Citral data for the various products were calculated by means of Equation 2.

Results and Discussion

Experimental data summarizing the analysis of natural and artificial lemon extracts are given in Table I. They illustrate that of nine genuine samples received from seven different manufacturers, three showed citral contents of less than 0.2% and hence failed to meet the requirements of the Canadian Food and Drugs Act (*5*). Similar analyses of five artificial preparations obtained from four different suppliers revealed that without exception these products contained, at best, only trace amounts of the flavomatic.

Examination of both types of compositions by the Association of Official Agricultural Chemists' procedure yielded invariably higher results because of the inferior specificity of the *m*-phenylenediamine reaction (*14*).

Citral data for official flavorings are reproduced in Table II. Standards and criteria of identity for these products have been recognized by the British and United States Pharmacopeia, respectively, as well as other reference compendia (*1–5*, *13*). However, because of the lack of suitable assay procedures, requirements for citral content have not yet been established. Displaying a high degree of sensitivity and selectivity, the present method should prove of value for this purpose. Experimental results are reproducible, and their comparison with citral values as deduced from the composition of these products will readily demonstrate whether or not the citral content of a given preparation falls within the anticipated range (Table II, columns 3 and 4). None of the products tabulated, if genuine, contain any constituents capable of significantly interfering in the reaction.

Experimental data illustrating the analysis of single-strength and concentrated canned citrus juices are given in Table III. Extensive researches on the composition of these products have been carried out during the past quarter of a century. Wilson and Hall, processing a total of 10,000 gallons of California Valencia orange juice demonstrated the presence of three carbonyl compounds, acetone, acetaldehyde, and citronellal in the volatile oil (*7*). More recently, Kirchner and Miller, in continuation of their long-term studies of citrus products, combined vacuum fractionation, extraction, and column chromatographic techniques to determine no less than 29 constituents, nine of which they identified as carbonyl components (*9*). Three thousand gallons of freshly reamed, 2500 gallons of freshly canned, and 1520 gallons of stored, canned juice, respectively, were used for this work. Citral was not detected in any of the products examined, although its occurrence in grapefruit juice—approximately 0.1 mg. per kg. as based on the analysis of a 2500-gallon sample—had previously been established (*8*).

Experimental data on jams, marmalades, conserves, jellies, puddings, and soft drink powders are given in Table IV. These products were found to differ in many respects and slight procedural variations—e.g., solvent ratio, period of extraction, and size of analytical sample—had to be adopted to secure meaningful results. Some measure of the efficiency and selectivity of the assays was established by means of recovery experiments (Table V). As far as the authors are aware no data which could be used for comparison purposes have as yet been reported in the literature.

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PEA PROTEIN EVALUATION

Correlation between Alcohol-Insoluble Substances and Lysine Availability in Canned Peas

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APPROXIMATELY 47% of the protein in the average Israeli diet is derived from grain products (*9*), mainly wheat, which is poor in lysine. A popular vegetable protein source relatively rich in lysine (*16*) is canned peas (*Pisum sativum*). The variety grown for canning is Perfection.

Canned peas are commonly evaluated on acceptability criteria related to the organoleptic properties of the product, such as alcohol-insoluble substances (AIS) (*10*). No relationship, however, has hitherto been established between these criteria and a chemically determinable protein quality value (*13*).

The nutritive value of the protein in a food may be reduced by procedures involving heat (*24*). This reduction can often be compensated for to a large extent by supplementing the processed material with lysine, although analysis by conventional means shows little actual change in lysine concentration as a

The object of this study was establishment of a relation between alcohol-insoluble substances (AIS) in canned peas of different grades and their chemically determined nutritive value as a protein food relatively rich in lysine. A close negative correlation was found between AIS, an established acceptability criterion, and available lysine determined as ε-DNP-lysine by reaction with 2,4-dinitrofluorobenzene. The concentration of available lysine in canned peas as such and in their protein can be predicted from the AIS content in peas of the Perfection variety according to a significant regression equation.

result of the heat treatment. It has been concluded, therefore, that the lysine may in some way become bound or enzymatically unavailable through blocking of its ε-amino group by the Maillard reaction or otherwise (*12*). Tsien, Beeson, and Bolin (*23*) showed that chemical or microbiological procedures for the estimation of lysine in green peas, based on acid hydrolysis as a first step, are inadequate. The free ε-amino group lysine in the peas was determined by the authors according to the principle of Sanger (*19*) by reaction with 2,4-dinitrofluorobenzene (DNFB). In this report, the lysine containing a free ε-amino group is called "available," as suggested by Bruno and Carpenter (*5*) for the evaluation of different animal and vegetable protein foods.

The work was done to determine whether any relation exists between the usual acceptability measurements for canned peas of different grades and their chemically determined value as a source of protein rich in available lysine.

Materials and Methods

Five samples (five cans each) of three grades of peas (fancy, standard, and reconstituted dried) were taken during the canning season at regular intervals at a cannery known for its strict quality control.

The contents of each sample were drained, carefully mixed, and comminuted in a blender (John Oster Manufacturing Co., Milwaukee, Wis.). Samples for the determination of moisture (*1*, Method 30.2) and AIS (*1*, Method 30.14) were taken from the comminuted material. The rest was carefully dried in thin layers under forced ventilation at a temperature of 70° C. for 5 hours and under a vacuum pressure not exceeding 100 mm. of Hg. The dried material was ground in a hammer mill (Culatti Hammermill, Zürich, Switzerland) fitted with a 1-mm. sieve.

Crude protein was determined according to Method 2.23 (*1*) but with a mixture of Se/K_2SO_4 as catalyst. For the reaction between the ε-amino group of lysine and DNFB the procedure of Baliga, Bayliss, and Lyman (*2*) for preparation was followed by that of Bruno and Carpenter (*5*) permitting isolation of DNP-lysine as described by Biserte, Holleman, Holleman-Dehove, and Sautière (*4*), absorbance at 3600 A. being read on a spectrophotometer (Beckman DU). Pure 2,4-DNP-L-lysine for calibration was prepared according to Porter and Sanger (*17*).

Each result given in the tables is the mean of five samples analyzed in two replications.

Results and Discussion

Table I gives the averages of ordinary acceptability measurements of the canned peas. The results, showing statistically significant differences in all cases, exemplify the known fact of a negative correlation between moisture and AIS, reported by Makower (*14*) and Schuphan and Weinmann (*21*).

In Table II, the averages for the crude protein and available lysine content are reported. The results show statistically significant differences in all cases. The crude protein content of the canned peas in the material as such and in the dry matter increases with decreasing grade. Such a trend, related to maturity, was also reported by Raake (*18*). The crude protein contents of the three grades of canned peas parallel those reported by Schuphan (*20*) for raw peas, but are lower. This was to be expected, the findings of Chitre, Williams, and Elvehjem (*7*) having shown that canning of immature or nearly mature raw peas (comparable with local fancy and standard grades, respectively) decreases the protein content by 20 to 25% compared with the raw peas, including the losses during blanching as determined by Kramer and Smith (*11*).

Available lysine per 100 grams of canned peas and per 100 grams of crude protein therein decreases with decreasing grade, as shown in columns 4 and 5 of Table II. This decrease is even more significant considering (*7*, *20*) that the lysine content is higher in the protein of the lower grade peas and is increased by processing in higher grade peas.

The importance of peas in the diet in Israel lies in their supplementary value as a lysine source. That the DNFB procedure indicates the availability of lysine was shown by Carpenter (*6*), who found a clear correlation between the gross protein value for chicks and available lysine in fish and milk products as well as in leaf, cereal, and oilseed products. Mauron (*15*) reported that lysine may be made nutritionally unavailable in dried milk, and the extent of this damage can be measured by determining available lysine by the DNFB procedure. Bensabat, Frampton, Allen, and Hill (*3*) showed a significant negative correlation between available lysine (determined by a chemical procedure based on the DNFB reaction) and the curing time at 110° to 135° C. of peanut cotyledons, cake, and meal. Conkerton and Frampton (*8*) demonstrated a close relationship between the availability of lysine after reaction with gossypol, measured by one of the procedures mentioned above, and the nutritive value of cottonseed meal determined biologically.

The authors' results show a decrease in the concentration of free ε-amino groups of lysine, correlative with its availability

Table I. Characterization of Three Grades of Canned Peas by Different Chemical Acceptability Measurements

Grade	Moisture, %	Dry Matter, %	AIS, %
Fancy	79.03	20.97	12.75
Standard	76.04	23.96	19.34
Reconstituted	64.64	35.36	29.11
L.S.D. (*22*)	1.31	0.83	1.07

Table II. Crude Protein and Available Lysine in Canned Peas of Different Grades

Grade	Crude Protein, %		Available Lysine, %	
	Canned peas	Dry matter of canned peas	Crude protein in canned peas	Canned peas
Fancy	5.91	28.18	4.90	0.289
Standard	6.23	26.24	4.11	0.256
Reconstituted	8.57	24.20	2.78	0.239
L.S.D. (*22*)	0.30	0.15	0.41	0.016

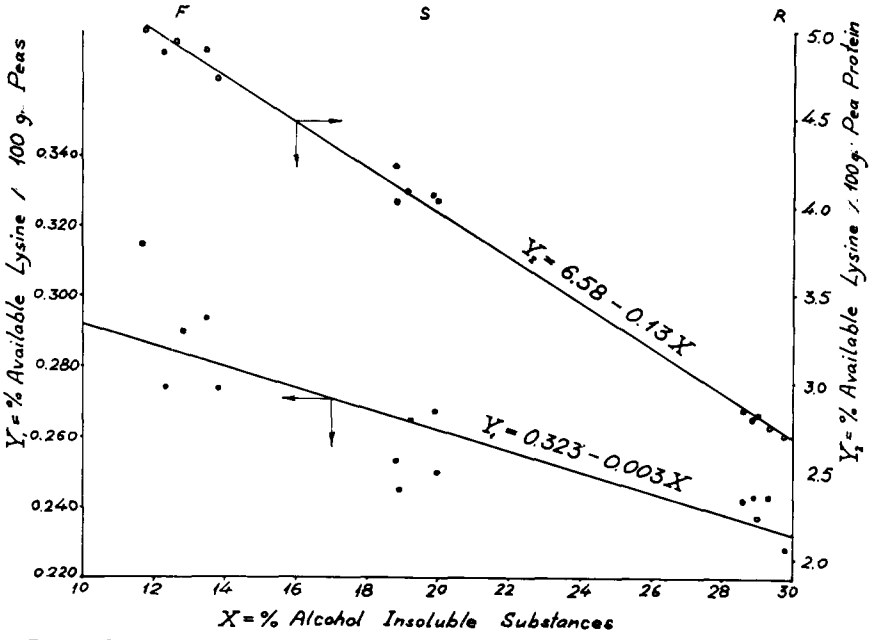

Figure 1. Relation of available lysine in peas and pea protein to alcohol-insoluble substances in peas

in canned peas and their protein, with decreasing grade (Equations 1 and 2).

$$Y_1 = 0.323 - 0.003\,X \quad (1)$$

$$Y_2 = 6.58 - 0.13\,X \quad (2)$$

where

Y_1 = concentration of available lysine in 100 grams of peas
Y_2 = concentration of available lysine in 100 grams of pea protein
X = AIS content in peas

It may be assumed, therefore, that the nutritive value of canned peas as a source of available lysine decreases with increasing maturity of the peas.

Figure 1 shows the regression lines and their equations between available lysine in the canned peas and their protein and the AIS content in the different grades. The correlation coefficient used as a measure of significance for the equations is 0.83 for the first equation (Y_1) and 0.99 for the second (Y_2), as against 0.641 at the 1% level of significance. The close negative correlation between both these parameters for the samples analyzed by the authors shows that the nutritive value of canned peas as a supplementary source of available lysine can be predicted from the AIS content. Reduced acceptability rating of canned peas corresponds to a reduced supplementary protein value.

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CAROTENOIDS DETERMINATION

Influence on the Estimation of β-Carotene by Other Carotenoids in Butternut Squashes at Harvest and during Storage

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The procedure of the Association of Official Agricultural Chemists (*1*), designed primarily for the rapid analyses of leaf and stem tissues, has been widely used to determine the β-carotene content of plant materials. The validity of this method depends upon the assumption that the major portion of the carotenoid hydrocarbons is in the form of β-carotene. However, if other carotenoids are present, they may be reflected in the values obtained.

Fujita and Ajisaka (*4*) reported only the α- and β-carotene content of *Cucurbita moschata* (Kintônasu) and *C. moschata* var. melonaeformis (Kabotya). To our knowledge the other carotenoids of *C. moschata* Duch., which include the hydroxycarotenoids eluted by the 10% acetone-hexane, have not been identified.

An early attempt at identification of the carotenoids of squashes using *C. moschata* Duch. (Giant squash) was made by Suginome and Ueno (*10*). They reported two carotenoids, "cucurbiten" and "cucurbitaxanthin," which they