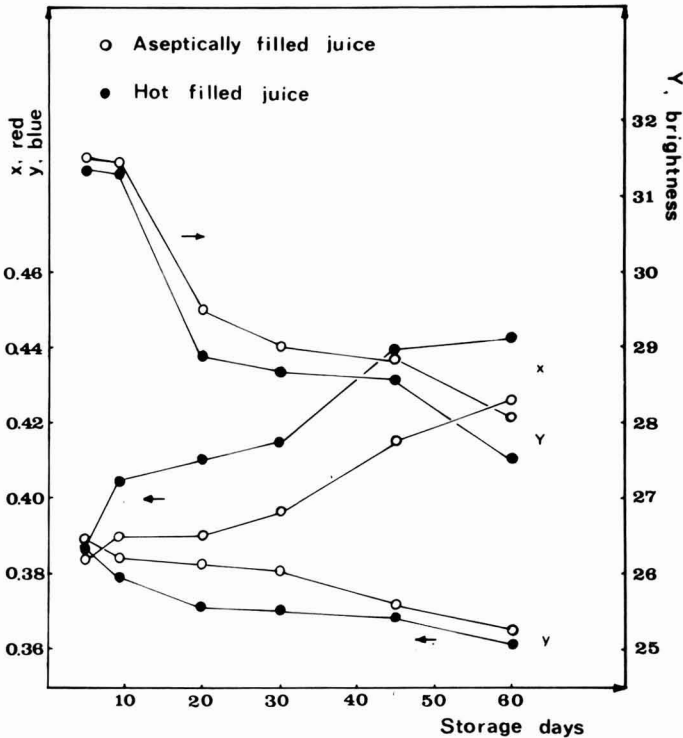


FIG. 4. TRISTIMULUS COLOR CHANGES OF ASEPTICALLY AND HOT FILLED ORANGE JUICE STORED AT 25°C

aseptically and hot filled juices stored at the same temperature.

Triangular taste comparisons between aseptically and hot filled juices are given in Fig. 5.

The data not shown in the figure indicated that initially there was a significant difference between samples and the aseptic juices were preferred. However, already after 8 days at 4°C and even at 4 days at 15°C the panel was no longer able to differentiate between the two types of juices (Fig. 5).

Both type of juices stored at 4°C and 15°C maintained good organoleptic qualities for a period exceeding 4 months. However, at 25°C, which represents ambient temperature in most places, the juices were judged still acceptable after 30 days and unacceptable after 40 days. At an accelerated storage temp of 35°C both types of juices became unacceptable after less than one month.

In summary, we have shown that aseptic packaging of orange juice as compared to hot fill causes less damage to vitamin C destruction and



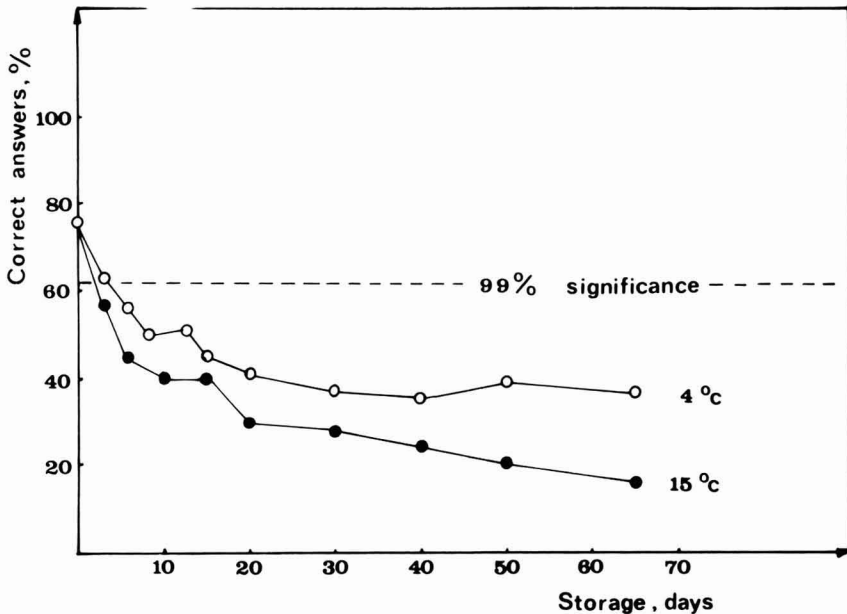


FIG. 5. TRIANGULAR COMPARISONS BETWEEN ASEPTICALLY AND HOT FILLED ORANGE JUICES STORED AT 4°C AND 15°C

browning. However, as far as the customer is concerned sensoric taste and visual color evaluations do not bear out this advantage even after a very short storage period. On the other hand good quality of hot filled juice can be maintained for relatively long periods (4 months or more) provided juices are stored at low temperatures.

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# OPTIMIZATION OF PROTEIN EXTRACTION FROM PASTURE HERBAGE QUANTITATIVE AND QUALITATIVE CONSIDERATIONS

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## ABSTRACT

*An attempt has been made to develop a mathematical model in which by using herbage dry matter (DM) and crude protein (CP) analysis it would be possible to predict the yields of protein which may be recovered from pasture herbage during large-scale protein extraction operations. The nutritional characteristics of the leaf protein concentrates (LPC) recovered from pasture have also been studied in relation to the optimum regrowth stage as determined by the use of the designed mathematical model.*

*The relationship between both herbage DM and CP content and protein yields, obtained during protein extraction procedures, was found to be statistically highly significant. Therefore, a simple mathematical model was designed for predicting protein yields from pastures based on these two routine analysis of herbage. It appears that a fair prediction of the protein extraction efficiency from pasture can be made before the start of a large-scale extraction operation. A three-dimensional model of protein extraction from pasture also has been built.*

*The nutritional quality of LPC extracted from pasture in its "optimal" regrowth stage (as indicated by the mathematical model) was significantly higher than the LPC recovered from herbage cut in its earlier regrowth stages. Delay in the protein extraction, beyond the herbage regrowth considered as optimum, decreased the yields of recoverable protein but did not affect the nutritional quality of the recovered protein.*

*The relationships presented in this paper were found to be significant only in the case of herbage processing after its previous treatment with water, i.e. when herbage was processed in the state of its physiological moisture saturation.*

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## INTRODUCTION

In previous papers of this series (Ostrowski 1979a,b) it has been shown that protein extraction from pasture herbage in temperate and subtropical climatic conditions has great potential in the production of unconventional protein concentrates for both human and animal consumption. The protein concentrates recovered from green herbage in leaf protein concentrate form (LPC), after supplementation with synthetic methionine and lysine, represent protein of nutritive value similar to that observed in casein (Ostrowski 1979c,d).

The quantities of protein which can be extracted from grasslands and various green crops depends on a number of factors such as the botanical composition of herbage, its regrowth stage, topdressing, system of extraction and the like, all of which limit protein recovery from green vegetation (Arckoll and Festenstein 1971; McKenzie 1977; Oke 1973; Ostrowski 1975; Ostrowski *et al.* 1975; Pirie 1971). Also, the protein fractions of feed- and food-grade which are recoverable from the herbage grown and/or processed in various conditions vary in both quantities and proportions (Ostrowski 1976).

Pastures with a higher participation of clover in the sward did yield higher quantities of LPC as compared to those pastures with a high proportion of perennial grasses (McKenzie 1977). Again, pasture herbage cut at earlier stages of development, when the nitrogen to dry matter ratio was high, yielded substantially more LPC recoverable from the unit of dry matter as opposed to the more matured herbage (McKenzie 1977; Ostrowski 1975). The significant differences in the protein yields recoverable from pasture herbages which were used for the protein extraction purpose cannot be accepted in practice due to the economy of the whole protein extraction operation. Also differences in the chemical composition of LPC's obtained at various pasture regrowth stages (Ostrowski 1976) may indicate differences in the nutritive value of LPC. This means that LPC produced by the same protein extraction plant, from the same pasture but processed in various stages of its regrowth may represent a product which might not have standard nutritional quality. It was therefore necessary to establish the method to be used which technologists responsible for the efficiency of the protein extraction operation could apply in practice in order to optimize the protein recovery process from pasture herbage in both quantitative and qualitative terms.

In this paper an attempt has been made to develop a simple mathematical model by which to predict the degree of protein recovery from certain pasture herbage and to gauge the optimum time when herbage should be used for the purpose of protein extraction — this in reference

to both the quantity and nutritional quality of the final product — LPC.

### MATERIALS AND METHODS

A permanent stand of irrigated pasture growing on a mixture of sandy loam and clay soil types was used as a source of herbage. The botanical composition of the pasture is given in Table 1, the climatic conditions in which the pasture grew is summarized on Fig. 1 and the herbage processing technique used in this study has been schematically depicted on Fig. 2. A typical efficiency of protein extraction operation conducted on herbage at its 5th week of regrowth is given on Fig. 3. Average annual herbage dry matter production as recorded on the pasture involved in the study was  $1.5 \times 10^4 \text{ kg ha}^{-1}$ .

Table 1. Botanical composition of pasture<sup>1</sup>

|  | Percent Dry Matter |
|--|--------------------|
| Perennial ryegrass ( <i>Lolium perenne</i> ) | 1                  |
| <i>Paspalum dilatatum</i>                    | 8                  |
| Mercer grass ( <i>Paspalum paspaloides</i> ) | 23                 |
| White Clover ( <i>Trifolium repens</i> )     | 55                 |
| Other grass species                          | 0                  |
| Weeds  | 0                  |
| Dead Matter                                  | 13                 |

<sup>1</sup> Determined in 5th week of pasture regrowth

Herbage samples were obtained from the pasture in the summer season during six consecutive weeks the herbage was in its 1st, 2nd, 3rd, 4th, 5th and 6th week of regrowth (Trial 1). The results obtained from the series of 44 individual protein extractions conducted at each of the six different pasture regrowth stages provided the basis for the designing of the mathematical model to gauge the possible optimum extraction time of the pasture stand. To check the validity of the prediction of LPC production efficiency made on a basis of a laboratory-scale process on three occasions (Trial 2), a pilot-scale operation was performed in weekly intervals (28th, 35th and 42nd day of pasture regrowth). The pasture herbage was harvested after previous projection of the LPC yields which were predicted with the use of the mathematical model designed in Trial 1. In Trial 2 those irrigated pasture areas not involved in the study within Trial 1 were used.

In order to reduce "climatic" variability in protein extraction from

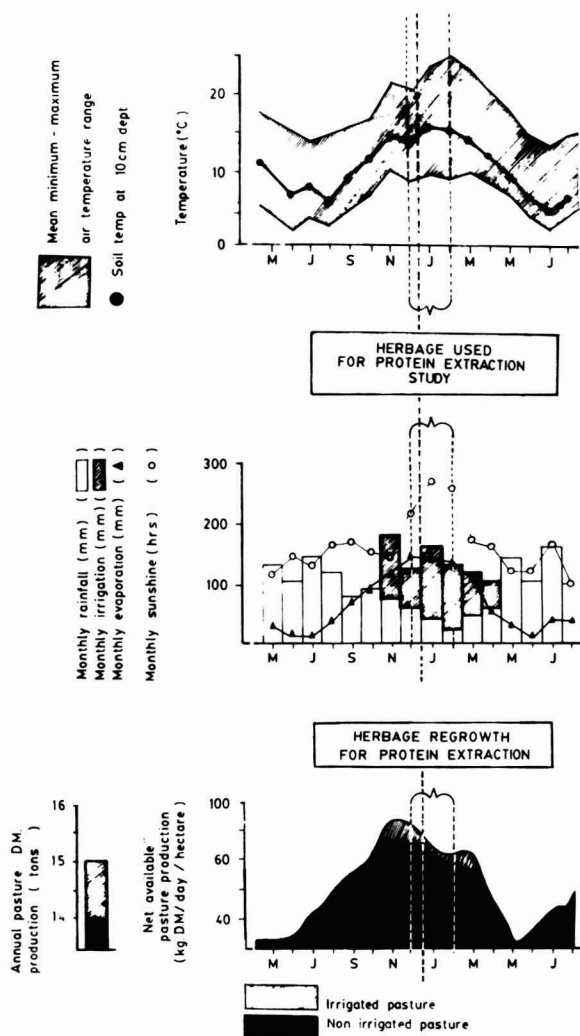


FIG. 1. CLIMATIC CONDITIONS IN WHICH PROTEIN RECOVERY FROM PASTURE HERBAGE HAS BEEN INVESTIGATED AND PASTURE PRODUCTIVE CHARACTERISTICS

Shaded area closed within two solid lines indicate difference between monthly mean highest and lowest recorded air temperature. Black columns built on rainfall figures, indicate irrigation (mm).

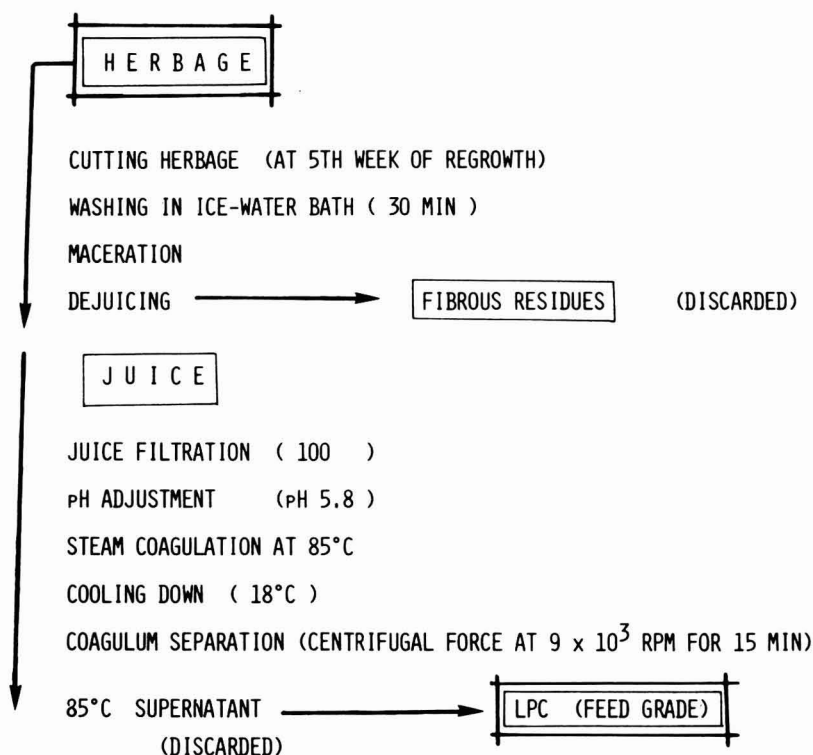


FIG. 2. DIAGRAM SHOWING INDIVIDUAL STAGES OF PROTEIN EXTRACTION FROM PASTURE HERBAGE AND THE PROCEDURE USED FOR THE RECOVERY OF THE FEED-GRADE LEAF PROTEIN CONCENTRATE (LPC)

herbage sampled in various climatic conditions during the trials, the freshly cut herbage leaves were processed after previous saturation with moisture, i.e. at the stage of complete herbage moisture capacity. To achieve this, the herbage was allocated in a water bath for 30 min immediately after being harvested, and then after drainage, a portion of the herbage was used for dry matter and crude protein ( $N \times 6.25$ ) analysis and the remaining bulk of herbage was macerated followed by dejuicing. Herbage was processed within 30 to 45 min of harvesting.

Protein concentrates obtained from extractions in Trials 1 and 2 were freeze-dried and used for further chemical and nutritional evaluation.

Total dry matter (DM) content in herbage and juice was determined after heating in a forced-draught oven at  $110^\circ$  for 24 h. Total nitrogen (TN) was determined using the Kjeldahl method as described by the Association of the Official Agricultural Chemists (AOAC 1965). Protein

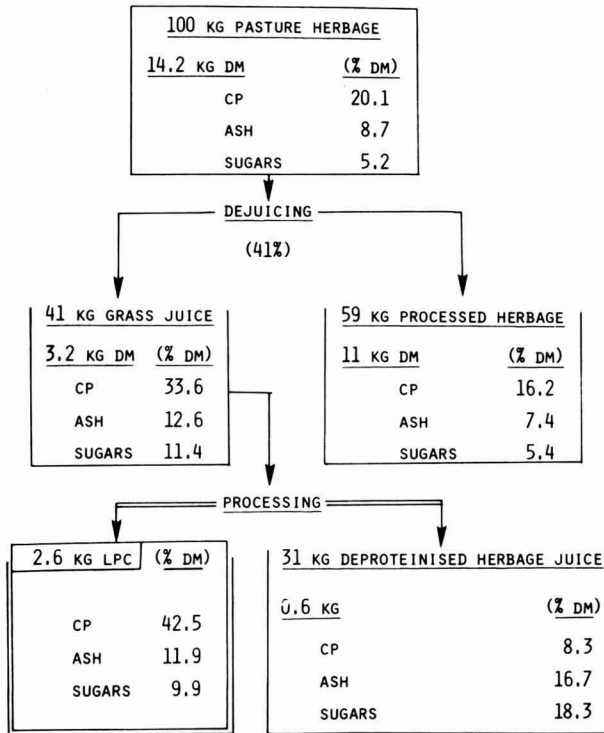


FIG. 3. EFFICIENCY OF PROTEIN EXTRACTION OPERATIONS CONDUCTED AT THE 5TH WEEK OF HERBAGE REGROWTH, AND AVERAGE DISTRIBUTION OF DRY MATTER, CRUDE PROTEIN ASH AND SOLUBLE SUGARS IN TYPICAL EXTRACTION PROCEDURE WITH HEAT COAGULATION AT 85°C USED FOR PROTEIN RECOVERY FROM THE EXTRACTED JUICE

nitrogen was determined by mixing freshly-extracted juice with an equal volume of a solution of 20% (w/v) trichloro-acetic acid (TCA) and 2% (w/v) silicotungstic acid (STA). The precipitant curd layer was separated by centrifugation for 15 min at 3,000 rpm at 5°C. After centrifugation the TCA/STA precipitants were used for precipitable dry matter determinations. TCA/STA soluble nitrogen in the supernatants was determined as TN by the use of the Kjeldahl procedure. Precipitable nitrogen was calculated from total juice nitrogen by subtracting TCA/STA soluble nitrogen.

Ash was determined as the residue remaining after heating in muffle oven for 18 hours at 600°C. Total soluble sugars were analyzed using a procedure with an anthrone indicator (Yemm and Willis 1954).



Methionine was determined by the Lorenzo-Andreu (1961) procedure and methionine availability by the method described by Pieniazek *et al.* (1965). Total and available lysine were estimated as described by Ostrowski *et al.* (1970). Tryptophan was measured by using the procedure described by Matheson (1964) and tryptophan availability was determined using *Tetrahymena pyriformis* according to Boyne *et al.* (1975). *In vitro* digestibility was determined by a two-stage pepsin-trypsin digestion procedure according to Saunders *et al.* (1973). Amino acids were determined using the analytical and sample preparation procedures as recommended by Byers (1971a, b) for protein concentrates analysis. The nutritional value of protein in LP's was determined using rats (AOAC 1965).

In designing a mathematical model for protein extraction from pasture herbage, a number of curves and surfaces were fitted to the data obtained from Trial 1. The two qualities of the functions sought were goodness of fit and simplicity.

Calculations were initially restricted to polynomial models and were done with the SPSS package (Nie *et al.* 1975). For each equation a range of independent variables has been included so that they may be used within the model.

## RESULTS AND DISCUSSION

With maturity the dry matter content in the herbage increased with a simultaneous gradual decrease in crude protein concentration (Fig. 4).

This was associated with a significant reduction in the precipitable protein (g/kg herbage DM) recovered from more mature herbage (Table 2). On the other hand, the optimum regrowth stage during which time the maximum yield of protein from the unit of pasture area was achieved has been estimated as the 36th day.

Table 2. The efficiency of protein extraction as achieved at different pasture regrowth stages

|  | Pasture Regrowth Stage (Days) |    |    |    |     | S.E. ( $\pm$ )<br>of Mean |     |
|--|-------------------------------|----|----|----|-----|---------------------------|-----|
|  | 7                             | 14 | 21 | 28 | 35  |                           | 42  |
| <b>PROTEIN RECOVERY</b>                        |                               |    |    |    |     |                           |     |
| (% of the total N $\times$ 6.25<br>in herbage) | 32                            | 40 | 48 | 46 | 42  | 36                        | 2.4 |
| <b>YIELD OF PROTEIN</b>                        |                               |    |    |    |     |                           |     |
| — g/kg herbage dry matter                      | 44                            | 49 | 81 | 80 | 66  | 41                        | 6.7 |
| — g/10m <sup>2</sup> pasture area              | 16                            | 37 | 59 | 97 | 101 | 86                        | 8.1 |

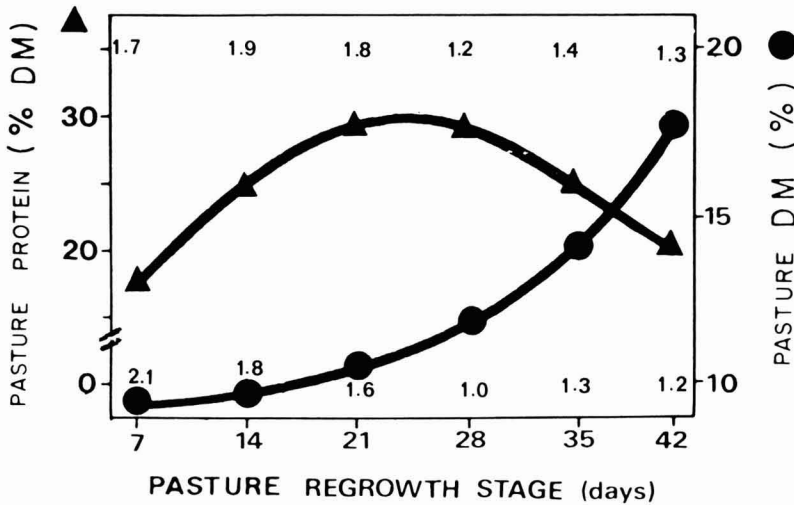


FIG. 4. CHANGE IN CRUDE PROTEIN ( $N \times 6.25$ ) AND DRY MATTER CONTENT IN HERBAGE CUT FROM PASTURE AT DIFFERENT REGROWTH STAGES

Figures in brackets indicate standard error of mean of 35 determinations: upper row — SE for pasture protein, bottom row — SE for pasture dry matter.

The observed reduction in both extractability and yields of protein from herbage of increasing maturity is consistent with earlier observations (Ostrowski 1979b) and results reported by other workers (Arkcoll and Festenstein 1971; Pirie 1971; Oke 1973), who were using different pure crops of herbage. Higher fiber content with increasing maturity reduced the efficiency of cell rupture in the macerator and therefore cellular protein may be trapped by fibrous material during juice extraction in the press (Arkcoll and Festenstein 1971; Pirie 1971). However, despite the decrease in yield of protein extracted from the unit of herbage DM weight with longer periods of pasture regrowth, more matured herbage produced higher herbage dry matter yields. Thus, the decision about the optimum herbage stage for the purpose of protein extraction has to be a compromise between the yield of dry matter and the diminishing protein extraction rate (as measured by yield or protein per unit of processed herbage dry matter). A delay in pasture cutting beyond the established "optimum" regrowth stage resulted in a substantial reduction in the quantities of protein recovered from the unit of pasture area irrespective of the fact that there was a simultaneous increase in yield of pasture dry matter. At the same time, the protein yield expressed per unit of processed herbage dry weight decreased. This indicates that the maturity of herbage is one of

the most important factors to consider from an extraction efficiency point of view.

Using the data collected from a series of protein extraction procedures, the relationships were established between pasture regrowth stage, dry matter, crude protein and efficiency of the protein extraction process. This in order to prepare a mathematical model of protein extraction operation.

The relationship between the maturity of herbage, expressed as herbage regrowth stage in days (D), and protein recovery, expressed as g of protein extracted from 1 kg of herbage DM (Y), was characterized by the regression equation:

$$Y = 120.6 - 0.227 D^2 \quad (R^2 = 67) \text{ for } 7 \leq D \leq 42 \quad (1)$$

Despite a certain convenience in using formula (1) in agriculture practice, without the necessity of analyzing pasture herbage before processing it, it is, however, much more accurate and therefore more appropriate to conduct a simple analysis for dry matter content using, e.g. an infra red moisture tester. This measure of herbage maturity done in field conditions could be completed with 15 to 20 min (Ostrowski-Meissner 1980). Thus, in taking herbage dry matter results (%) determined in herbage after being kept for some 30 min in water bath as an independent variable X, a yield of protein (g) from the unit of DM (1 kg) could be calculated (Y) from the Eq. (2):

$$Y = -219 + 39.08 X - 0.08 X^3 \quad (R^2 = 0.84) \text{ for } 10 \leq X \leq 17 \quad (2)$$

Predictions of the efficiency of protein extraction from the fresh crop based on the crop DM has been already considered by Heath and King (1977) as a reliable proposition for the commercial extraction operation. Also Jones and Houseman (1975) stressed the dependence of both DM and protein extractability on the stage of herbage maturity and its moisture content; the relationship expressed by the following equation:

Crude Protein extraction (%) =  $0.42 - 0.63 X$  of grass (R.S.D.  $\pm 10.62\%$ ) where X is herbage DM.

Many factors however may change the herbage DM within a similar maturity stage and this includes the herbage juice to fiber ratio, as well as the protein nitrogen (PN) to nonprotein nitrogen (NPN) ratio at given herbage dry matter (Arckoll and Festenstein 1971). Therefore, it appeared to be more appropriate to use herbage crude protein (N  $\times$  6.25) content (Z) for the calculation of yields of recoverable protein from the unit of herbage DM.

$$Y = -25.38 + 5.6Z \quad (R^2 = 0.959) \quad \text{for } 22.2 \leq Z \leq 32.5 \quad (3)$$

The Eq. (3) provided a more accurate measure of the efficiency of protein extraction from herbage as compared to the values calculated from the Eq. (1) and (2). When predictions of the protein yields which could possibly be recovered from pasture herbage in an "on-the-farm" extraction operation were made by the use of Eq. (1), (2) and (3) the obtained values underestimated the yields by 29%, 14% and 9%, respectively, as compared to those yields practically achieved in large-scale operations. The closest agreement between projected and practically-achieved values was observed with the use of Eq. (3) when protein content ( $Z$ ) was used as an independent variable. On the other hand, the predicted protein yields were overestimated (by 37%, 21% and 15%, respectively) when pasture herbage was processed without previous washing (moisture saturation) in water. It appears therefore that herbage washing and treatment with water for approximately 15 to 30 min before processing was advantageous from the point of view of standardizing the extraction process. Despite the discrepancy between predicted and practically achieved protein yields, both crude protein and dry matter analysis in pasture herbage were significantly correlated with protein yields (g/kg herbage DM) as recovered in LPC form, (Eq. 2 and 3). It was reasonable to suppose that the simultaneous use of these two analyses in one equation could result in the formula which may provide a more accurate prediction of the protein yields in large scale extraction operations.

A close relationship which was observed between herbage crude protein ( $Z$ ) and herbage dry matter ( $X$ ) may be expressed by the Eq. (4).

$$Z = 43.86 - 1.342 X \quad (R^2 = 0.897) \quad \text{for } 12.6 \leq X \leq 17.8 \quad (4)$$

Taking into account the previously established relationships between protein yield expressed as g/kg herbage DM ( $Y$ ) and both herbage dry matter ( $X$ ) and herbage crude protein ( $Z$ ) contents as well as the relationship between the  $X$  and  $Z$  (Eq. 4) the three-dimensional model of protein extraction from pasture has been built so that both dry matter and crude protein concentration in herbage can be used as independent variables for the projection of protein extraction efficiency. This being measured by g of protein recovered from 1 kg of dry weight of pasture herbage (Fig. 5). Therefore, by knowing the crude protein ( $Z$ ) and the herbage dry matter concentration ( $X$ ) a prediction of the protein extraction efficiency can be made from the Fig. 5 or by using the regression Eq. (5) characterizing the surface type response of protein yield (g/kg DM) as expected to be achieved ( $Y$ ) in the protein extraction operation from pasture.

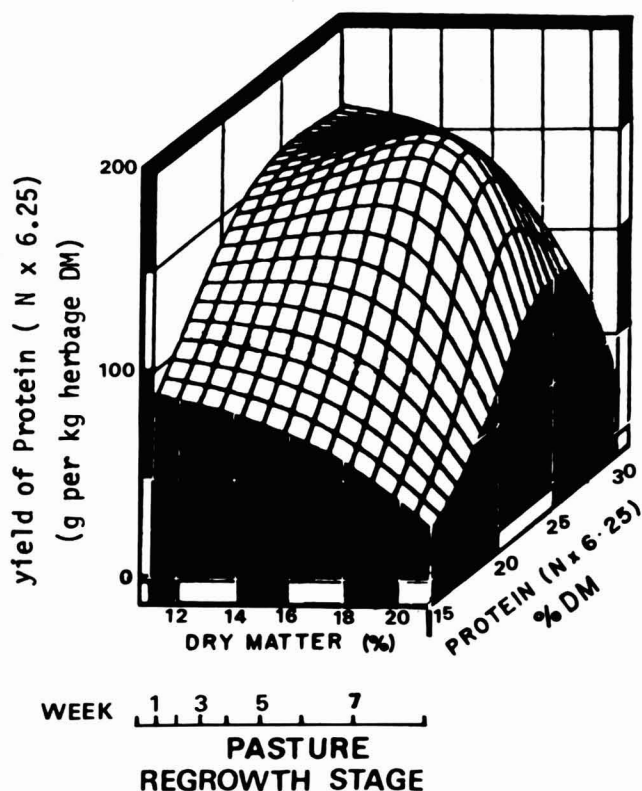


FIG. 5. GRAPHIC REPRESENTATION OF THE RELATIONSHIPS BETWEEN DRY MATTER AND CRUDE PROTEIN IN PASTURE HERBAGE IN RELATION TO HERBAGE REGROWTH STAGE (DAYS AFTER LAST HARVEST AND/OR GRAZING)

The graph allows projection of the yield of protein possible to be removed from pasture during its processing from routine DM and nitrogen analysis of herbage

$$Y = -63.5 + 6.98X + 5.38Z + 0.08XZ - 0.02Z^2 - 0.35X^2 \quad (R^2 = 0.96) \quad (5)$$

In practical conditions when herbage is saturated with water (herbage moisture holding capacity) prior to processing, dry matter of herbage taken for protein extraction ( $X$ ) can be predicted from the maturity stage expressed as days of regrowth ( $D$ ). The relationship between  $X$  and  $D$  may be characterized by the regression Eq. (6);

$$Y = 9.60 + 0.13X \quad (r = 0.943) \quad (6)$$

The protein extraction model presented in Fig. 5 refers conveniently to both herbage DM and/or regrowth stage, the former of course, being more appropriate for routine use, and with the latter giving a prediction of herbage DM in the case of lack of analytical facilities on farms where processing is carried out without outside analytical assistance.

If the optimum point (maximum yield of extracted protein) on Fig. 5 has not yet been reached in relation to the pasture regrowth stage, then a decision can be made as to how much longer the herbage should be left on the pasture before it will produce the maximum yields of extracted protein in LPC form. This will ensure a most efficient protein extraction process in terms of LPC being recovered from the unit of pasture dry weight and/or from the related pasture area.

By using regression Eq. (7) it is possible to predict with significant accuracy ( $R^2 = 0.901$ ) the yield of protein concentrate-LPC ( $Y_a$ ) which can be obtained from the unit of pasture area ( $g/10m^2$ ); independent variables being herbage dry matter content ( $X$ ) and protein concentration in herbage ( $Z$ ).

$$Y_a = 25.1X + 0.524Z - 321 \quad (R^2 = 0.901) \quad \text{for } 12.6 \leq X \leq 17.8 \quad (7)$$

In order to check the validity of a proposed mathematical model of protein extraction efficiency as depicted on Fig. 5, three series of large scale protein extraction operations were conducted in which a pasture herbage was analyzed for DM and CP prior to protein extraction process. From these analyses predictions as to the protein yields anticipated to be achieved in large-scale operations were made. There was significant agreement between both the predicted and practically-achieved LPC yields in the certain herbage regrowth stages projected and found as optimal for the purpose of protein extraction (Table 3).

The surface of the function characterized by the Eq. (5) and depicted on Fig. 5 indicates that the dry matter concentration in processed herbage has a major influence on the herbage protein which can be extracted as LPC. It is known that moisture in herbage provides the carrier for protein during the extraction process (Heath and King 1977). Therefore it explains why herbage washed in water before processing, simultaneously absorbing as much water as is determined by the physiological plant moisture capacity, can yield much more protein as compared to herbage processed without any water treatment before or during the extraction process.

At present, research is being concentrated on the development of the

Table 3. Comparison of the protein yields achieved from pasture at the three regrowth stages with yields calculated by the use of mathematical protein extraction model and projected and practically estimated pasture regrowth stages — optimal for protein extraction purpose

|   | Yield of Protein (3) Re-<br>covered From 1 kg Herbage DM |   | S.E.<br>of Mean<br>Difference | Signifi-<br>cance |    |
|---|--|---|-------------------------------|-------------------|----|
|   | Predicted <sup>1</sup>                                   | Achieved<br>in the<br>Extraction<br>Operation |                               |                   |    |
| Stage of regrowth (Days)  |  |   |                               |                   |    |
|   | 28   | 87  | 82                            | 3.4               | NS |
|   | 35   | 71  | 70                            | 3.1               | NS |
|   | 42   | 36  | 30                            | 2.8               | NS |
| Optimum regrowth stage (days)<br>for protein extraction<br>purpose when maximum<br>yield from pasture<br>area were achieved | 36   | 39  | 1.9                           | NS                |    |

<sup>1</sup> From the regression equation:

$$Y \text{ (g/kg DM)} = -63.5 + 6.98X + 5.38Z + 0.08XZ - 0.02Z^2 - 0.35X^2$$

(X — herbage crude protein, % DM; Z — dry matter, %)

computerized model of the protein extraction operation involving various ecological and agronomical (soil, botanical composition, topdressing, irrigation, season of the year) and technical (system of protein extraction, protein recovery method) factors as well as detailed meteorological data from the herbage regrowth period so as to accurately predict and control the efficiency of the large-scale protein extraction operation. The complicated model for the protein extraction efficiency evaluation based on the various factors having been assessed by multiple regression analysis does not increase the accuracy of predictions as compared to expectations based on the CP content in herbage DM Eq. (3). This was, however, the case only in the practical situations when pasture herbage was subjected to water treatment prior to protein extraction process. Multiple regression analysis with the involvement of the various factors affecting the protein recovery in protein extraction process is necessary for the development of the general model when pasture herbage is not washed in water prior to processing and when processing conditions as well as both ecological and agronomical factors affecting herbage during its regrowth are variable and cannot be standardized. The question arises however, how optimization of protein extraction in quantitative terms affects the quality of the final product — LPC.



Table 4. Chemical and nutritional characteristics of LPC obtained from pasture herbage in three various regrowth stages in plant-scale extraction process using heat coagulation at 85°C

| Measurement   | Stage of Regrowth (Day) <sup>1</sup> |          |          | S.E.<br>of Mean<br>Difference | Signifi-<br>cance |
|---|--------------------------------------|----------|----------|-------------------------------|-------------------|
|   | 25                                   | 35       | 42       |                               |                   |
| <b>CHEMICAL CHARACTERISTIC OF<br/>LPC</b>   |                                      |          |          |                               |                   |
| Crude protein (N X 6.25) (% DM)   | 43.7b                                | 44.5a    | 45.8a    | 1.1                           | NS                |
| Total essential amino acids <sup>2</sup><br>(g per 100g recovered)                        | 46.8b                                | 49.4a    | 50.3a    | 1.2                           | NS                |
| <b>BIOLOGICAL CHARACTERISTIC<br/>OF LPC</b>   |                                      |          |          |                               |                   |
| Protein digestibility <i>in vitro</i> (%)   | 83 b                                 | 87 a     | 88 a     | 1.0                           | *                 |
| Amino acid concentration (g/16gN)<br>and (availability - %) <sup>3</sup>                  |                                      |          |          |                               |                   |
| — Lysine  | 5.2(81)b                             | 5.9(80)a | 6.3(82)a | 0.14(1.3)                     | * (NS)            |
| — Methionine  | 1.7(78)a                             | 2.0(77)a | 2.1(77)a | 0.15(1.7)                     | NS (NS)           |
| — Tryp <sub>1</sub> tophan  | 1.7(80)b                             | 2.2(78)a | 2.4(76)a | 0.11(2.9)                     | * (NS)            |
| <b>Protein Efficiency Ratio (PER)<sup>4</sup></b>   |                                      |          |          |                               |                   |
| Diet supplemented with DL<br>methionine (0.2%)  | 1.2b                                 | 1.6a     | 1.7a     | 0.13                          | *                 |
| Diet supplemented with DL<br>methionine (0.2%) and L-lysine<br>(0.5%)                     | 2.0a                                 | 2.4a     | 2.4a     | 0.14                          | NS                |
| Diet supplemented with DL<br>methionine (0.2%) L-lysine (0.5%)<br>and L-tryptophan (0.3%) | 2.7a                                 | 2.8a     | 2.8a     | 0.08                          | NS                |
| Hemolytic activity  | 3.8a                                 | 3.6a     | 3.5a     | 0.14                          | NS                |

<sup>1</sup> Each value represents mean of the three extraction procedures. Values in the same line with unlike superscripts show significant differences at P<0.05 level as determined using Duncan's multiple range test.

<sup>2</sup> Cystine excluded

<sup>3</sup> In brackets availability values (%) and (SE) and (significance) related to availability of amino acids

<sup>4</sup> Casein control group PER: 2.8; All groups of rats fed LPC without amino acid supplementation gave negative weight gains

### Quality of LPC

Table 4 demonstrates the chemical and nutritional characteristics of LPC obtained by one step heat coagulation procedure at 85°C as a result of extraction made at the 28th, 35th and 42nd day of herbage regrowth stage.

While there were no significant differences in the crude protein and total essential amino acid concentrations in the LPC's due to the regrowth stage at which herbage was used for protein extraction, there was a significantly higher *in vitro* digestibility of protein concentrates recovered from herbage in their later growth stages. There was also a significantly higher concentration of lysine and tryptophan in the LPC extracted from more matured herbage which was accompanied by significantly higher PER values of protein after supplementation of the diets with

synthetic methionine considered in earlier studies to be the first amino acid limiting biological value of LPC proteins (Ostrowski 1979a, b). In order to achieve in all LPC's the PER values similar to casein, the latter being used as a reference protein, both synthetic *L*-lysine and *L*-tryptophan supplements were necessary, in addition to *DL*-methionine.

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# TOTAL AND INDIVIDUAL GLYCOALKALOID COMPOSITION OF STORED POTATO SLICES

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## ABSTRACT

*The quantitation of  $\alpha$ -chaconine and  $\alpha$ -solanine by thin-layer chromatography agreed with the total glycoalkaloid (TGA) content as determined by titration. The TGA level of potato slices approximately doubled to 26–30 mg/100g fresh weight during the 7-h incubation period. Soaking of potato slices resulted in lower increases. Incubation at 25°C resulted in higher TGA levels than at 5°. The initial  $\alpha$ -chaconine/ $\alpha$ -solanine ratio was 1.42 but with time and temperature changed to 2.4–2.8 in unsoaked slices and 1.5–1.6 in soaked slices.*

## INTRODUCTION

A class of nitrogen-containing steroidal glycosides, which are commonly called glycoalkaloids, are found in many *solanum* species. The facts that these compounds have toxicological properties (Jadhav and Salunkhe 1975) and can influence flavor (Sinden *et al.* 1976) have made them an important research topic relative to common potatoes, *Solanum tuberosum*.

Numerous glycoalkaloids have been identified in different species of potatoes (Osman *et al.* 1978), however, it would appear that except for incubated slices of the Kennebec variety (Shih and Kuc 1974) the major glycoalkaloids associated with *S. tuberosum* are  $\alpha$ -solanine and  $\alpha$ -chaconine (Maga 1980).

Usually the total glycoalkaloid (TGA) level has been used as a measuring tool (Fitzpatrick and Osman 1974; Fitzpatrick *et al.* 1978; Mackenzie and Gregory 1979). However, there are instances when individual glycoalkaloid levels are of interest relative to quantitation. In this case, thin-layer chromatography (Shih and Kuc 1974; Jeppsen *et al.* 1975; Ahmed and Muller 1978; Cadle *et al.* 1978; Osman *et al.* 1978) and gas-liquid chromatography (Herb *et al.* 1975; Zacharius *et al.* 1975; Fitzpatrick *et al.* 1977; Osman *et al.* 1978) have been utilized.

The question of glycoalkaloid quantitation is a controversial area in

that extraction and measurement techniques are suspect (Mackenzie and Gregory 1979). For example, data published by Herb *et al.* (1975) indicated that significantly higher levels of  $\alpha$ -chaconine and  $\alpha$ -solanine, as determined by gas-liquid chromatography, were present as compared to the TGA level as quantitated by titration. In contrast, the recent work of Cadle *et al.* (1978) would indicate that TGA content and quantitation of  $\alpha$ -chaconine and  $\alpha$ -solanine levels by thin-layer chromatography can be reasonably consistent.

Tuber damage has been reported to increase TGA synthesis (Salunkhe *et al.* 1972; Ahmed and Muller 1978; Mondy and Chandra 1979) and it has been postulated (Salunkhe *et al.* 1972) that delays in the processing on nonintact potatoes can result in increased TGA levels.

Thus, this study was designed to measure both total and individual glycoalkaloid levels in sliced potatoes as influenced by various pre-chipping storage conditions. Potential glycoalkaloid buildup in potato slices used in the manufacture of potato chips is especially important in light of the fact that during the chipping process TGA content becomes larger due to the removal of water from the chip (Sizer *et al.* 1980). Also, increased acceptance of natural style chips, which are usually made from nonpeeled potatoes, can be important since glycoalkaloids are more prevalent in the skin portion as compared to the interior portion of a potato (Baerug 1962).

## MATERIALS AND METHODS

Potato slice preparation: Commercially available Russet Burbank potatoes of uniform size were washed and sliced, unpeeled, through an electric, laboratory potato slicer to a thickness of 2mm to simulate commercially available "thick slice natural chips." Storage conditions: Immediately after slicing 2kg units were stored under the following conditions:

- (1) Placed in 3 liters of tap water and held in the dark at 5°C for 1, 3, 5 and 7 h and manually stirred every 30 min.
- (2) The same as number 1, except held at 25°C.
- (3) Placed in a container without water and stored in the same manner as number 1.
- (4) The same conditions as number 3, but held at 25°C.

The factors of light, temperature and soaking in water have previously been reported as influencing glycoalkaloid levels in potato slices (Salunkhe *et al.* 1972; Fitzpatrick *et al.* 1977; Ahmed and Muller 1978; Mondy and Chandra 1979).

TGA analysis: TGA analyses were performed utilizing the nonaqueous titration method introduced by Fitzpatrick and Osman (1974) as modified

by Fitzpatrick *et al.* (1978). Three samples were taken from each storage condition for analysis. In addition, 5 samples were taken and analyzed immediately after slicing to serve as a control. Individual glycoalkaloid analysis: Glycoalkaloids were extracted with methanol-acetic acid (95:5 vol. %) as described by Ahmed and Muller (1978) and subjected to their thin-layer chromatography separation and spot development techniques. Quantitation was accomplished by spotting known quantities of  $\alpha$ -solanine and  $\alpha$ -chaconine and measuring the developed color intensities relative to the samples using a Transidyne Model 2955 scanning densitometer equipped with a Model 2967 computing integrator. Three samples from each variable along with freshly sliced samples were subjected to this procedure.

It should be noted that the data are presented on the basis of mg/100g fresh potato weight since preliminary analyses revealed that water uptake did not amount to more than a 4% increase in potato slice weight, while loss of starch, determined by the measurement of total solids in the soak water, resulted in a maximum potato weight loss of 7.5%. Both values are well within the range of experimental error and thus no corrections were made for these variables.

## RESULTS AND DISCUSSION

It should be noted that these analyses were performed with only one potato variety and that differences in both the ratio and amount of individual as well as TGA levels among varieties apparently exist (Ahmed and Muller 1978; Mondy and Chandra 1979).

Previous studies have reported increases in glycoalkaloid concentration after storage times of 1 to 7 days, which are not comparable to normal processing conditions (Ahmed and Muller 1978; Herb *et al.* 1975; Fitzpatrick *et al.* 1977; Salunkhe *et al.* 1972; Mondy and Chandra 1979). This study was done at times of 0 to 7 h, which is more likely in actual commercial operations.

Several interesting observations can be noted from the data presented in Table 1. In viewing the TGA quantitation data, it can be seen that TGA content increased with time in all conditions. This is in agreement with previous investigators (Ahmed and Muller 1978; Fitzpatrick *et al.* 1977; Mondy and Chandra 1979; Salunkhe *et al.* 1972). However, as mentioned earlier, their studies involved longer storage times before measurements were taken. In this study, increases in TGA levels were noted after one hour of storage and in light of the concern for TGA levels in excess of 20mg/100g (Jadhav and Salunkhe 1975; Sinden *et al.* 1976) it is apparent

Table 1. Influence of time, temperature and presence of water on the total and individual glycoalkaloid levels in potato slices

| Hours of Storage | Presence of Water | Temperature of Storage |                      |       |                       |                      |                      |       |                       |                      |                      |       |                       |
|------------------|-------------------|------------------------|----------------------|-------|-----------------------|----------------------|----------------------|-------|-----------------------|----------------------|----------------------|-------|-----------------------|
|                  |                   | 5°                     |                      |       |                       |                      | 25°                  |       |                       |                      |                      |       |                       |
|                  |                   | $\alpha$ -chaconine    | $\alpha$ -solanine   | Ratio | TGA                   | $\alpha$ -chaconine  | $\alpha$ -solanine   | Ratio | TGA                   | $\alpha$ -chaconine  | $\alpha$ -solanine   | Ratio | TGA                   |
| 0 (control)      | --                | 7.1±0.4 <sup>1</sup>   | 5.0±0.5 <sup>1</sup> | 1.42  | 11.8±0.9 <sup>1</sup> | 7.1±0.4 <sup>1</sup> | 5.0±0.5 <sup>1</sup> | 1.42  | 11.8±0.9 <sup>1</sup> | 7.1±0.4 <sup>1</sup> | 5.0±0.5 <sup>1</sup> | 1.42  | 11.8±0.9 <sup>1</sup> |
| 1                | +                 | 6.3±0.3                | 6.2±0.5              | 1.02  | 12.3±0.4              | 7.0±0.2              | 6.1±0.3              | 1.15  | 12.9±0.6              | 7.0±0.2              | 6.1±0.3              | 1.15  | 12.9±0.6              |
| 1                | -                 | 8.7±0.3                | 4.8±0.3              | 1.81  | 13.2±0.5              | 11.0±0.3             | 5.2±0.2              | 2.11  | 15.9±0.8              | 11.0±0.3             | 5.2±0.2              | 2.11  | 15.9±0.8              |
| 3                | +                 | 8.2±0.2                | 6.8±0.4              | 1.21  | 14.9±0.7              | 9.2±0.5              | 7.1±0.2              | 1.30  | 16.1±0.3              | 9.2±0.5              | 7.1±0.2              | 1.30  | 16.1±0.3              |
| 3                | -                 | 13.8±0.6               | 7.2±0.3              | 1.92  | 19.6±1.0              | 18.3±0.4             | 7.2±0.5              | 2.54  | 25.3±0.6              | 18.3±0.4             | 7.2±0.5              | 2.54  | 25.3±0.6              |
| 5                | +                 | 9.4±0.4                | 7.0±0.5              | 1.34  | 16.2±0.8              | 10.3±0.2             | 7.0±0.3              | 1.47  | 17.4±0.5              | 10.3±0.2             | 7.0±0.3              | 1.47  | 17.4±0.5              |
| 5                | -                 | 15.3±0.2               | 7.6±0.4              | 2.01  | 23.1±0.6              | 20.5±0.4             | 7.5±0.4              | 2.73  | 27.7±1.1              | 20.5±0.4             | 7.5±0.4              | 2.73  | 27.7±1.1              |
| 7                | +                 | 10.5±0.6               | 7.2±0.4              | 1.46  | 17.0±1.1              | 11.4±0.5             | 7.3±0.2              | 1.56  | 18.9±0.5              | 11.4±0.5             | 7.3±0.2              | 1.56  | 18.9±0.5              |
| 7                | -                 | 18.3±0.5               | 7.6±0.5              | 2.41  | 26.4±0.8              | 21.9±0.3             | 7.9±0.4              | 2.77  | 29.1±0.7              | 21.9±0.3             | 7.9±0.4              | 2.77  | 29.1±0.7              |

<sup>1</sup> mg/100g fresh weight ± the standard error of the mean



from these data that this level can easily be exceeded. With most processed potato products, the majority of the peel is removed. However, with natural style chips, for example, the presence of peel and processing delays in excess of several hours can result in significant increases in TGA concentration.

The influence of soaking potato slices in water versus storage without water on TGA concentration can also be seen. In all instances, unsoaked slices had higher TGA levels than soaked slices. Similar observations were reported by Mondy and Chandra (1979). Also, as would be expected, TGA levels were higher at the 25° incubation as compared to 5°. Thus, the temperature at which chipped or damaged potatoes are held before frying can also influence TGA levels. This would be especially true in potatoes that are tempered at room temperature for several days before frying to alter the starch/reducing sugar ratio.

It can also be noted that the combined  $\alpha$ -chaconine and  $\alpha$ -solanine quantitative values are in close agreement with TGA values. This has not been the case in other reported studies (Fitzpatrick *et al.* 1977; Herb *et al.* 1975). These discrepancies reflect differences in methodology since other investigators (Cadle *et al.* 1978) have found close quantitative agreement between individual and total glycoalkaloid levels.

Another interesting facet of the data in Table 1 related to the  $\alpha$ -chaconine/ $\alpha$ -solanine ratio as influenced by the variables investigated. The control potato slices (immediately after slicing) has a ratio of 1.42. In the presence of water this value decreased to 1.02 at 5° and 1.15 at 25° after one hour and then gradually returned with increased storage time to values approximating the original. Thus, it would appear that in the presence of water,  $\alpha$ -solanine synthesis is more active than that of  $\alpha$ -chaconine. However, in the absence of added water, the opposite apparently is true since data from this study demonstrate that a higher ratio is present after one hour of storage independent of temperature with this ratio increasing with further storage time.

Leaching of TGA in the water soaked samples was not thought to be a strong possibility since glycoalkaloids have very limited solubility in water, and preliminary analyses of the water employed in the soaking process resulted in no detectable levels of TGA.

Mondy and Chandra (1979) have proposed that glycoalkaloid synthesis in a water state may be impaired by the leaching of water soluble TGA precursors such as alanine and leucine or water inactivation of certain enzymes. This study would indicate that more than one mechanism may be dominant in the synthesis of  $\alpha$ -chaconine and  $\alpha$ -solanine.

This study also demonstrated that with the potato variety employed,  $\alpha$ -chaconine was the predominant glycoalkaloid present, especially with

increased storage time. For example, storage at 25°C for 7 h without soaking resulted in approximately a 60% increase in  $\alpha$ -solanine but over a 200% increase in  $\alpha$ -chaconine content.

In conclusion, it has been demonstrated that relatively short storage of unpeeled sliced potatoes can result in increased TGA levels, as well as major changes in glycoalkaloid composition.

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# THE EFFECT OF AN INDIVIDUAL QUICK BLANCHING METHOD ON ASCORBIC ACID RETENTION IN SELECTED VEGETABLES<sup>1</sup>

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## ABSTRACT

*Method of blanching (including cooling) was found to have a significant effect on residual ascorbic acid level in peas ( $P < 0.01$ ) and broccoli ( $P < 0.001$ ), but not cut green beans ( $P > 0.05$ ). The separate parts of the procedure (heating and cooling) had an additive effect but acted independently of one another. In each case where a difference was observed the experimental procedure (individual quick blanch/evaporative or "air" cool) was found to yield a product higher in ascorbic acid than the conventionally processed product (water blanch/water cool). The practical implication is that adoption of specialized steam blanch procedures or evaporative cooling will result in improved nutrient levels in sensitive vegetables (e.g. peas and broccoli) as compared to conventional product. The experiments also indicated that any contact of heat and water with susceptible vegetable materials will cause a marked reduction in ascorbic acid levels. For the vegetables studied, the K-1 individual quick blanch system yielded fully blanched product which retained mean ascorbic acid levels between 82 and 91 % of raw values, depending on the specific vegetable.*

## INTRODUCTION

The origination of the blanching process can be attributed to two independent studies performed in the late 1920's (Kohman 1929; Joslyn Cruess 1929). Both studies demonstrated the beneficial aspects of inactivating enzyme systems within the vegetable material being processed, by the ap-

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plication of a short heat treatment. In the intervening years much has been published on the subject. Tressler and Evers (1957) gave a fairly complete review of work performed, up to 1957. Joslyn and Hyde (1964) and Tressler *et al.* (1968) provided an overview of the activity since 1957. In more recent times, novel approaches to the blanching process have been reported by Lazar *et al.* (1971); Bomben *et al.* (1973); Bomben (1976); Limandri and Robe (1976) and Cumming *et al.* (1978). These last five studies have described individual quick blanch (IQB) processes which utilize steam as the heating medium. In three of the units cooling is accomplished by a flow of air using the principle of evaporative cooling.

Ascorbic acid retention in vegetables has been studied at some length. However, the authors believe that insufficient attention has been directed to the blanch-cool sequence as an entity. The experiment reported here involved use of the unique blanching system described by Cumming *et al.* (1978) and deals with the performance of the K-1 unit and, with the effect of the entire blanch-cool system on the retention of ascorbic acid in selected vegetables. Ascorbic acid was chosen for testing, both for its intrinsic importance as a nutrient and as an indicative component of the process material, demonstrating a high degree of water solubility and heat lability.

## METHODS

Blanching was accomplished by one of two processes. The first which approximated common commercial practice was to immerse the product with periodic agitation in water maintained between 94° and 99°C. The alternate procedure employed the experimental K-1 Blancher designed and built by the Engineering and Statistical Research Institute (Ottawa, Ontario) and the Food Processing Section of the Agriculture Canada Research Station (Kentville, Nova Scotia). This unit operates on the IQB principle, wherein a monolayer of product is exposed to live steam for a short period of time, followed by a longer holding time amassed on a slow moving belt. The combination of heating and holding allows minimum application of heat to attain the required center temperature for enzyme inactivation.

Cooling of blanched product was achieved either by total immersion in cold water or by use of the evaporative cooler considered to be an integral part of the K-1 unit. Table 1 lists the various residence times employed. After cooling, the product was frozen by the IQF method for peas and beans while broccoli was bagged (approx. 2 kg per bag) using heat sealed 51  $\mu$  polyethylene bags and blast frozen. Peas and beans were bagged in

Table 1. Standard blanch/cool regimes employed for the vegetables studied

| Product  | Blanch Times (S) <sup>a</sup> |      |       | Cool Time (S) |       |
|----------|-------------------------------|------|-------|---------------|-------|
|          | Steam Heat                    | Hold | Water | Air           | Water |
| Peas     | 35                            | 55   | 90    | 120           | 180   |
| Beans    | 35                            | 55   | 90    | 120           | 180   |
| Broccoli | 40                            | 70   | 90    | 180           | 120   |

<sup>a</sup>Blanch times determined in preliminary studies using enzyme test of Pinsent (1962)

the same fashion after freezing. All product was stored at  $-26^{\circ}\text{C}$  for later evaluation.

For the blanched samples, ascorbic acid determinations were performed immediately after cooling. For each experiment performed, a raw sample was tested, in order that the percent retention could be determined. The determination employed gave the amount of reduced form of *L*-isomer of ascorbic acid present; this was a slightly modified version of the method reported by Lundergan and Moore (1975). In the present method, the spectrophotometric determination employed a blank which was zeroed for sample background. The subsequent sample reading was then taken directly. A standard curve was produced daily to adjust for changes in the indophenol dye solution.

A preliminary study was undertaken to determine optimum sample size for reproducible extraction, and to yield a sufficient amount of ascorbic acid to give reliable readings. Table 2 shows the proportions of vegetable to the extraction medium. For each batch processed, duplicate samples were taken and subsequent readings on these samples performed in duplicate. Each series was replicated a minimum of three times. Vacuum-oven moisture determinations were performed in duplicate on each process batch in order that ascorbic acid might be expressed on a dry weight basis. The percent retention of ascorbic acid was calculated on the dry weight basis. The standard curve and ascorbic acid levels were calculated with a computer. Input data consisted of the spectrophotometric reading, sample moisture, sample weight, and amount of oxalic acid used.

Peroxidase activity was determined using the method of Pinsent (1962) and reported as Willstatter purpurogallin units (PE units). In all treatments reported, the process was adequate to be considered a complete blanch.

## RESULTS AND DISCUSSION

In the first part of the experiment all three vegetables were exposed to



Table 2. Relative amounts of sample and solvent employed in the extraction of ascorbic acid from the vegetables studied

| Sample   | Weight (g) | 1% Oxalic Acid (ml) |
|----------|------------|---------------------|
| Peas     | 50         | 100                 |
| Beans    | 50         | 100                 |
| Broccoli | 50-60      | 400                 |

four "optimal" treatments making use of various combinations of steam and water blanch and air and water cool. Mean values for ascorbic acid retention are found in Table 3. The actual conditions for these treatments can be extracted from Table 1. Statistical analysis revealed that steam blanching gave a highly significant improvement in ascorbic acid retention for both peas ( $P < 0.01$ ) and broccoli ( $P < 0.001$ ) when compared to water blanching. It was also found that air cooling gave a highly significant ( $P < 0.01$ ) improvement in ascorbic acid retention over water cooling ( $P < 0.01$  for peas and  $P < 0.001$  for broccoli). There was no significant interaction between blanching and cooling ( $P > 0.05$ ). In the case of cut green beans there were no significant differences ( $P > 0.05$ ) for blanching or cooling.

Table 3. The effect of blanch method (optimized by method) on ascorbic acid retention in selected vegetables

| Treatment <sup>1</sup> | Mean Retention Ascorbic Acid (%) |          |          |
|------------------------|----------------------------------|----------|----------|
|                        | Peas                             | Broccoli | Beans    |
| Steam/Air (1)          | 86.0                             | 91.0     | 81.4     |
| Steam/Water (2)        | 75.8                             | 77.8     | 82.3     |
| Water/Air (3)          | 75.0                             | 69.4     | 80.5     |
| Water/Water (4)        | 70.9                             | 61.3     | 84.9     |
| SEM (df) <sup>2</sup>  | 1.6 (24)                         | 2.1 (12) | 4.4 (14) |
| Sig. Effects           | B**, C**                         | B**, C** |          |

<sup>1</sup> Optimal blanch/cool treatments were determined for each vegetable in preliminary experiments

Sig. Effects (\*5%, \*\*1%).

B = Steam vs water blanch (1, 2 vs. 3, 4)

C = Air vs water cooling (1, 3 vs 2, 4)

<sup>2</sup> SEM = Standard error of the mean

Interpretation of these results indicates that the blanch/cool system has a profound effect on ascorbic acid content in some vegetables but not in others. Using broccoli and green beans as examples, this point can be made clear. Raw broccoli was found to have a mean (wet weight basis) ascorbic

acid content of 114.3 mg/100 g while steam blanched/air cooled and water blanched/water cooled product had 104 mg/100 g and 68.4 mg/100 g, respectively. Green beans had a mean raw ascorbic acid of 11.7 mg/100 g and showed no response to differences in the blanch/cool method.

It is clear that contact with water in either the heating or cooling phase causes a reduction of ascorbic acid content in both peas and broccoli. This fact is of great importance in view of common blanch/cool procedures employed in the industry. The results indicate that heat, water and product in the same location at the same time results in loss of ascorbic acid in certain vegetables. Interestingly, no significant interaction ( $P > 0.05$ ) was found between blanching and cooling methods in relation to ascorbic acid level. This indicates that the effects are additive in nature only. The practical implication is that the use of either steam blanch or air cool will improve ascorbic acid retention over water blanch or water cool, respectively.

It was not immediately clear why green beans were unaffected by the differences in blanch/cool systems. In a test designed to delineate the relative quantities of ascorbic acid in specific tissues it was found that the ascorbic acid is concentrated in the seeds (38.0 mg/100 g wet wt basis) which are protected by the pod (8.4 mg/100 g wet wt basis).

Where a sensitivity to the blanch treatment does exist it seems that a benefit can be derived from the avoidance of water and heat in combination with the product. In addition to peas and broccoli, cauliflower and Brussels sprouts appear to be sensitive to blanch method. However, insufficient quantities have been processed to present this as a statistically significant result.

A second phase of the experiment was to evaluate the effect of blanching time on the ascorbic acid content of peas. Table 4 presents the findings of this study. All samples were adequately blanched by the procedures employed. When these results were analyzed statistically, it was found that the steam blanch yielded product with significantly ( $P < 0.001$ ) higher ascorbic acid levels than water blanched product. Blanch time also showed a significant ( $P < 0.05$ ) effect on ascorbic acid content. As might be expected, increased time resulted in reduced ascorbic acid levels.

At this point, it is important to reiterate that the so-called steam blanch is not a conventional steam blanch procedure, wherein the blanch is achieved in a steam atmosphere before the cooling step begins. The procedure employed here involves a relatively short exposure to steam followed by a holding step where blanching is finally accomplished. Conventional steam blanching may not yield the highly significant improvements observed here when compared to water blanch procedures.

Table 4. The effect of blanch time and method on the retention of ascorbic acid in peas

| Method <sup>2</sup>      | Heat<br>(S) | Hold<br>(S) | Cool<br>(S) | Mean<br>Enzyme<br>Activity <sup>1</sup><br>(PE units<br>× 10 <sup>-4</sup> ) | Mean<br>% Retention<br>Ascorbic Acid |
|--------------------------|-------------|-------------|-------------|--|--------------------------------------|
| S/A                      | 35          | 55          | 120         | 1.34   | 86.5                                 |
| W/A                      | 90          |             | 180         | .78  | 74.5                                 |
| S/A                      | 60          | 60          | 120         | .04  | 80.2                                 |
| S/A                      | 90          | 60          | 120         | .08  | 78.4                                 |
| W/A                      | 120         |             | 120         | .06  | 70.6                                 |
| W/A                      | 150         |             | 120         | .08  | 70.2                                 |
| SEM (df) <sup>3</sup>    |             |             |             |  | 2.2 (21)                             |
| Sig. Effects (*5%, **1%) |             |             |             |  | B**, D*                              |

<sup>1</sup> Values below 5.00 indicate no practical enzyme activity

<sup>2</sup> S = steam, W = water, A = Air

B = Blanch method S/A vs W/A

D = Duration of blanch

<sup>3</sup> SEM = Standard error of the mean

## CONCLUSIONS

It has been shown that the ascorbic acid content of certain vegetables, specifically peas and broccoli, can be very much affected by blanching. Method of both blanching and cooling were shown to have statistically significant influences on ascorbic acid retention in both peas ( $P < 0.01$ ) and broccoli ( $P < 0.001$ ). The relationship of blanch and cool methods were found to be noninteractive but rather, additive in nature. Thus, it may be possible to exploit to advantage either a steam blanch or an evaporative cool relative to water blanch and water cool. For these vegetables it can be said that the coincident presence of product, heat and water under the conditions of an adequate blanch/cool sequence serves to cause significant reductions in ascorbic acid content.

Interestingly, green beans showed no response to blanch or cool method. This fact was attributed to the localized concentration of ascorbic acid in the seed which was protected from leaching by the pod.

Retention of ascorbic acid varied inversely with duration of blanch ( $P < 0.05$ ) for peas blanched both in water and in steam. This points to the need for careful control of the blanch procedure in order to achieve an adequate blanch while maintaining an optimum nutrient level.

Finally, for certain selected vegetables the new blancher-cooler system of this experiment provides a method of blanching superior to conven-

tional water blanch-water cool processes when ascorbic acid retention is used as an indicator.

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# MODIFICATION OF CORN WET-MILLING STEEPING CONDITIONS TO REDUCE ENERGY CONSUMPTION

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## ABSTRACT

*Laboratory corn wet-milling experiments were conducted with modified operating conditions. Mill-starch yield obtained from corn steeped at reduced steep solution volume was comparable to normal operation as long as the absolute amount of steeping agent, SO<sub>2</sub>, remained the same. Increasing the SO<sub>2</sub> concentration or removing the mass transfer barriers between the endosperm and the steep solution by degerminating between two steeping periods improved mill-starch yield. These modifications have potential for saving energy consumed in steeping and in concentrating residual steep solution.*

## INTRODUCTION

The purpose of corn wet-milling is to separate and recover the corn kernel's major components: germ, hull, gluten and starch (Anderson 1970; Watson 1967). The wet-milling process consists of four stages: steeping, germ separation and recovery, fiber (mainly hull) separation and recovery, and starch-gluten separation.

During steeping, dry corn (about 10–15% moisture) is soaked in twice as much water by weight for 40 to 50 h at a temperature of about 50°C to a moisture of 40 to 50%. Commercial steeping is a counter-current operation which normally occurs in a battery of 8–12 tanks. Rather than using fresh water, steeping utilizes water originally introduced at the final starch washing step. This starch washing water then works its way in counter-current fashion through the corn wet-milling operation to the steep tanks. Before entering the steeping operation, 0.1–0.2% SO<sub>2</sub> is added to the water to prevent growth of putrefactive organisms and aid in milling. At that point in the steeping battery where the SO<sub>2</sub> concentration drops to 0.04%, *Lactobacillus* activity can proceed to form lactic acid

(Watson *et al.* 1951). The lactic acid restricts growth of other organisms, and aids in softening of the corn (Watson 1967).

After steeping, the corn is ground in a cracking-mill, which tears apart the kernel and releases the germ. Germ is removed by density difference, and the rest of the kernel is fine milled to further release starch. Fibrous material is screened out, and the starch is finally separated from the gluten by centrifugation.

In the wet-milling process, steeping appears to be the most important step (Cox *et al.* 1944). In the corn kernel, the starch granules within the endosperm cells are embedded in a protein matrix. The smaller starch granules and a heavy protein matrix lie predominantly in the cells under the aleurone layer, which is the outer layer of the endosperm. The larger starch granules and a less massive protein matrix lie nearer the center of the kernel. These protein matrixes, which hold the starch granules within the endosperm cells, must first be broken down before the starch can be liberated. The SO<sub>2</sub> which is dissolved in the steep solution has been found indispensable to the release of starch from the endosperm. Sulfurous acid apparently is able to disintegrate the protein matrixes to release the starch granules and consequently facilitate complete starch-gluten separation. Steeping also serves to leach out soluble substances (sugars and minerals) which are located predominantly in the germ. The loss of soluble substances lowers the density of the germ and provides a better germ separation. Furthermore, during the long steeping period, the germ cells lose viability and the germ becomes more plastic, which protects the germ from breaking in the degerminating step.

Since ease of starch recovery depends on the degree of endosperm protein disintegration, the path by which water and sulfurous acid enter the kernel and the permeability of the enveloping membrane to passage of water and solute both into and out of the kernel are important in steeping. The corn kernel is covered by a) the pericarp (hull) and tip cap, b) the seed coat and hilar layer and c) the aleurone layer (Wolf *et al.* 1952). Water must pass through these three enveloping layers to reach the corn germ and endosperm. Water is taken up through the tip cap since the outer layer of the pericarp is cutinized (Wolf *et al.* 1952). Capillary force moves water rapidly through the spongy layer of the pericarp to the top of the kernel. Water then slowly diffuses across the seed coat and the aleurone layer. The rate of uptake of water and sulfurous acid by the germ and endosperm is hence controlled by the resistance of the seed coat and the aleurone layer to water and solute penetration.

The wet-milling process is an energy intensive operation (Krochta and Look 1978). Energy is spent mainly in achieving and maintaining steep temperature, concentrating the steep water remaining after the steep for use as a fermentation medium or feed component, and in drying the separated germ, fiber, gluten and starch. If it would be possible to reduce steep time and/or to reduce the volume of residual steep water, a consid-

erable amount of energy could be saved. One approach to either reducing steep time or reducing steep solution volume is increasing the concentration of steeping agent,  $\text{SO}_2$ . This would produce a larger flux of  $\text{SO}_2$  across the seed coat and aleurone layer into the endosperm, and still provide sufficient  $\text{SO}_2$  dispersion while reducing steep solution volume.

Another approach involves eliminating the slow diffusion step by placing the endosperm in direct contact with the steeping agent. In this way,  $\text{SO}_2$  might disperse the protein matrixes holding starch granules in the endosperm cells more completely and/or more quickly, and possibly with less steep solution. This could be accomplished by degerminating: i.e., steeping the corn for several hours to soften it, breaking the corn into pieces with a cracking-mill, and then steeping for the rest of the required time. The barriers to absorption of the steep solution are thus removed, and the  $\text{SO}_2$  solution is placed in direct contact with the endosperm.

To explore these approaches, experiments were conducted which studied the effects of: (1) reducing the steep solution volume and increasing the concentration of steeping agent, and (2) removing the mass transfer barriers between the endosperm and the steep solution by degerminating between two steeping periods.

### EXPERIMENTAL PROCEDURES

Most laboratory steeping studies have been done batchwise, with no addition or development of lactic acid. Nonetheless, it appears that starch yields comparable to those of industry are obtained by steeping in this manner (Zipf *et al.* 1950). Thus, a bench-scale experimental method similar to those previously described (Cox *et al.* 1944; Watson *et al.* 1955; Zipf *et al.* 1950) was used. Normal dent corn (10–15% moisture) was screened on a U.S. standard #5 sieve to remove chaff, dust, and broken kernels. Desired concentrations of dilute sulfurous acid were prepared by dissolving sodium bisulfite in distilled water. A 300 g sample of screened corn and the required amount of sulfurous acid were placed in a 1 liter bottle, which was immersed in a temperature bath set at 50°C. The steep solution was recirculated continuously from the bottom to the top of the bottle by a pump operating at a rate of 150–200 ml/min. This ensured an even concentration in the steep solution. After the corn was steeped for 48 h, the steep solution was drained off, measured, and analyzed. A 200 g portion of the steeped corn was ground with an equal weight of water in a Waring blender with the leading edges of the blades filed down. Blending time was 2 min at one-third speed. A 100 ml volume of distilled water was added to the slurry, and the slurry was poured into a 600 ml



beaker. To facilitate flotation of the germ, 30 g of corn starch was also added to the slurry. A magnetic stirrer was used to agitate the slurry gently, and the floating germ was skimmed by hand using a piece of perforated sheet metal until removal was completed. Recovered germ was washed with 100 ml of distilled water, and the wash water was returned to the slurry. Degermed slurry was ground once more in the Waring blender at full speed for 2 min to release more starch from the broken kernels. Coarse and fine fiber fractions were removed from the ground mass by screening the slurry through U.S. standard #100 and #200 mesh sieves stacked together on a mechanical shaker. Both fractions were washed twice with 100 ml of distilled water and screened. The wash solution was combined with the screened slurry, and the final slurry containing starch and gluten was settled in a 1 liter graduated cylinder for 3 h. This starch and gluten fraction is known as mill-starch. Mill-starch yield is an index of millability, and was used in this study as a measure of steeping effectiveness. Germ, coarse fiber, fine fiber, and mill starch were put in separated trays and dried overnight at 80°C in a forced-air oven and analyzed. Solid extracted in steeping was determined by evaporating a steeping solution sample to dryness in a forced air oven at 50°C followed by drying in a vacuum oven at 65°C for 16 h.

In the steep-degerminate-steep process, corn was steeped for 24 h. Portions using one-half of the steeped corn with one-half of the steep solution at a time were ground for 2 min in a Waring blender at one-third speed. The portions were then combined and returned to the bottle for further steeping. Germ recovery, fiber removal and recovery, and mill-starch separation proceeded as described earlier.

When using the 1:2 ratio of water to corn, the corn was not fully covered by the steep solution. In those cases, steeping was done by revolving the corn in a jar, equipped with a baffle to ensure even contact of steep solution and corn, until most of the steep solution was taken up by the corn (6--7 h). The wetted corn and the remaining steep solution were then transferred to the temperature bath for the rest of the steeping.

All experiments were performed in duplicate, and average results are shown with average variability.

## RESULTS AND DISCUSSION

Table 1 shows the effect of varying steep solution volume and steeping agent concentration on mill-starch yield, residual steep solution volume, and soluble substance extracted. Results show that as long as the absolute amount of steeping agent in water remained the same, the volume of steep

Table 1. Effect of the steep solution volume and concentration on mill-starch yield (48 h steep)

| Water:Corn<br>g:g | Steeped Corn                  |             | Mill-Starch Yield<br>% <sup>a</sup> | Residual Steep |                      |
|-------------------|-------------------------------|-------------|-------------------------------------|----------------|----------------------|
|                   | SO <sub>2</sub> Conc.<br>wt % | Weight<br>g |                                     | Volume<br>ml   | Solid Extracted<br>g |
| 2:1               | 0.2                           | 443         | 70.3 ± 0.1                          | 442            | 11.71                |
| 1:1               | 0.2                           | 433         | 67.5 ± 0.5                          | 162            | 6.77                 |
| 1:1               | 0.4                           | 439         | 72.1 ± 0.6                          | 150            | 7.16                 |
| 1:2               | 0.4                           | 420         | 63.6 ± 1.7                          | 30             | 2.15                 |
| 1:2               | 0.8                           | 420         | 69.6 ± 1.8                          | 30             | 2.51                 |

<sup>a</sup>% of total products: mill-starch, germ, and fiber

water could be reduced without significantly affecting the mill-starch yield. For example, the mill-starch yield for the 1:2 ratio of water to corn steep at 0.8% SO<sub>2</sub> is comparable to the 2:1 ratio of water to corn steep at 0.2% SO<sub>2</sub>. The 1:1 ratio of water to corn steep at 0.4% SO<sub>2</sub> gave slightly improved yield compared to the 2:1 ratio of water to corn steep at 0.2% SO<sub>2</sub>. The slight decrease in mill-starch yield from the 1:2 ratio of water to corn steep at 0.8% SO<sub>2</sub> may be due to slightly lower corn moisture content in this steep. In the 1:1 ratio of water to corn steep, the corn was completely immersed in the steep solution during the entire steep period. Hence, the corn was in equilibrium with the steep solution. However, in the 1:2 ratio of water to corn steep, most of the steep solution was absorbed in the first 6 h, and only a small amount of steep solution was left. Therefore, the corn was not completely saturated with the steep solution at the end of the steep.

Therefore, somewhere between the 1:1 and 1:2 ratio of water to corn steeps may be optimum for good starch yield. However, the reduction in the amount of soluble substances extracted at lower water to corn ratio indicates a possible difficulty in germ separation. Since one function of steeping is to leach out soluble substances from the germ and lower the density of the germ, this indicates that the germ density would be higher in the reduced volume steep. In fact, greater difficulty in germ separation was encountered for the lower water to corn ratios.

Nonetheless, if germ recovery is not shown to be seriously affected in larger scale studies, it appears that steeping solution volume can be reduced significantly without reducing mill-starch yield. The result is a 65–90% reduction in energy consumption for concentration of the steep residual solution.

Table 2 is a more extensive comparison of mill-starch yields obtained with increased SO<sub>2</sub> concentration. Mill-starch yield increased by 6% when

Table 2. Effect of the steep agent concentration on mill-starch yield (48 h steep)

| Water:Corn<br>g:g | Steeped Corn                  |             | Residual Steep                      |              |                      |
|-------------------|-------------------------------|-------------|-------------------------------------|--------------|----------------------|
|                   | SO <sub>2</sub> Conc.<br>wt % | Weight<br>g | Mill-Starch Yield<br>% <sup>a</sup> | Volume<br>ml | Solid Extracted<br>g |
| 2:1               | 0.1                           | 454         | 66.6 ± 0.9                          | 423          | 10.02                |
| 2:1               | 0.2                           | 443         | 70.3 ± 0.1                          | 442          | 11.71                |
| 2:1               | 0.3                           | 444         | 71.3 <sup>b</sup>                   | 447          | 13.10                |
| 2:1               | 0.4                           | 445         | 72.6 ± 0.5                          | 450          | 13.05                |

<sup>a</sup>% of total products: mill-starch, germ and fiber

<sup>b</sup>One experiment

the concentration of SO<sub>2</sub> was doubled in the 2:1 ratio of water to corn steeps. These results agree with those of Cox *et al.* (1944), who found that a more dispersed protein matrix resulted when the concentration of SO<sub>2</sub> in the steep solution increased. As a result, utilization of a larger SO<sub>2</sub> concentration in the steep solution could allow reduced steeping time without reducing mill-starch yield.

Table 3 is a comparison of mill-starch yield obtained by normal steeping and the steep-degerminate-steep process. For all the steep-degerminate-steep cases, there is a 3–4% increase in mill-starch yield. This indicates that more effective protein disintegration is obtained when the mass transfer barriers between the steep agent and the endosperm are removed. Thus, one could reduce steeping time at a given steep solution volume and concentration while not adversely affecting mill-starch yield, if a steep-degerminate-steep were used. It also appears that it might be possible to both reduce steep time and steep residual volume by combining the use of the steep-degerminate-steep process with higher SO<sub>2</sub> concentration in a smaller steep solution volume. If higher mill-starch yield is the desired end, the steep-degerminate-steep process could be combined with higher SO<sub>2</sub> concentration and/or normal steeping time and volume.

## CONCLUSIONS AND RECOMMENDATIONS

Although the experimental procedure used in this study was simple, the results do give a comparison of mill-starch yield obtained under different operating conditions. Results show the potential for decreasing the steep solution volume (water to corn ratio), as long as the absolute amount of steeping agent remains the same. This indicates the potential for considerable reduction in energy expended for concentrating the residual steep solution. However, reducing steep water may cause difficulty in germ separation.

Table 3. Effect of removal of the mass transfer barriers between the endosperm and the steep solution on mill-starch yield

| Water: Corn<br>g:g | Steeped Corn                  |             | Steeping Time<br>hr  | Mill-Starch Yield<br>% <sup>a</sup> | Residual Steep<br>Volume<br>ml | Solid Extracted<br>g |
|--------------------|-------------------------------|-------------|----------------------|-------------------------------------|--------------------------------|----------------------|
|                    | SO <sub>2</sub> Conc.<br>wt % | Weight<br>g |                      |                                     |                                |                      |
| 2:1                | 0.2                           | 443         | 48                   | 70.3 ± 0.1                          | 442                            | 11.71                |
| 2:1                | 0.2                           | 445         | 24-D-24 <sup>b</sup> | 72.2 ± 0.4                          | 445                            |                      |
| 1:1                | 0.4                           | 439         | 48                   | 72.1 ± 0.6                          | 150                            | 7.16                 |
| 1:1                | 0.4                           | 441         | 24-D-24 <sup>b</sup> | 75.2 ± 0.1                          | 154                            |                      |

<sup>a</sup>% of total products: mill-starch, germ and fiber

<sup>b</sup>24 h steep-degerminate-24 h steep

The water that is used in the steeping operation enters the corn wet milling process at the starch washing step and works its way through the process to the steep tanks. Thus, achievement of water-use reduction in steeping will have to include efficient water re-use throughout the corn wet milling process. The results found in this study provide an incentive to study and achieve such water-use efficiency.

Additional results show that increasing the  $\text{SO}_2$  concentration while maintaining the steep time and steep solution volume (water to corn ratio) improves mill-starch yield. This indicates the potential for maintaining normal mill-starch yields at reduced steep time when one increases  $\text{SO}_2$  concentration at normal steep solution volumes. Degerminating the corn between two steeping periods while maintaining steep time and steep solution volume (water to corn ratio) also improves mill-starch yield. Thus, this approach also has potential for maintaining normal mill-starch yields at reduced steep times. Both of these approaches to reducing steep time would reduce the energy expended for holding steeps at high temperature for long periods of time.

More elaborate, large-scale pilot plant testing is needed to verify and optimize these results and conclusions.

Finally, Watson and Sanders (1961) have concluded that the counter-current flow of corn and steeping solution in the batteries of corn steep tanks used commercially makes impossible the use of  $\text{SO}_2$  concentration higher than 0.2%, since the delicate balance of lactic acid fermentation necessary for optimum steeping would be destroyed. However, high  $\text{SO}_2$  concentration could be confined to the water inlet end of the battery by selective circulation of high  $\text{SO}_2$  steep solution among a few tanks, with only a certain amount transferred to the remaining tanks. Steep solution for the remaining tanks could be made up with  $\text{SO}_2$ -free water. Thus, the advantages of steeping with higher  $\text{SO}_2$  concentrations could likely be achieved.

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# NON-ENZYMATIC BROWNING OF HYGROSCOPIC WHEY POWDERS IN OPEN VERSUS SEALED POUCHES

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## ABSTRACT

*The moisture sorption behavior and non-enzymatic browning of hygroscopic whey powders stored in open and sealed conditions were examined. The water released from crystallization of the amorphous lactose in the whey powders was entrapped within sealed pouches, resulting in an increased local moisture content as well as  $a_w$  for products initially humidified in the 0.33 to 0.44 range before packaging. Thus, the rate of non-enzymatic browning reaction during storage at 35° was greatly increased in the sealed samples compared to samples stored open to the environment. In fact, at 0.33  $a_w$  a maximum was observed for the sealed storage samples, while a minimum was shown in the open stored samples. Because of this, precautions should be taken in using the results of storage studies of open systems to predict kinetics of deteriorative reactions during shelf-life tests of sealed food systems, especially if amorphous to crystalline change can occur.*

## INTRODUCTION

Dried whey powders, a major by-product of cheese manufacturing, have attracted attention for use in the food industry due to their low price, versatility with respect to functionality, and nutritive value as a food ingredient. The major component of whey powders is lactose, amounting to about 70% by weight. Whey powder can be either hygroscopic or non-hygroscopic, depending on the processing conditions (Saltmarch *et al.* 1980). Both types are utilized by the food industry

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today; however, their physicochemical properties are very different and may affect the quality of the finished food product differently during storage or distribution.

In hygroscopic whey powders, most of the lactose molecules are in the amorphous state, while in non-hygroscopic powders, crystalline forms are predominant (Nickerson 1974). Due to the high hygroscopicity of amorphous lactose, hygroscopic whey and milk powders very easily take up moisture from the surrounding air, resulting in solubilization of the amorphous lattice of lactose in the system (Heldman *et al.* 1965). At a certain extent of water absorption, amorphous molecules acquire sufficient mobility and space to rearrange themselves into a regular crystalline lattice (Berlin *et al.* 1968; Buma 1966). Once crystallization is initiated, the water bound on the hygroscopic sites of the amorphous lactose matrix will be released, since the lactose molecules form stronger intermolecular bonds and are so tightly packed in the crystal form that water cannot fit between. Moreover, this crystallization with simultaneous liberation of moisture causes step discontinuities in the adsorption isotherm (Saltmarch and Labuza 1980).

With respect to the water activity ( $a_w$ ) range at which this isotherm discontinuity occurs, past results show different values; the earlier workers such as Suplee (1926); Troy and Sharp (1930); Sharp and Doob (1941) noted discontinuities in various milk products in the 0.5 to 0.6  $a_w$  range at 25°C; Berlin *et al.* (1968) and Berlin and Anderson (1975) reported  $a_w$  0.35 to 0.52 for whey powders at 25°C; Warbuton and Pixton (1978) showed the initiation of lactose crystallization in milk powders at 0.42  $a_w$  when stored at 25°C. Recently, scanning electron microscopy was employed to evaluate more exactly the effect of  $a_w$  on lactose crystallization (Warbuton and Pixton 1978; Saltmarch and Labuza 1980). Saltmarch and Labuza (1980) found that in hygroscopic whey powders, lactose crystals quickly formed by recrystallization during storage at an  $a_w$  of 0.33. The moisture adsorption isotherm had a discontinuity in the 0.33 to 0.44  $a_w$  range which was time dependent.

Non-enzymatic browning through the Maillard reaction is a major deteriorative factor in the storage of dehydrated dairy food products (Choi *et al.* 1949; Loncin *et al.* 1968). In general, the non-enzymatic browning reaction rate increases above the BET monolayer moisture content as  $a_w$  increases. At about  $a_w$  0.6 to 0.8 the rate reaches a maximum, and then decreases again as  $a_w$  increases (Labuza 1970; Loncin *et al.* 1968; Sharp 1957). However, under certain conditions this maximum can be shifted. The presence of humectants such as propylene glycol or glycerol shifts the browning maxima to lower  $a_w$ 's (Eichner and Karel 1972; Warmbier *et al.* 1976 a,b.; Labuza 1980). Hygroscopic whey powders show a maxi-

imum value of browning at 0.44  $a_w$  which is due to the moisture released during recrystallization of the hygroscopic lactose as reported by Saltmarch *et al.* (1980). This situation is analogous to the presence of liquid humectants, since the water released during recrystallization is entrapped in the viscous matrix during collapse of the amorphous lactose. Based on the relative rate of recrystallization (increases with increased  $a_w$ ) and relative rate of diffusion of water out of the sample (decreases with increased  $a_w$ ) a maximum should be present in the rate of browning as was found by Saltmarch *et al.* (1980).

The method of storage can thus be very important from the standpoint of quality loss in hygroscopic food products. When impermeable packaging is employed, the extra amount of water released during recrystallization will be entrapped within food itself as well as the gas space of the package. This can thus lead to an increase in  $a_w$  of the food product which could result in acceleration of deteriorative reactions.

The objectives of the present study were to examine the moisture sorption behavior of hygroscopic whey powders stored under open and sealed conditions, and to determine the effect of open versus sealed packaging on non-enzymatic browning development during storage.

## MATERIALS AND METHODS

Two-hundred gram samples of hygroscopic whey powder (Mid America Farms, Minneapolis, MN) were weighed into petri dishes and placed to desiccators containing saturated salt solutions ranging from  $a_w$  0.11 to 0.65. They were evacuated and covered with aluminum foil to prevent light penetration, and equilibrated at room temperature ( $\sim 21^\circ\text{C}$ ) for one week. The sample from each desiccator was then divided into two portions. One portion was stored open as such over the saturated salt solution in the same desiccator at  $35^\circ\text{C}$ . Two gram samples of the other portion were sealed in aluminum foil-laminated pouches and also stored at  $35^\circ\text{C}$ . For the isotherms, the moisture content was measured during storage at about 30 day intervals for a total storage of 145 days at  $35^\circ\text{C}$ .

## MOISTURE AND $a_w$ DETERMINATION

One gram samples were vacuum-dried at  $60^\circ\text{C}$  for 24 h and 28 in. Hg to determine moisture content during storage.

Water activities were determined using the vapor pressure manometer (VPM) according to the method described by Labuza *et al.* (1976).

## BROWNING ASSAY

The extent of brown pigment formation was determined by the procedures based on Choi *et al.* (1949) as adapted by Saltmarch *et al.* (1980). Two grams of each sample were weighed into a 125 ml Erlenmeyer flask and 50 ml distilled, deionized water added to disperse the whey powders with shaking in a 37°C water bath at 120 oscillations per minute for 15 min. After the suspension was adjusted at pH 8.0 by using 3N NaOH solution, 5 ml of enzyme mixture was added containing 8 mg trypsin (Type IX, Sigma T-1034), 15 mg  $\alpha$ -chymotrypsin (Type II, Sigma C-4129) and 6.5 mg peptidase (Grade I, Sigma P-7625) previously to pH 8.0 with 0.1N NaOH. After one hour digestion with shaking under the same conditions as above, 5 ml of trichloroacetic acid (50% w/3) was added to each flask with swirling. The samples were then filtered gravimetrically into large test tubes through three Whatman No. 5 filter papers.

The color intensity of the filtrate was read at 420 nm using the Beckman UV-VIS Spectrophotometer Model 26 and is expressed as OD/gram solids. A blank was prepared without use of whey powder.

## RESULTS AND DISCUSSION

## Moisture Sorption Isotherm

Figure 1 shows sorption isotherms of the hygroscopic whey powders at 35° after 120 days storage. The plotted  $a_w$ 's are based on the initial  $a_w$  of humidification. As seen, the moisture contents of the sealed samples at all humidification  $a_w$ 's above 0.11 were higher than those of open samples. Furthermore, a discontinuity (i.e. a drop in moisture at increased  $a_w$ ) was observed in the range of 0.33 to 0.52  $a_w$  for both systems. As noted previously, amorphous lactose when picking up enough water can acquire sufficient mobility to rearrange into a crystalline lattice. Once the crystallization is initiated, the bound water is released, which causes the discontinuity in the isotherm in the 0.33 to 0.52 range. What also is obvious is that the open samples have a lower moisture content than the sealed samples. This must be due to the fact that the water released during recrystallization diffuses out of the whey when left open, but could not in the sealed samples. It should be noted that the zero time water contents were measured after one week initial equilibration at 21°C. These values are exactly the same as for the stored samples in sealed pouches at 35°C, i.e. no change in moisture occurred for the sealed pouches for the total storage period of 145 days but the open samples lost water continuously over the whole period at the lower  $a_w$ 's.

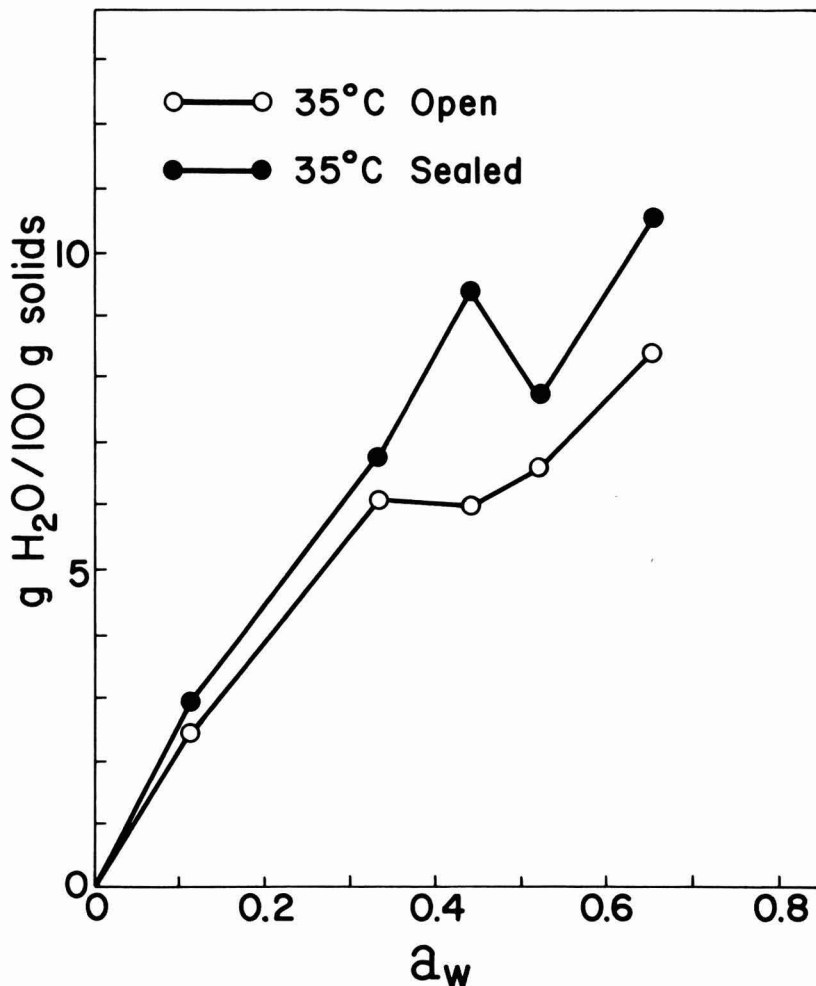


FIG. 1. ADSORPTION ISOTHERMS OF HYGROSCOPIC WHEY POWDERS AFTER STORAGE IN OPEN VERSUS SEALED SYSTEMS

When the samples were sealed in the pouches, the moisture released due to the continuing crystallization of lactose would be entrapped within the pouches and reabsorbed by the protein and remaining uncrystallized lactose. This should lead to an increase in the  $a_w$  of the sample. The samples exposed open to the salt solution should remain at the same  $a_w$ . Table 1 shows the changes in  $a_w$  of the hygroscopic whey powders stored open vs. those sealed in the pouches at 35°C. After 145 days storage there were no changes found in the open stored samples. As expected, in the sealed samples an increase in the water activity was exhibited in the  $a_w$  range of 0.33 and 0.44. Based on this, one could expect a different rate of reaction for browning in the open vs. closed

Table 1. Changes in  $a_w$ 's of hygroscopic whey powders stored at 35°C open versus sealed in pouches

| Expected | 0 Day | After 145 Days |        |
|----------|-------|----------------|--------|
|          |       | Open           | Sealed |
| 0.11     | 0.12  | 0.13           | 0.14   |
| 0.33     | 0.32  | 0.32           | 0.40   |
| 0.44     | 0.44  | 0.43           | 0.53   |
| 0.52     | 0.51  | 0.51           | 0.54   |
| 0.65     | 0.65  | 0.65           | 0.67   |

samples. Only a small change occurred at higher  $a_w$ 's since most of the recrystallization had already occurred during pre-equilibration at 25°C (Saltmarch and Labuza 1980).

Another possibility for the increase in  $a_w$  in sealed samples could be that it was due in part to production of three moles of water per mole of reducing sugar during the reaction (Hodge 1953). However, this is probably insignificant until a high extent of reaction is achieved.

The results for non-enzymatic browning reaction are shown in Fig. 2. Overall, it can be seen that the rate of browning for samples that were sealed is higher than for open storage. In addition, the open samples exhibited a maximum in browning rate at 0.44  $a_w$ , while the sealed samples showed a maximum at 0.33  $a_w$ . A shift in the maximum to a lower  $a_w$  range compared with browning in other dry foods can be correlated to the onset of extensive crystallization, as noted by Saltmarch *et al.* (1980). The water released from the amorphous lactose during crystallization can act as a solvent to mobilize the reactants. The viscous nature of the collapsed lactose which precedes crystallization would entrap water, and thus would allow the reactants to be in closer contact for reaction. Even though the lactose is crystallizing out, enough is still available for reaction in the collapsed structure. Crystalline lactose should not be reactive since it is out of solution. Moreover, the loss of water by diffusion or evaporation from this collapsed structure is probably slow enough so as to allow the reaction to proceed, as found by Saltmarch *et al.* (1980). As noted in Table 1, the actual  $a_w$  of the sealed sample originally humidified to 0.33 is 0.40, while that humidified to 0.44 is 0.53. Thus, the sealed system shows a maximum in browning rate at a true  $a_w$  close to 0.44, which is the same as for the open system. However, as noted, the rate of browning is still higher for the closed systems. This may be due to the relative driving force differences for water diffusion in the system.

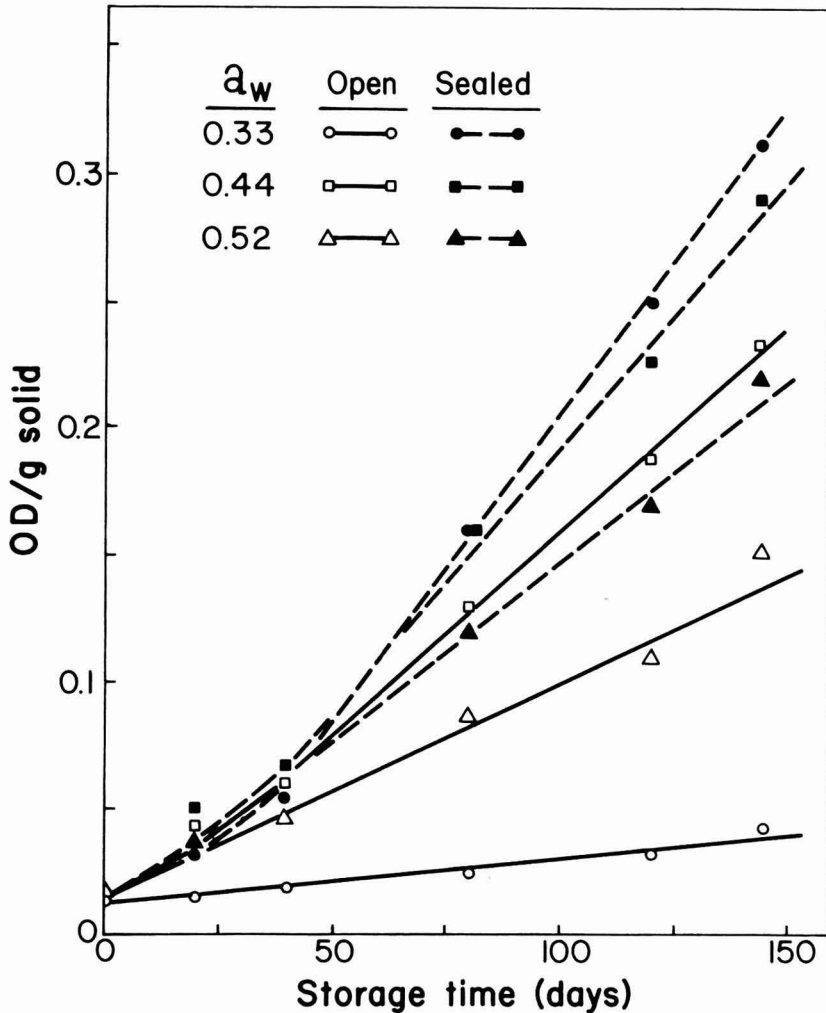


FIG. 2. COMPARISON OF BROWNING DEVELOPMENT IN HYGROSCOPIC WHEY POWDERS STORED AT 35°C. OPEN VERSUS SEALED IN POUCHES

The results of this study thus suggest a problem in reviewing previous storage studies of food systems. The fact remains that the actual physical chemical condition of a food sealed in a pouch may not be the same as when prepared, especially if an amorphous to crystalline change can occur releasing water. Thus, caution is recommended in using such results to predict kinetics of reactions.

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## BOOK REVIEW

**Applied Cook-Freezing**, P. Glanfield. Applied Science Publishers Ltd., Ripple Road, Barking, Essex, England. 200 pp. 1980. \$35.00.

The title accurately describes this book which deals with how the University of Keele in England instituted a cook-freeze system to alleviate foodservice operating cost problems in the early 1970's. The preface to the book states that there has been "little attempt to provide scientific or technical details on the cook-freezing process" and this is certainly true; but, the book serves another purpose. It provides foodservice persons with a clear historical summary of how one operation serving approximately 15,000 meals per week established a successful cook-freeze foodservice system.

Part I discusses very systematically the stages of the feasibility study leading to the selection of the cook-freeze system, and is applicable to the analysis of any new moderate size foodservice system. Changes in the production kitchen and staff are discussed and indicate that the system that was developed could produce about 110 portions per labor hour. Changes in cooking procedures necessitated by the cook-freeze system are briefly outlined.

In Part II, 90 recipes are listed that were developed for the cook-freeze system. Some of the descriptions are in English terminology such as with Irish stew (p. 62) "Put the potatoes, onions and celery into a bratt pan. Stir gently to stop the vegetables from catching." Also, oven temperatures are in the European regulator settings. While these terms might cause one to reread a sentence, the procedures are all sufficiently common so most Americans will have no problem applying the recipes to their systems.

Part III of the book describes the computer food accounting system that was developed to gain more timely management control of the financial part of the operation. Input documents are described and examples of output are presented. While there is nothing new included, the explanations are clear and would be useful to a person interested in developing their own computer based food accounting system.

This is a good book for beginners in food-freezing systems and would also be of interest to persons wanting to produce traditional English institutional recipes.

O. P. SNYDER



## ERRATA

In the paper: Prediction of Ascorbic Acid Retention During Drying. II. Simulation of Retention in a Model System by R. Villota and M. Karel which was published in the *Journal of Food Processing and Preservation* Vol. 4, No. 3, pages 141–159, there were errors in Table 3 (Models of two independent variables) as printed on page 151. The correct version of Table 3 is given below.

Table 3. Models of two independent variables

|                | Reduced Ascorbic Acid     |       |       | Total Ascorbic Acid      |       |       |
|----------------|---------------------------|-------|-------|--------------------------|-------|-------|
|                | Coeff.                    | R     | S     | Coeff.                   | R     | S     |
| a <sub>1</sub> | +17.936                   | 0.884 | 0.801 | +7.878                   | 0.893 | 0.893 |
| a <sub>2</sub> | - 2.245 × 10 <sup>8</sup> | 0.976 | 0.384 | +7.512 × 10 <sup>8</sup> | 0.956 | 0.531 |
| a <sub>3</sub> | -33.33                    | 0.996 | 0.159 | -2.503 × 10 <sup>8</sup> | 0.979 | 0.377 |
| a <sub>4</sub> | +5920.67                  | 0.998 | 0.096 | -2.934 × 10 <sup>6</sup> | 0.996 | 0.151 |
| a <sub>5</sub> | - 1.585 × 10 <sup>6</sup> | 0.999 | 0.081 | -9.878 × 10 <sup>7</sup> | 0.997 | 0.153 |
| a <sub>6</sub> | + 4.711 × 10 <sup>8</sup> | 1.000 | 0.044 | -1.907                   |       |       |
| a <sub>7</sub> | - 2.339                   |       |       |                          |       |       |

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