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Potential of Oxidised Phenolics as Food Colourants

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ABSTRACT

A range of phenolic compounds was oxidized using the enzyme polyphenoloxidase (E.C. 1.14.18.1). Mixtures of phenolics and mixtures of phenolics with amino acids and peptides were also oxidised. The oxidised products exhibited red, yellow, blue, green and orange colours but many of them turned to brown or black as further reactions occurred. The colours were measured in terms of Hunter L,a,b coordinates and the stability of the colours to heat, pH and sulphur dioxide was measured. The majority of colours had insufficient stability or tinctorial power to be considered for use as food colourants. The exceptions were the orange products formed on oxidation of catechin and the yellow products from dihydroquercetin.

INTRODUCTION

The enzyme polyphenoloxidase is widely distributed in plants and is responsible for enzymic browning when fruits and vegetables are damaged as well as the flavour and colour development during the fermentation of tea. Its ability to form a red colour when tyrosine is oxidised or when catechol is oxidised in the presence of amino acids has been recognised for many years (Horowitz, Fling & Horn, 1970; Platt & Wormall, 1927). The formation of coloured products when catechin was the substrate has also

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been documented (Hathway & Seakins, 1957; Brown & Whiteoak, 1964). There have been a number of reviews on the enzyme (see especially Mason, 1955) which have detailed the reactions of the quinones with other naturally occurring molecules to give coloured products, and there have been suggestions that such colours may be useful for colouring food (Kharlamova & Kafka, 1981; Bokuchava *et al.*, 1978; Craven *et al.*, 1981).

The enzyme catalyses two reactions: the ortho hydroxylation of aromatic nuclei already containing one hydroxyl group and the further oxidation of ortho dihydroxy groups to *o*-quinones. In this latter form a number of reactions can occur. Dimerisation or polymerisation is common as is reaction with amino or sulphydryl compounds. Colours form when the double bond structure becomes sufficiently conjugated to absorb in the visible region of the spectrum and this depends on the type of phenolics present.

While there is information on the chemistry of these systems and their relation to biological processes such as sclerotisation of insect cuticles little is known about the properties of the colours especially their potential for use as food colourants. The purpose of this paper was to identify which of the selected phenolic compounds produced coloured oxidation products and to determine the suitability of each colour for food use by a series of simple tests. Basic criteria such as pH sensitivity, heat stability, reaction with sulphur dioxide and tinctorial power were used to screen the compounds. Phenolics were oxidised by themselves, mixed with other phenolics and mixed with some amino acids and peptides.

MATERIALS AND METHODS

Materials

Reagents were analytical grade. Amino acids, tyrosinase and gelatin and casein hydrolysates were obtained from Sigma Chemical Company, St Louis, MO, USA. Phenolic compounds were purchased in the USA as follows:

Catechin	Sigma Chemical Company
Catechol, resorcinol, gallic acid	Fisher Scientific Company Fairlawn, NJ
Phloroglucinol	Merck & Co., Rahway, NJ
Protocatechuic acid	Pflatz & Bauer, Stanford, CT
Quercetin	Eastman Kodak, Rochester, NY
Dihydroquercetin, Hyperoside	K & K, Plainview, NJ
Caffeic acid	Aldrich Chem. Co. Milwaukee, WI

Apart from quercetin and hyperoside (quercetin-3-galactoside) which were yellow in solution, the other phenolics were colourless. Cyanidin-3-glucoside was extracted from blackberries and partially purified by chromatography on Amberlite CG-50 after the method of Fuleki & Francis (1968). The concentration of the extracted anthocyanin was calculated using the molar absorption coefficient for cyanidin-3-glucoside (25, 740; Francis, 1982) and spectrophotometry of a diluted solution.

Methods

Assays were performed at pH 6·5 in phosphate buffer (0·023 M) and at a substrate concentration of 1 mM. Total volume was 6·5 ml and each assay contained 2000–3000 U (expressed as polyphenoloxidase units) of enzyme. Tubes were incubated at room temperature (20°C). Typically an assay contained phosphate buffer (0·05 M, 3 ml), enzyme (1 ml), substrate (6·5 mM, 1 ml) and water (1·5 ml). Poorly soluble compounds such as catechin were dissolved in the 3 ml of buffer as a 2·16 mM solution and additional water added to maintain the volume at 6·5 ml. Enzyme was added to the assays last, the tubes were mixed and colour development observed.

Colour measurement

Values for the Hunter coordinates were obtained on a Gardner XL-23 colorimeter (Gardner Laboratory Inc., Bethesda, MD, USA) using the transmittance accessory and zeroed on a distilled water blank to L = 100, $a = 0$, $b = 0$ in a 1 cm path length cell (volume 9 ml).

Spectral measurements

Spectra were recorded on a double beam spectrophotometer (Lambda 3; Perkin Elmer, Norwalk, CT, USA).

Stability test

Heat
After measuring the colour at 24 h, tubes were immersed in a boiling water bath for 15 min where they achieved an internal temperature of 75°–80°C. After cooling, the colour was measured again and any precipitation or turbidity noted.

pH
Portions (1 ml) of the assay were taken after heating and added to 5 ml of citric/phosphate buffer (0·05 M) at pH 2·6, 3·6, 5·0, 6·3 and 8·0. A further

TABLE 1
Hunter Values for Oxidised Phenolics during Reaction and after Heating

Substrate	*0·25 h*[a]			*24 h*[a]			*After heating*[b]		
	L	*a*	*b*	L	*a*	*b*	L	*a*	*b*
Catechol	82·8	−1·4	24·1	58·0	7·0	19·2	59·8	6·5	20·4
Catechin	73·3	38·5	49·5	60·4	43·7	40·8	61·2	31·3	40·7
Caffeic acid	83·4	7·6	18·2	75·0	6·4	21·6	65·1	8·8	26·0
Dihydroquercetin	84·7	1·9	42·5	69·7	6·6	40·6	81·7	−2·3	34·9
Cyanidin-3-glucoside	62·0	21·0	4·9	56·4	10·7	23·6	48·0	12·2	21·9
Protocatechuic acid	97·6	1·9	12·5	82·4	8·9	15·1	84·7	5·3	17·0
Gallic acid	97·5	−0·2	1·2	74·5	−1·8	18·2	69·1	1·8	17·3

[a] Mean of five determinations.
[b] Mean of two determinations.

portion was added to 5 ml of pH 6·3 buffer containing 100 ppm sulphur dioxide. The resulting colours were measured on the Gardner XL-23.

Tinctorial power

The L,*a*,*b* values were compared with those designated as acceptable (Taylor & Clydesdale, 1987).

Inhibitor studies

Ascorbic acid and sulphur dioxide were used to inhibit the reaction using the conditions recommended by Augustin *et al.* (1985). Inhibitor solution was added to the assays 15 min after addition of enzyme to give a concentration of 0·4 mM. The samples were then heated as described previously to inactivate the enzyme.

RESULTS AND DISCUSSION

Single compounds

Using the assay conditions described, the phenolic compounds were incubated with polyphenoloxidase and the colour measured at 0·25 and 24 h (Table 1). The simple phenolics which contained no *o*-dihydroxy group, namely, resorcinol, phloroglucinol and tyrosine, did not react in this system. Subsequent experiments with tyrosine showed that a detectable reaction took place only with higher levels of enzyme.

Although both quercetin and hyperoside contained *o*-dihydroxy groups, the colour measurements (and scanning spectra of control and test samples) indicated that oxidation had not occurred. Dihydroquercetin, however, was readily oxidised to a yellow compound and thus it appears that the double bond in the middle ring affects the reactivity of these molecules with regard to polyphenoloxidase. In the dihydro form, the two planar aromatic rings (A and B) are 'kinked' round the middle ring. When a double bond is introduced, the whole molecule becomes planar and this change in shape, coupled with the requirements of the enzyme's active site, may be responsible for the different reactions.

In that case, the anthocyanin would not be expected to react although the values in Table 1 indicate a slow reaction as the initial purple/red colour changed to a more brown colour. This apparent contradiction was explained by the impure nature of the cyanidin-3-glucoside used. It has been reported that pure anthocyanins will not react with polyphenoloxidase but, in the presence of trace amounts of phenolics, indirect oxidation occurs readily

(Eskin, 1979). The purification process used was not sufficiently rigorous to free the anthocyanin from all traces of phenolics and it is these that cause the apparent reaction.

Gallic acid reacted slowly to give a pale green colour after 24 h but control tubes containing no enzyme also gave the same colour showing the oxidation was non-enzymic.

The remaining compounds were oxidised by the enzyme to give coloured compounds. In the case of catechol, a light yellow colour formed after 15 min but after 24 h a brown colour was present. This was due to the quinone dimerising and then polymerising with a concomitant shift in the visible spectrum. Neither colour was particularly strong although the brown colour was quite dark (L = 58·0). With catechin, colour development was obvious from the first addition of enzyme and the bright, orange colour became more saturated with time due to the formation of dimer (Hathway & Seakins, 1957). Caffeic acid was oxidised to a brown compound but the colour was light as evidenced by the high L values at 15 min and 24 h. Protocatechuic acid also gave a light colour which was reminiscent of rose wine. Dihydroquercetin which was colourless at the start, was rapidly oxidised to a bright yellow compound which persisted at 24 h. The colour changes observed with cyanidin-3-glucoside were associated with the oxidative degradation of the molecule and the original red/purple colour turned to brown at 24 h.

When the assays were heated, cooled and the colour remeasured, the results shown in the third column of Table 1 were obtained. There was little change in the colours of the catechol, caffeic acid, cyanidin-3-glucoside, protocatechuic acid and gallic acid assays. The redness of the catechin colour was reduced (43·7–31·3) and the lightness of the dihydroquercetin colour increased (69·7–81·7).

The colours were assessed at this stage to determine whether they possessed sufficient tinctorial power to be of use as food colourants. Hunter values were compared with the 'acceptable region' defined by Taylor & Clydesdale (1987). Of the colours formed, only those of catechin and dihydroquercetin were 'acceptable' in the aqueous model system, while cyanidin-3-glucoside was marginal although in the unattractive brown region.

The pH and sulphur dioxide sensitivity of all the colours were measured but only those pertaining to catechin and dihydroquercetin are reported here (Table 2). They reflect the general trend of these oxidation products in that sulphur dioxide has a negligible effect on colour while pH causes changes in L values and some individual *a* and *b* values. The compounds all darken as the pH rises from 2·6 to 8·0 although to different extents. These

TABLE 2
Hunter Values for Catechin and Dihydroquercetin Oxidation Products at Different pH Values and with Sulphur Dioxide

pH	*Catechin*			*Dihydroquercetin*		
	L	*a*	*b*	L	*a*	*b*
2·6	91·1	−3·1	24·7	95·3	−0·5	11·2
3·6	90·9	−2·9	25·9	95·1	−0·9	12·5
5·0	89·3	−1·2	26·4	93·8	−0·9	14·8
6·3	87·9	1·6	29·0	93·9	−0·5	15·3
8·0	86·7	2·4	38·2	91·6	0·1	18·1
6·3 + SO_2	91·5	0·6	26·7	95·5	−0·7	12·0

Values are means of duplicate determinations.

tests involved dilution of the original assays and values in Tables 1 and 2 are not directly comparable.

Catechol mixtures

From the previous experiment it was clear that catechol was easily oxidised in the assay system. Mixtures of catechol with other phenolics (in equimolar proportions) were reacted in the system to study the effect of one oxidised phenolic species on another. The Hunter values are shown in Table 3.

The colours produced suggested that, in some cases, true interaction had taken place rather than two colours combining to make a different shade. For example, the catechol/dihydroquercetin mixture was initially blue, whereas the two compounds had both given yellow colours at the same time interval when reacted singly with the enzyme.

The values in Table 3 indicate that the initial reaction which gave rise to some attractive colours at 15 min continued slowly and generally produced 'dirty' colours after 24 h. Resorcinol, catechin and hyperoside initially formed a green colour which then turned green/brown for the first two compounds and yellow/green for the hyperoside mixture. Blue was the initial colour produced by phloroglucinol and dihydroquercetin but these gave way to blue/green and green shades respectively at 24 h. Protocatechuic and gallic acids gave yellow colours similar to that of catechol at 15 min and the brown colour of the former compound at 24 h was again comparable with the catechol situation, but the gallic acid sample remained yellow at 24 h and turned brown only on heating. Caffeic acid and quercetin formed unattractive brown colours while cyanidin-3-glucoside was black initially

TABLE 3
Hunter Values for Colours formed with Catechol

Substrate	*0·25 h*			*24 h*			*After heating*		
Catechol plus:	L	*a*	*b*	L	*a*	*b*	L	*a*	*b*
Nothing	82·8	−1·4	24·1	58·0	7·0	19·2	59·8	6·5	20·4
Resorcinol	76·0	−7·3	21·7	75·1	−0·5	17·8	81·5	3·1	17·7
Phloroglucinol	61·2	−10·3	1·0	41·7	−6·0	10·5	55·6	−1·8	15·6
Protocatechuic acid	89·3	−3·3	25·5	71·2	4·5	17·1	69·7	5·0	18·1
Gallic acid	75·2	0·7	26·5	68·8	5·0	30·5	58·3	10·3	23·0
Caffeic acid	79·9	3·0	17·3	73·3	2·4	18·9	64·2	4·4	20·6
Catechin	42·0	−6·3	18·9	23·4	1·8	12·1	32·7	4·4	17·6
Quercetin	74·5	−1·2	14·0	60·1	4·0	17·4	63·6	5·0	21·1
Dihydroquercetin	26·0	−3·5	−8·1	37·3	−0·2	11·8	68·4	−3·4	26·1
Hyperoside	62·7	−11·2	25·8	60·4	0·4	24·1	64·9	2·3	26·0
Cyanidin-3-glucoside	25·1	4·4	1·0	27·6	11·4	14·5	34·8	14·6	18·5

Values are means of duplicate determinations.

and then browned. The heating regime had little effect on the final colours: only the catechol/dihydroquercetin mixture showed a large thermal change with the colour turning from green to yellow.

At the concentrations used, only the catechol/gallic acid sample had Hunter coordinates in the 'acceptable' region of the colour solid and this colour faded on heating. Stability tests on the heated colours showed little variation with pH and negligible changes in the sulphur dioxide solution.

Attempts were made to control the reaction and retain the initial attractive colours by adding inhibitors of the reaction (ascorbic acid and sulphur dioxide) and then inactivating the enzyme by heat. The catechin/catechol and dihydroquercetin/catechol mixtures were studied. In the former case, an olive green colour was observed after 15 min. Addition of ascorbic acid rendered the tubes colourless while sulphur dioxide turned them a light orange colour. The effects of ascorbic acid were reversible as some colour reappeared after standing for 24 h but the sulphur dioxide-treated tubes remained light orange/yellow.

The dihydroquercetin/catechol mixture was blue/black initially but ascorbic acid bleached it to a faint straw colour while sulphur dioxide changed it to pale yellow. Again ascorbic acid had reversible effects while sulphur dioxide was irreversible.

These experiments showed that mixtures of phenolics did react together when oxidised by polyphenoloxidase. Under the conditions used, the initial attractive colours were often replaced by muddy browns after 24 h and the use of different ratios of phenolics or more complex mixtures may prove useful. Similarly, more refined attempts to halt the reaction in the initial stages may give more attractive colours.

Catechin mixtures

Catechin was reacted in equimolar ratio with the other phenolics and the colours observed. Whereas catechol had produced a range of colours, catechin gave rise to orange colours with all but gallic acid, cyanidin-3-glucoside and, as noted above, with catechol. These results suggest that the predominant reaction is still catechin dimerisation although the possibility of other interactions cannot be ruled out. The values in Table 4 are all similar and the trend is for the a values to be lower in the mixtures than when catechin is reacted alone. All the colours after heating were 'acceptable' with the exception of the catechin/cyanidin-3-glucoside sample, and all showed similar behaviour in the pH test: the samples became darker (L values decreased) and yellower (b value increased) as the pH was raised from 2·6 to 8·0. Sulphur dioxide had a negligible effect on the colours at pH 6·3. Further studies are required to determine the exact nature of the compounds formed.

TABLE 4
Hunter Values for Catechin Mixtures

Substrate	*0·25 h*			*24 h*			*After heating*		
Catechin plus:	L	*a*	*b*	L	*a*	*b*	L	*a*	*b*
Nothing	73·3	38·5	49·5	60·4	43·7	40·8	61·2	31·3	40·7
Resorcinol	91·9	2·7	34·8	74·7	29·7	50·3	78·0	11·9	47·8
Phloroglucinol	76·4	30·7	51·6	69·3	35·9	46·9	72·8	17·7	47·6
Gallic acid	74·5	0·4	16·0	74·2	1·8	32·5	64·5	8·4	30·7
Caffeic acid	78·3	23·6	46·9	73·2	27·3	47·6	73·6	10·8	39·3
Protocatechuic acid	69·7	29·3	46·8	57·5	32·0	38·8	66·5	15·4	41·8
Quercetin	72·7	34·4	49·1	64·5	35·8	43·6	66·6	23·1	43·1
Dihydroquercetin	75·7	32·1	51·0	65·1	34·5	43·9	69·0	13·8	42·7
Hyperoside	75·4	28·5	50·4	60·0	36·6	40·5	63·0	25·9	41·1
Cyanidin-3-glucoside	48·8	11·9	21·6	30·1	22·0	19·6	25·1	15·6	16·0

Values are means of duplicate determinations.

Dihydroquercetin mixtures

The mixtures were made up as above and all (with the exception of the catechol and catechin samples discussed previously) gave yellow colours after 24 h. Again all bar the resorcinol and gallic acid colours were 'acceptable' and the products demonstrated the same behaviour as the catechin products when subjected to the pH and sulphur dioxide tests.

Mixtures with peptides and amino acids

Catechol, catechin, dihydroquercetin and gallic, caffeic and protocatechuic acids were mixed in equimolar proportions with PRO, hydroxyPRO, LYS, ALA, GLU, ARG, CYS, urea and gelatin and casein hydrolysates in the assay system. The reaction of phenolics with amino acids and their derivatives is well documented (Mason, 1955) and more recently, Igarishi *et al.* (1982) reported the formation of a green colour when quercetin and cysteine ethyl ester were oxidised non-enzymically.

Catechin gave the typical orange colour with all the mixtures. The Hunter values and colours of all samples after boiling were identical with the control sample containing catechin alone. Only the sample containing cysteine showed a difference, being less yellow (lower *b* value) and therefore appearing redder than the other samples.

Catechol formed a number of brightly coloured products with these compounds. Proline and hydroxyproline produced deep purple colours but they were destroyed on heating and an orange/brown colour resulted. LYS, ALA, GLU, ARG, gelatin and casein hydrolysate all gave orange/brown colours which lightened on heating. CYS and urea formed very light colours which were heat-stable.

Gallic acid samples reacted slowly, being colourless at 15 min. They all showed an olive green colour at 24 h which browned on heating. Caffeic acid also produced a heat-stable light brown colour with all the samples and protocatechuic acid samples were all rose. All dihydroquercetin samples were yellow with comparable values to the control. The addition of CYS caused a lag phase in the reaction which was overcome by heating and urea had no effect on colour production despite its amino groups.

Thus, only catechol reacted with the amino acids to give novel coloured compounds. These proved to be relatively unstable and not suitable for food use. The well-known tanning properties of certain phenolics indicated that, under the correct conditions, stable coloured compounds can form. Of all the colours formed in this section, only the catechin and dihydroquercetin mixtures produced 'acceptable' colours and the pH and sulphur dioxide stability of these were identical to that reported above.

CONCLUSION

Even in the simple system used in this paper, the oxidation of phenolics by polyphenoloxidase produced a wide range of colours. With different proportions in the mixtures, possibly more colours could be formed. The purpose of this study was to identify materials with potential for colouring food and the rudimentary stability tests allowed rapid screening of a large number of samples and gave basic information on the suitability of each sample. On the whole, the products were tolerant of pH and sulphur dioxide. The tinctorial power of individual colours was assessed by comparison with the colours that exist in model food systems at typical usage levels. In the case of beverages, synthetic colour usage is typically 75 ppm whereas, in the assays, the 1 mM substrate concentration is equivalent to 110 ppm for the simple phenolics and about 300 ppm for the flavonoids. The latter compounds approach their limit of solubility around 500 ppm and, apart from this consideration, it was thought that compounds that do not give acceptable colours between 100 and 300 ppm would probably be unsuitable for general food use.

The above findings confirm and extend previous reports of coloured phenolic oxidation products. With the exception of the catechin and dihydroquercetin products, however, none of the colours produced was suitable for consideration as a food colourant due to poor tinctorial power and/or poor stability. Although only the technical problems associated with these compounds have been studied, the authors acknowledge the considerable legislative problems that their use as pure compounds would entail.

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REFERENCES

Augustin, M. A., Ghazali, H. M. & Hassim, H. (1985). Polyphenoloxidase from guava. *J. Sci. Food Agric.*, **36**, 1259–65.

Bokuchava, M. A., Pruidze, G. N. & Ul'yanova, M. S. (1978). Biochemistry of the production of natural colourings of vegetable origin. *Food Sci. Technol. Abs*, **10**, 6T224.

Brown, B. R. & Whiteoak, R. J. (1964). Polymerisation of flavans. Part VII: Oxidative polymerisation of catechin. *J. Chem. Soc.*, 6084–90.

Craven, M. R., Ledward, D. A. & Taylor, A. J. (1981). The production and analysis of food colourants derived from vegetable material. *J. Sci. Food Agric.*, **32**, 847–8.

Eskin, N. A. M. (1979). *Plant pigments, flavors and textures: the chemistry and biochemistry of selected compounds*, New York, Academic Press, 28–42.

Francis, F. J. (1982). Analysis of anthocyanins. In: *Anthocyanins as food colors* (Markakis, P. (Ed.)), New York, Van Nostrand, 182–207.

Fuleki, T. & Francis, F. J. (1968). Quantitative methods for anthocyanins. 3: Purification of cranberry anthocyanins. *J. Food Sci.*, **33**, 266–74.

Hathway, D. E. & Seakins, J. W. T. (1957). Enzymic oxidation of catechin to a polymer structurally related to some phlobotannins. *Biochem. J.*, **67**, 239–45.

Horowitz, N. H., Fling, M. & Horn, G. (1970). Tyrosinase. In: *Methods in enzymology, XVIIa* (Tabor, H. & Tabor, C. W. (Eds)), New York, Academic Press, 615–32.

Igarashi, K., Furukawa, Y., Arai, H. & Yasui, T. (1982). Formation of green pigment by reaction of quercetin with cysteine ethyl ester. *Agric. Biol. Chem.*, **46**, 3089–91.

Kharlamova, O. A. & Kafka, B. U. (1981). Natural food colourant. *Food Sci. Technol. Abs*, **13**, 10T558.

Mason, H. S. (1955). Comparative biochemistry of the phenolase complex. In: *Advances in enzymology, 16* (Nord, F. F. (Ed.)), New York, Interscience, 105–84.

Platt, B. S. & Wormall, A. (1927). A note on plant oxidation: the nature and reactions of the substance 'Tyrin'. *Biochem. J.*, **67**, 239–45.

Taylor, A. J. & Clydesdale, F. M. (1987). Assessment of tinctorial power of food colourants. *Food Chem.*, in press.