developing high-strength blending flour fractions from U. S. air classified flour.

Industrial uses of wheat constitute a long-range market not competitive with food uses. Increasing efficiency of production and changing economic patterns make this a challenging possibility. We are studying the composition of gluten proteins, how their unique properties derive from their structure, and how they can be chemically modified.

Another approach is to use flour as a chemical raw material, for it is available in large quantities at relatively low cost. Flour consists essentially of starch and gluten protein; both constituents have uses as binders, adhesives, and thickeners.

Fruits and Vegetables

More than half of the fruits and vegetables now marketed in the United States are in processed form. Indications for the future point to an increasing proportion of processed products. Despite the gains in quality realized by freezing, many unsolved problems remain. One of these occurs in peaches, which undergo enzymatic browning in the frozen condition. In studies at the Western Division, an enzyme has been separated from several plant tissues which, when applied to peaches, prevents surface enzymatic browning. This enzyme has been identified as 3-*O*-methyl transferase; it acts by causing the methylation of catechol derivatives, which in their natural form undergo browning.

Interestingly enough, one of the plant sources of this enzyme is apple cambium, but it is well known that a cut apple exhibits rapid enzymatic browning. This apparent paradox was explained by the discovery that the enzyme had optimum activity at high pH, and adjustment of acidity of the cut apple permanently prevented browning.

The introduction of gas-liquid chromatography has rapidly advanced the chemistry of volatile flavor components of fruits and vegetables during the past few years. Chemical studies of flavor components must, of course, be correlated with subjective evaluations by trained taste panels. Such panels can determine odor thresholds of aqueous solutions of individual components of fruit or vegetable volatiles which appear to be related to product aroma. Chemists at the Western Division have demonstrated that an additive relationship exists between total concentration of the mixture and threshold olfactory response. Thus the aroma threshold of a 10-component mixture was identified by a taste panel when each of the compounds was present at one tenth of its individual threshold concentration. This finding would appear to be an important one and an early step toward interpretation, in terms of flavor, of the complicated chromatograms obtained in chemical studies.

Tobacco Research

Prior to January 1964, emphasis was on the relationship between tobacco leaf and smoke composition and smoking quality. Since publication of the Surgeon General's report on "Smoking and Health" last January, the tobacco program has been largely reoriented to health-related problems. Congress has provided increased funds in current appropriations for investigations in this field. More should be known about the composition of tobacco leaf and smoke and the properties of individual components. Arrangements have been made with the U. S. Department of Health, Education, and Welfare to study the medical implications of such information as it becomes available.

Conclusions

The problems that the Utilization Research Divisions seek to solve are the practical ones facing agriculture, but solutions will be reached most rapidly through a blend of basic and applied research, with increasing emphasis on the basic as the program expands. This research and that conducted at universities and other institutions will provide a firm basis for advances in the better utilization of agricultural products, both here and abroad.

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The following additional papers were presented orally as part of the symposium:

"Causes and Control of Darkening of Plant Tissues with Especial Reference to the Potato," E. C. Bate-Smith, J. C. Hughes, J. B. Pridham, L. W. Mapson, D. A. Robb, and T. Swain, Low Temperature Research Station, Cambridge, England;

"Antioxidant Components of Wood Smoke Used in the Curing of Meat," D. J. Tilgner and Z. Sikorski, Technical University, Politechnika Gdanska, Gdansk, Poland;

"The Amination of Starch," M. L. Wolfrom, Ohio State University, Columbus, Ohio;

"Effect of Synthetic Chelates on the Autoxidation of Unsaturated Fatty Acids," Giovanni Jacini and Enzo Fedeli, Stazione Sperimentale Olii e Grassi, Milano, Italy;

"Studies in the Hydroboration of Terpenes," H. C. Brown, Purdue University, Lafayette, Ind.

END OF SYMPOSIUM

ORANGE PEEL CONSTITUENTS

Flavones of the Neutral Fraction of the Benzene Extractables of an Orange Peel Juice

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The proximate analyses of orange peel juice samples collected over a period of 2 seasons are given in another paper (*4*). Among the properties determined on the whole juices were the taste thresholds when added to commercial orange juice and the amounts of benzene-extractable material present. The benzene extracts were then separated into acidic, neutral, and lactonic fractions. Since the neutral fractions were usually the largest and known to be bitter, their taste thresholds were also determined. These considerations seemed to warrant an investigation of the nature of the neutral fraction in greater detail. The approach chosen to the problem of separation was by column chromatography

The constituents of the neutral portion of the benzene extractables of orange peel juice were separated chromatographically. Several constituents were isolated and identified, accounting for about 80% of the sample. Among these substances were nobiletin, tangeretin, 3,5,6,7,8,3′,4′-heptamethoxyflavone, sinensetin (5,6,7,3′,4′-pentamethoxyflavone), and tetra-O-methylscutellarein (5,6,7,4′-tetramethoxyflavone).

on a fairly large scale, in the hope that sufficient amounts of substances could be isolated for individual taste evaluation of the isolated substances and possible development of an analytical method for their determination.

Experimental

At the time this sample was taken, a local plant recovered orange peel oil by pressing peel between Pipkin rolls, passing the undiluted liquid so obtained through a finisher, and finally centrifuging. This aqueous effluent was obtained and promptly frozen until needed. At that time, two cans of this lot were thawed and filtered through Büchner funnels with filter aid, and the filtrate was extracted in two 11-liter batches four times with about one fifth their volumes of benzene for each extraction. The extractables were combined and reduced to 500-ml. volume, which was found to contain 16.8 grams of solute by aliquot residue weight. A 450-ml. portion of this solution (15.2 grams of solute) was extracted with 13 liters of 1% aqueous sodium hydroxide to remove acidic substances. The solvent was then evaporated under vacuum in a rotary evaporator. Since it was desired to deal only with nonvolatile neutral substances, 150 ml. of water was added to the residue and then distilled to rid it of volatile substances (*3*). The residue was treated with 100 ml. of ethanol, 100 ml. of water, and 10 grams of solid KOH and allowed to stand 2 hours with occasional shaking to decompose any lactones present. The mixture was then diluted with 400 ml. of water and extracted with ethyl ether as long as appreciable substance was removed. After the combined extractables had been washed with water, the solvent was evaporated. The neutral residue weighed 11.0 grams or 72.5% of the benzene extractables.

After considerable experimentation with various systems of column chromatography, the procedure adopted as giving the most successful separation was that used by Corbin, Scnwartz, and Keeney (*1*) in their work on dinitrophenylhydrazone separation, except that isooctane was used instead of hexane. Four hundred grams of analytical grade Celite was packed by the recommended procedure into a 5 × 120 cm. column to give a packed section 106 cm. deep.

One gram of the neutral fraction was then added by warming it repeatedly in a flask with equilibrated isooctane and pouring the solution onto the column. Development was continued with equilibrated isooctane. The eluate was collected in 100-ml. tubes mounted in an automatic apparatus for changing them. Each successive group of 10 tubes was combined and the solvent distilled off, the last stage of the distillation being conducted in a tared flask under vacuum. The residue weights were plotted on graph paper. Several peaks were obtained and the residues representing different portions of the same peak were combined. In some cases, fractions were made of transition areas between peaks. When 10 fractions had been collected and no further sharp peaks seemed forthcoming, a 97-liter fraction was taken as fraction 11. Fraction 12 consisted of 17 liters which contained a band which fluoresced pink under long-wave ultraviolet light. Finally, the column was stripped with 3 liters of methanol to give fraction 13.

Fraction 5 was found to be a mixture containing tangeretin, 3,5,6,7,8,3′,4′-heptamethoxyflavone, and at least one other compound. An aliquot was rechromatographed on a Magnesol (industrial regular) column developed with a benzene–ethyl acetate (3 to 1) mixture. The progress of the zones down the column was followed by their appearance under long-wavelength ultraviolet light and six subfractions were taken accordingly. From the weights of the aliquot subfraction residues and the total weight of the whole fraction, the subfraction percentages were calculated to the same total recovery basis as the main fractions and are listed with them in Table I.

Results and Discussion

When the tabular data (Table I) are summarized by adding the recovery percentages of each substance found, the results are tangeretin, 6.5; 3,5,6,7,8,3′,4′-heptamethoxyflavone, 6.2; tetra-*O*-methylscutellarein, 8.7; nobiletin, 36.0; sinensetin, 23.2; unidentified, 19.4.

The fruit on which the above data were obtained was processed in a central Florida plant early in January. Midseason fruits such as Seedling and Pineapple oranges usually predominate at this time, but it cannot be claimed that only these varieties were being processed nor that the season of harvest was especially typical. However, fruit to be utilized must pass legal standards of maturity. Presumably the proportions of the flavones in the neutral fractions would differ with variety, maturity, seasonal variations, and cultural practices. In this connection, enough work has been done on a thin-layer chromato-

Table I. Summary of Analytical Data on Neutral Fraction

Fraction	Vol. of Eluate, Liters	% of Total Recovery	Constituent	Method of Identification[a]
1	3	0.8	Not identified	...
2	41	1.5	Most tangeretin	UV
3	13	3.5	Tangeretin	Mixed m.p. and UV
4	17	1.4	Not identified	...
5	13			
		4.1	A. Not identified	...
		1.5	B. Impure tangeretin	UV
		6.2	C. 3,5,6,7,8,3′,4′-Heptamethoxyflavone	Mixed m.p. and UV
		6.9	D. Tetra-*O*-methylscutellarein	UV and IR
		1.8	E. Tetra-*O*-methylscutellarein	UV and IR
		1.1	F. Not identified	...
6	17	29.6	Nobiletin	Mixed m.p. and UV
7	33	4.7	Nobiletin	UV
8	37	1.0	Not identified	...
9	19	1.7	Nobiletin	Mixed m.p. and UV
10	51	11.2	Sinensetin	UV
11	97	12.0	Sinensetin	Mixed m.p. and UV
12	17	1.2	Not identified	...
13	3 (MeOH)	9.8	Not identified	...

[a] In addition to identification methods listed, each flavone was compared to an authentic sample chromatographically, usually on silica gel plates using a hexane-butanol solvent mixture.

graphic analytical method to make the qualitative statement that all the flavones listed have been found in neutral fractions throughout one entire season.

As far as the author knows, this is the first systematic attempt at analysis of such a fraction. It is hoped to devise a more practicable method of analysis and to apply it in further studies. Aside from the general qualitative and quantitative information presented in the present paper, this work led to the isolation of tetra-*O*-methylscutellarein (*5*). It is believed that this is the first isolation of this substance from natural sources, although it has long been known as a derivative of similar flavones. Tangeretin had not heretofore been known to occur in oranges. The other flavones mentioned have been found in this fruit and information concerning them has been summarized by Horowitz (*2*).

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BROWNING IN FRUITS

Nonvolatile Acids of Prunes

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Some nonvolatile acids of ripe prunes of the Italian, French, and Sweet Italian varieties were determined tentatively. Water extracts were fractionated by ion-exchange chromatography and the component acids identified on paper chromatograms. Quantitative data were obtained by titration of the acid fractions. Malic acid was the main acid of Italian and French prunes, but Sweet Italian prunes contained almost equal amounts of quinic acid. Traces of citric and, tentatively, benzoic acid were present in each variety. Phosphoric acid and possibly two isomers of chlorogenic acid were also identified.

Italian prunes (*Prunus domestica*), when harvested and marketed as fresh fruits, are commonly rejected because of a physiological disorder which has been arbitrarily called internal browning. This investigation is not related to the problem directly but was designed to be a comparative study of three varieties of prunes, in order to obtain more basic information about these fruits.

The acids of plums have been extensively studied. Prunes, however, have been studied only occasionally and a comprehensive study is not yet to be found.

Allen (*1*) observed that in plums the organic acids become localized in the flesh near the skin and around the pit, this condition becoming much more noticeable as the fruit approaches maturity on the tree.

Three isomers of chlorogenic acid were found in the prune, Imperial Epineuse, the neochlorogenic form being the most abundant, but caffeic acid was not detected (*18*). Mrak (*12*), however, found no chlorogenic acid but 0.30% caffeic acid in dried prunes. The presence of quinic acid (1%) and traces of benzoic acid have also been reported (*9*).

Procedure

Ripe fruits of Italian, French, and Sweet Italian prunes were harvested in 1963. Samples were destoned, chopped, and frozen. Later, each sample was allowed to thaw and the organic acids were extracted from 100 grams of tissue according to the methods of Dostal (*5*) and Markakis (*10*). No attempt was made to determine oxalic acid. Aliquots of each extract were titrated with standard NaOH to determine the acid content.

For fractionation, an aliquot equivalent to 1 meq. of the extract was put through a 20 × 0.7 cm. column of Dowex 1-X8 resin (100- to 200-mesh, acetate form) followed by 10 ml. of water. The gradient elution system consisted of two acid reservoirs connected to a 150-ml. mixing flask by a three-way stopcock. Nitrogen gas, at a pressure of 5 p.s.i., was used to maintain a steady rate of flow, and a magnetic stirrer ensured thorough mixing. The initial eluent was 75 ml. of 4.5*N* acetic acid, followed by 150 ml. of 8*N* acetic acid, 50 ml. of 8*N* formic acid, and 70 ml. of 10*N* formic acid. Several other elution systems were experimented with, but these concentrations gave the best resolution of our extracts. Sixty fractions of 5.7 ml. each were collected, using an automatic fraction collector.

The fractions were evaporated to dryness in vacuo at 40° C. and dissolved in 1 ml. of hot water. One milliliter of 0.002*N* NaOH, containing 0.01% phenolphthalein indicator, was added to each tube, using an automatic pipet (*6*). This served as a base line for titration, and those fractions which remained colorless (indicating the presence of acid) were titrated to the phenolphthalein end point with 0.01*N* NaOH. After titration, the fractions were dried in vacuo at 40° C. for qualitative determination by paper chromatography. The residues were dissolved in 0.5 ml. of 50% ethanol and 0.1 ml. of an aqueous slurry of Dowex 50-X8 resin (H^+ form, 50- to 100-mesh) was added to each fraction to remove excess sodium ions. The dissolved fractions were spotted on Whatman No. 1 16- × 46-cm. sheets, 2 cm. apart, and 5 cm. from the short edge of the sheet. The papers were held in a chromatography cabinet for

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