



Assessment of relative reduction of sugars, UV- absorbance and browning intensity of caramelized products (CPs)

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Abstract

The increased degradation of all sugars was observed as the heating time increased. The rate of sugar degradation was much greater under alkaline pH conditions, as compared to neutral pH. Among all of the sugars tested, glucose was degraded to a smaller extent, compared to the other. It was determined that the rate of degradation was dependent upon pH and the type of sugar involved. Regardless of sugar type, UV-absorbance of CPs prepared under alkaline conditions increased to a higher extent, as compared to those prepared at neutral pH. The results suggested that different intermediate products with different absorbance maxima were formed for different sugars. CPs from glucose had the lowest increase in absorbance at both 205 and 285 nm. Different patterns of changes in the UV-absorbance of CPs prepared under alkaline conditions were noted, as compared to those found under neutral conditions. Sharp increases in UV-absorbance at both 270 and 285 nm were found in all sugars within the first 60 min of heating. Continuous increases in absorbance at 270 nm were observed in glucose and D-Glucose heated up to 150 min, although no changes in absorbance at 285 were observed. D-Glucose showed the highest rate of degradation with the concomitant accumulation of intermediate products. Under neutral conditions, pH 7, both absorbance ratios (A₂₀₅/A₄₂₀ and A₂₈₅/A₄₂₀) for Dextrose, xylose and D-Glucose solutions increased within the first 10 min of heating with the development of browning. Conversely, no browning was formed with glucose solutions until 30 min of heating. The ratios for D-Glucose reached a maximum after 20 min of heating, whereas glucose and Dextrose solutions gave maximum ratios after 60 and 30 min of heating, respectively. For xylose, the highest A₂₀₅/A₄₂₀ and A₂₈₅/A₄₂₀ ratios were observed after 30 and 60 min, respectively. The increased absorbance ratios suggested that the intermediates were generated to a larger extent with lower transformation to brown polymers. From the result, it was suggested that the formation of brown polymers from different intermediates varied with the type of sugar. It is suggested that the caramelization occurred to a larger extent under alkaline conditions and that it depended upon type of sugar. Generally, hexose sugars, both glucose and D-Glucose, were more prone to caramelization than pentose, Dextrose and xylose as evidenced by the greater browning as well as intermediate format

Introduction

Caramelization is a type of non-enzymatic browning which is the result of thermal degradation of sugars at high temperature i.e. above 80°C at a pH range of 3-9 in the absence of amino acids (Davies and Labuza, 2005). Caramel coloring is one of the oldest and most widely-used food colorings and is found in almost every kind of industrially produced food, including: beer, brown bread, buns, chocolate, cookies, brandy, chocolate flavored flour-based confectionery, coatings, decorations, fillings and toppings, chips, dessert mixes, doughnuts, fish and shellfish spreads, frozen desserts, glucose tablets, cough drops, gravy browning, ice cream, jams, milk desserts, pancakes, pickles, sauces and dressings, soft drinks (especially colas), stouts, sweets This importance of caramels is due to there stabilizing, emulsifying, free radical scavenging, antimicrobial and anti-oxidative properties (El-Hassan et al., 2008). The process of caramelization produces large mixture of many species, which make up flavor, fragrance and color but 5-

Hydroxymethyl furfural (HMF) is the principal degradation product in caramel (Lamberts et al., 2008). Caramelization of various carbohydrates leads to a product with a high tinctorial strength provided by different additives catalyzing the process. As the caramel is a food additive, the catalysts for its manufacture under go regulations by food laws (Powrie et al., 2005). Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, where as natural antioxidants of plant region are classified as vitamins, phenolic compounds, or flavonoids (Osada, and Shibamoto, 2006).

Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant source food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. The main characteristic of an antioxidant is its ability to trap free radicals (Powrie et al., 2005). Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease (Yen & Lai, 2003).

Caramelization products (CPs) contain volatile and nonvolatile fractions of low and high molecular weights. CPs have shown antioxidant activity as acetone extracts from the glucose reduced the peroxide values of soybean and that from the D-Glucose inhibit lipid oxidation (Manisankar et al., 2004).

pH plays an important role in the strength of antioxidant activity (AO) of CPs as alkaline pH was found to be good for AO. And CPs from hexose sugars showed greater AO than that from pentose sugars (Jiang et al., 2008). There has been a growing interest in the search for natural antioxidants for the public perception that natural and dietary antioxidants are safer than synthetic analogues. Caramel color is also widely used in Pakistan but it is to be imported from the developed countries and thus it was the need of the time to utilize local resources and expertise to produce good quality caramel at an economical cost to be used as color, flavor and antioxidant additive. Thus the present study was designed to produce caramel having high tinctorial strength and high antioxidant activity by utilizing local resources to minimize cost for the commercial use.

Materials and Methods

Chemicals:

D- Glucose, potassium ferricyanide, ferric chloride, di-sodium phosphate, mono sodium phosphate trichloroacetic were purchased from Merck. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from sigma. Dextrose and liquid glucose were kindly supplied by Rafan maize products Faisalabad, Pakistan while sucrose was supplied by the Crescent Sugar mills Faisalabad, Pakistan.

Preparation of Caramelization products (CPs):

Solutions of sugars were prepared by mixing with 0.05 M phosphate buffer of a pH range of 3-9. 10 ml of each sugar solution was transferred to a screw-capped test tube and was subjected to heating for time duration range of 0-300 minutes. At the heating time designated the samples were taken out and cooled in ice water immediately and were stored at 4°C for further analysis (Benjakul *et al.*, 2005).

Ultraviolet absorbance and browning determination:

Ultraviolet absorbance was determined at wavelength of 270 and 285 nm by using UV-1601 spectrophotometer while browning intensity of the CPs was measured by the spectrophotometer at A_{420} (Benjakul *et al.*, 2005). Appropriate dilution was made for all the samples using distilled water. The absorbance showed the browning intensity of the caramelization products.

Results and discussion

The loss of sugars:

The increased degradation of all sugars was observed as the heating time increased ($P < 0.05$) (Fig. 1). The rate of sugar degradation was much greater under alkaline pH conditions, compared with at neutral pH. At pH 7, a slight decrease in sugar was found during the first 60 min of heating. Thereafter, sugars, especially D-Glucose and Dextrose, underwent more extensive degradation as evidenced by the marked decrease in reducing sugar content (Fig. 1.a). D-Glucose and Dextrose decreased to 73.5% and 75.4% after heating time for 150 min. At pH 10, sharp degradation was

observed in all sugars during the first 20 min of heating. Subsequently, sugars underwent degradation gradually up to 150 min. Among all of the sugars tested, glucose was degraded to a smaller extent, compared to the other. Higher levels of degradation of both D-Glucose and glucose occurred at 100 °C under alkaline conditions (Ajandouz *et al.*, 2001). After 180 min, Dextrose, D- Glucose, Liquid-Glucose and sucrose had degraded to 46.9%, 34.9%, 23.4% and 39.7%, respectively. From these results, it was determined that the rate of degradation was dependent upon pH and the type of sugar involved.

Table 1.a: Relative reduction of sugars with standard deviation at pH 3

Time (min)	% Relative concentration \pm SD			
	Dextrose	D- Glucose	L- Glucose	Sucrose
30	23 \pm 0.25	27 \pm 1.01	16 \pm 0.15	18 \pm 0.26
60	28 \pm 0.14	30 \pm 1.73	20 \pm 1.4	22 \pm 2.37
90	31 \pm 0.56	35 \pm 1.06	24 \pm 4.78	26 \pm 0.62
120	36 \pm 1.25	40 \pm 1.81	27 \pm 1.81	29 \pm 0.74
150	38 \pm 1.02	41 \pm 2.3	30 \pm 0.87	33 \pm 0.26
180	42 \pm 1.98	45 \pm 1.05	35 \pm 1.65	36 \pm 1.87

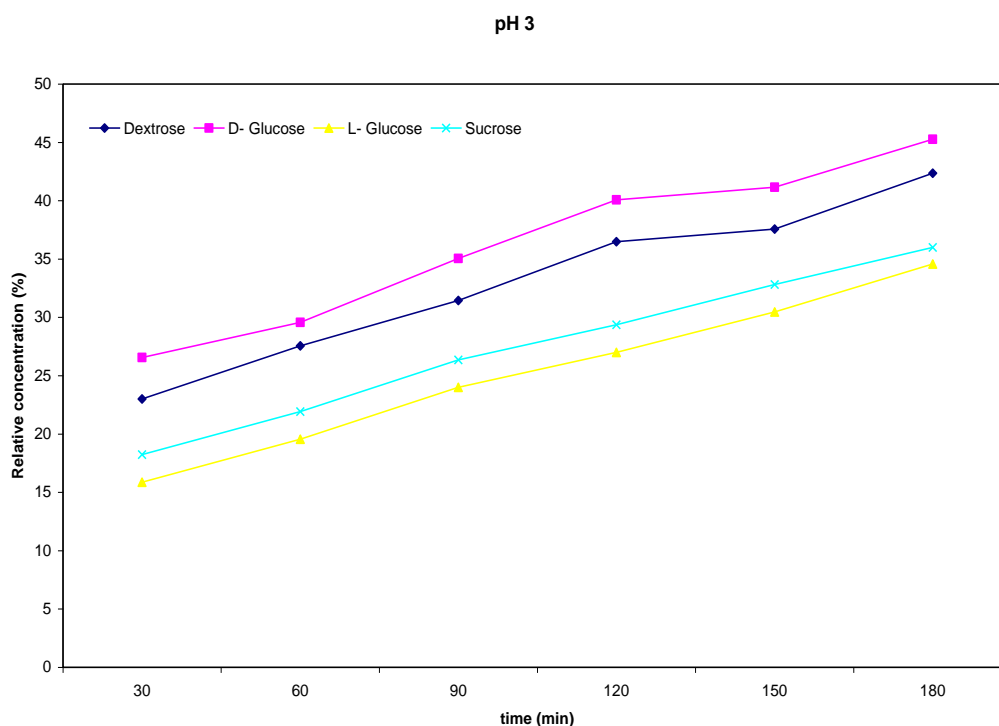


Figure (1.a)

Figure (1.a): Changes in reducing sugar contents of CPs from different sugars during heating at pH 3 for various times.

Table 1.b: Relative reduction of sugars with standard deviation at pH 7

Time (min)	% Relative concentration \pm SD			
	Dextrose	D- Glucose	L- Glucose	Sucrose
30	26 \pm 1.02	33 \pm 1.25	22 \pm 0.68	24 \pm 0.25
60	30 \pm 1.6	33 \pm 1.57	22 \pm 1.89	25 \pm 2.37
90	31 \pm 1.05	35 \pm 1.65	24 \pm 2.36	27 \pm 0.62
120	42 \pm 1.89	40 \pm 1.25	31 \pm 1.25	33 \pm 0.74

150	50±1.52	53±1.45	40±1.69	42±1.12
180	56±1.9	59±1.25	45±1.78	50±1.25

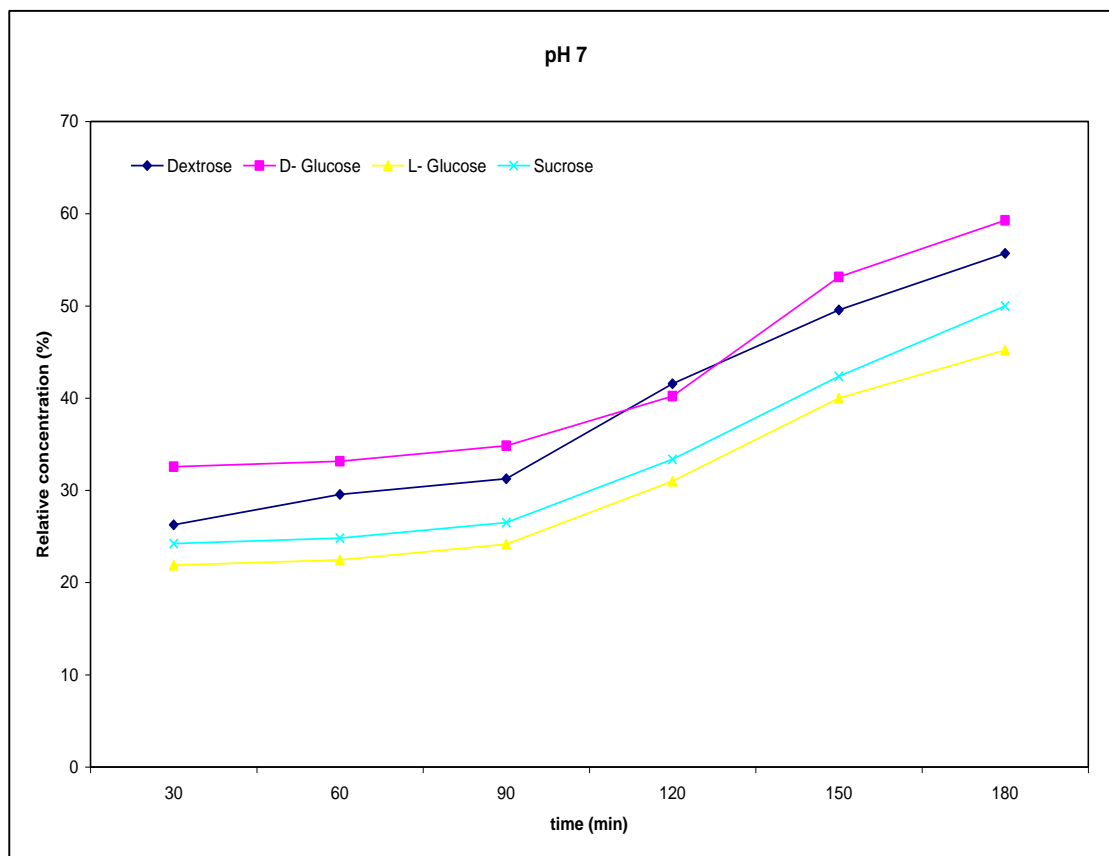


Figure (1.b)

Figure (1.b): Changes in reducing sugar contents of CPs from different sugars during heating at **pH 7** for various times.

Table 1.c: Relative reduction of sugars with standard deviation at **pH 10**

<i>Time (min)</i>	% Relative concentration ± SD			
	Dextrose	D- Glucose	L- Glucose	Sucrose
30	34±1.25	37±1.5	26±0.98	29±0.56
60	40±1.65	43±1.75	32±1.56	35±0.14
90	44±0.94	47±4.78	35±2.3	37±0.62
120	50±1.36	54±1.81	43±1.56	46±0.74
150	57±0.97	59±0.68	48±1.02	49±0.78
180	64±1.56	68±0.15	57±0.88	58±0.65

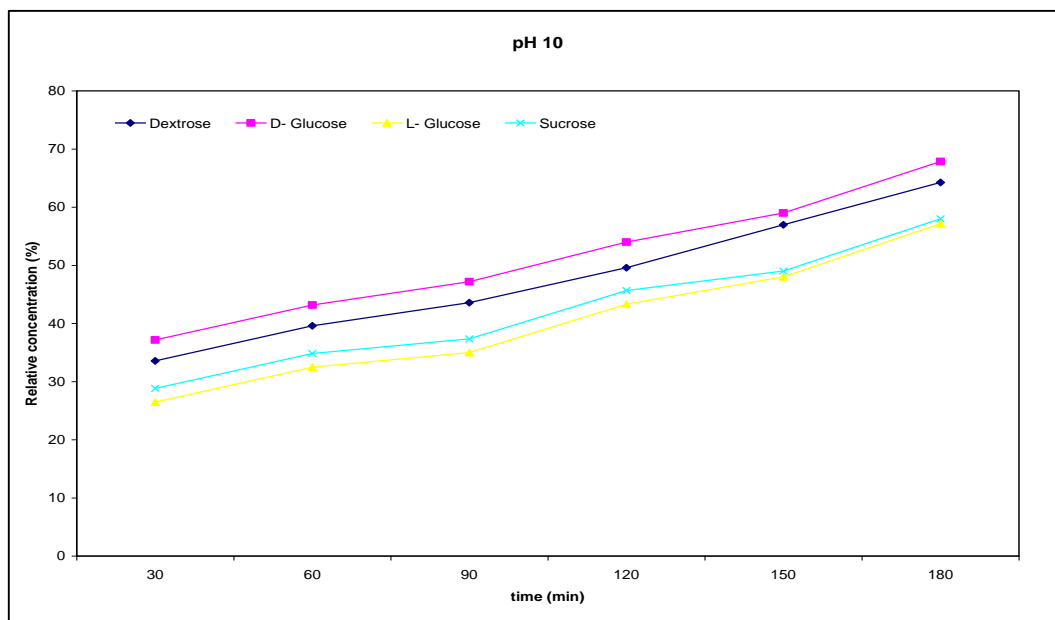


Figure (1.c)

Figure (1.c): Changes in reducing sugar contents of CPs from different sugars during heating at **pH 10** for various times.

UV- absorbance and browning of CPs:

UV-absorbance of CPs prepared from different sugars at pH 7 and 10 during heating is depicted in Fig. 2. Regardless of sugar type, UV-absorbance of CPs prepared under alkaline conditions increased to a higher extent, compared to those prepared at neutral pH. UV-absorbance was used to monitor the intermediate degradation products of nonenzymatic browning reactions (Ajandouz *et al.*, 2001). Under neutral conditions, UV-absorbance at both 205 and 285 nm of all sugars increased gradually as the heating time increased, and it was found that Dextrose showed higher UV-absorbance than the other sugars. The increase in absorbance at 205 nm due to CPs from xylose was higher than those from D-Glucose. Conversely, CPs from xylose showed lower absorbance at 285 nm than those from D-Glucose. The results suggested that different intermediate products with different absorbance maxima were formed for different sugars. CPs from glucose had the lowest increase in absorbance at both 205 and 285 nm. Different patterns of changes in the UV-absorbance of CPs prepared under alkaline conditions were noted, compared to those found under neutral conditions. Sharp increases in UV-absorbance at both 270 and 285 nm were found in all sugars within the first 60 min of heating. Thereafter, no marked changes in UV-absorbance at 270 and 285 nm were observed in Dextrose and xylose up to 150 min of heating. Continuous increases in absorbance at 270 nm were observed in glucose and D-Glucose heated up to 150 min, although no changes in absorbance at 285 nm were observed. From these results, the accumulation of intermediate degradation products was generally coincidental with the increase in sugar degradation (Fig. 1). D-Glucose showed the highest rate of degradation with the concomitant accumulation of intermediate products. Browning of CPs from different sugars prepared at pHs 7 and 10 as monitored by the increase in absorbance at 420 nm is shown in Fig. 3. The final stage of the browning reaction was monitored by the increase in absorbance at 420 nm (Ajandouz *et al.*, 2001). Increase in browning was generally observed as the heating time increased ($P < 0.05$). Regardless of sugar type, browning reactions occurred to a greater extent at alkaline pH, compared with at neutral pH. Browning at pH 7 increased continuously with increasing heating time, whereas browning occurred sharply within the first 20–30 min at pH 10. Subsequently, the browning was increased at a slower rate.

The absorbance ratios of sugar solutions heated for different times at pH 7 and 10 is shown in Tables 1 and 2. Under neutral conditions, pH 7, both absorbance ratios (A_{205}/A_{420} and A_{285}/A_{420}) for Dextrose, xylose and D-Glucose solutions increased within the first 10 min of heating with the development of browning (Table 1). Conversely, no browning was formed with glucose solutions until 30 min of heating. The ratios for D-Glucose reached a maximum after 20 min of heating, whereas glucose and Dextrose solutions gave maximum ratios after 60 and 30 min of heating,

respectively. For xylose, the highest A205/A420 and A285/A420 ratios were observed after 30 and 60 min, respectively. The increased absorbance ratios suggested that the intermediates were generated to a larger extent with lower transformation to brown polymers. The subsequent decrease in absorbance ratios suggested the formation of brown polymers from the intermediate. From the result, it was suggested that the formation of brown polymers from different intermediates varied with the type of sugar.

At pH 10, D-Glucose, Dextrose and xylose solutions showed a marked increase in the A270/A420 and A285/A420 ratios within the first 5 min of heating (Table 2). However, slight increase in both ratios was observed for glucose. Further heating resulted in a continuous increase in the ratios, suggesting more extensive formation of intermediates, compared to the development of brown polymers. The highest ratios of glucose and D-Glucose solutions were observed with the heating time of 90 min, whereas Dextrose and xylose had the maximum ratios when heated for 60 min. The subsequent decrease in ratio might indicate the transformation of intermediates into polymers. The slightly higher ratio for CPs prepared under neutral pH condition indicated that intermediates were generated to a larger extent, compared to final product formation. This was coincidental with the greater browning intensity of CPs prepared under alkaline condition, compared with CPs prepared under neutral condition (Fig. 3). It is suggested that the caramelization occurred to a larger extent under alkaline conditions and that it depended upon type of sugar. Generally, hexose sugars, both glucose and D-Glucose, were more prone to caramelization than pentose, Dextrose and xylose as evidenced by the greater browning as well as intermediate formation.

Table: 2.a: Changes in UV-absorbing compounds in CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in $A_{205\text{ nm}}$ at pH 3

Time (min)	UV-absorbance \pm SD			
	Dextrose	D- Glucose	L- Glucose	Sucrose
30	3.129 \pm 0.25	3.01 \pm 0.78	3.312 \pm 0.06	3.025 \pm 0.85
60	3.132 \pm 0.89	3.15 \pm 1.73	3.028 \pm 0.25	3.256 \pm 0.25
90	3.256 \pm 0.25	3.356 \pm 0.25	3.245 \pm 0.25	3.458 \pm 0.62
120	3.365 \pm 0.12	3.459 \pm 1.81	3.541 \pm 1.81	3.569 \pm 0.74
150	3.568 \pm 0.97	3.569 \pm 0.87	3.645 \pm 0.87	3.689 \pm 0.78
180	3.785 \pm 0.36	3.697 \pm 0.12	3.789 \pm 0.88	3.789 \pm 0.68

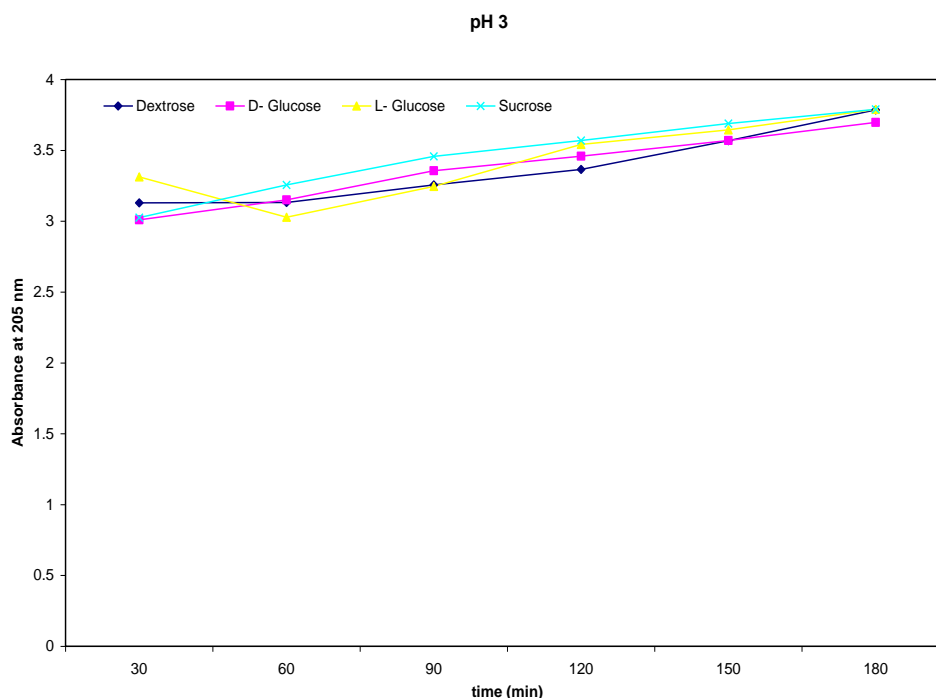


Figure (2.a)

Figure (2.a): Changes in UV-absorbing compounds in CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in A_{205} at pH 3

Table: 2.b: Changes in UV-absorbing compounds in CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in $A_{285\text{ nm}}$ at pH 3

Time (min)	UV-absorbance \pm SD			
	Dextrose	D- Glucose	L- Glucose	Sucrose
30	0.789 \pm 0.56	0.598 \pm 0.14	1.654 \pm 0.89	1.546 \pm 0.01
60	0.956 \pm 0.001	0.987 \pm 1.73	1.789 \pm 0.45	1.896 \pm 0.25
90	1.246 \pm 0.65	1.236 \pm 4.78	2.156 \pm 0.25	2.225 \pm 0.65
120	1.465 \pm 0.25	1.648 \pm 1.81	2.456 \pm 0.14	2.355 \pm 0.12
150	1.648 \pm 0.45	1.932 \pm 0.87	2.647 \pm 0.87	2.435 \pm 0.14
180	2.014 \pm 0.25	2.342 \pm 0.88	3.197 \pm 0.88	2.569 \pm 0.65

pH 3

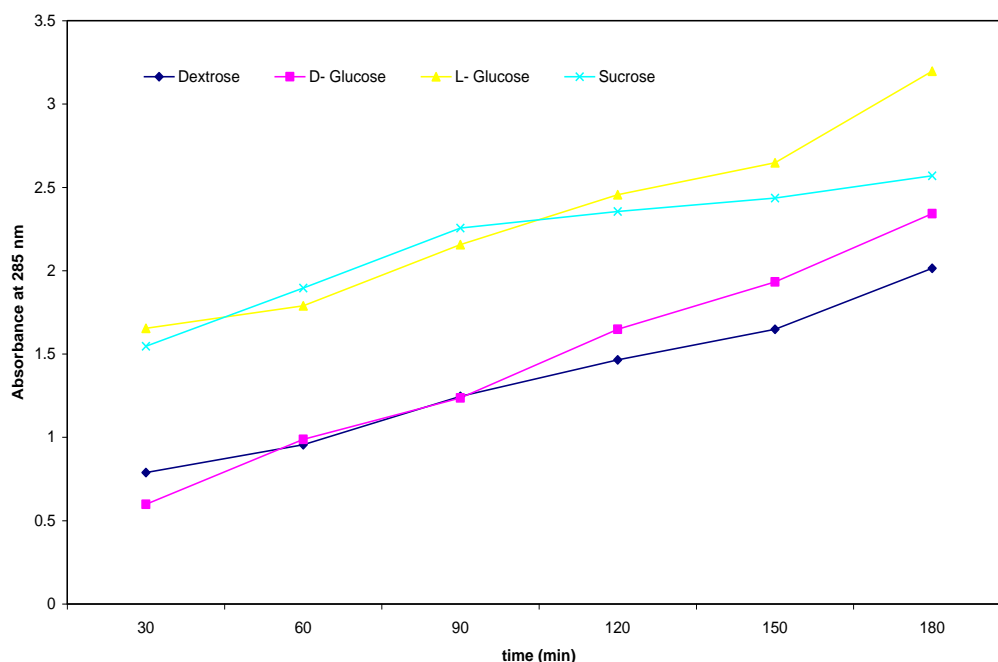


Figure (2.b)

Figure (2.b): Changes in UV-absorbing compounds in CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in A_{285} at pH 3

Table: 2.c: Changes in UV-absorbing compounds in CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in $A_{205\text{ nm}}$ at pH 7

Time (min)	UV-absorbance \pm SD			
	Dextrose	D- Glucose	L- Glucose	Sucrose
30	3.28 \pm 1.58	3.09 \pm 0.99	2.971 \pm 0.99	3.126 \pm 0.95
60	3.219 \pm 2.20	3.256 \pm 1.73	3.13 \pm 1.73	3.115 \pm 2.37
90	3.215 \pm 0.94	3.458 \pm 4.78	2.965 \pm 4.78	3.125.24
120	3.091 \pm 1.36	3.658 \pm 1.81	3.278 \pm 1.81	3.375 \pm 0.62
150	3.159 \pm 0.97	3.789 \pm 0.87	3.448 \pm 0.87	3.546 \pm 0.74

180	2.937±2.02	3.904±0.88	3.098±0.88	3.789±0.78
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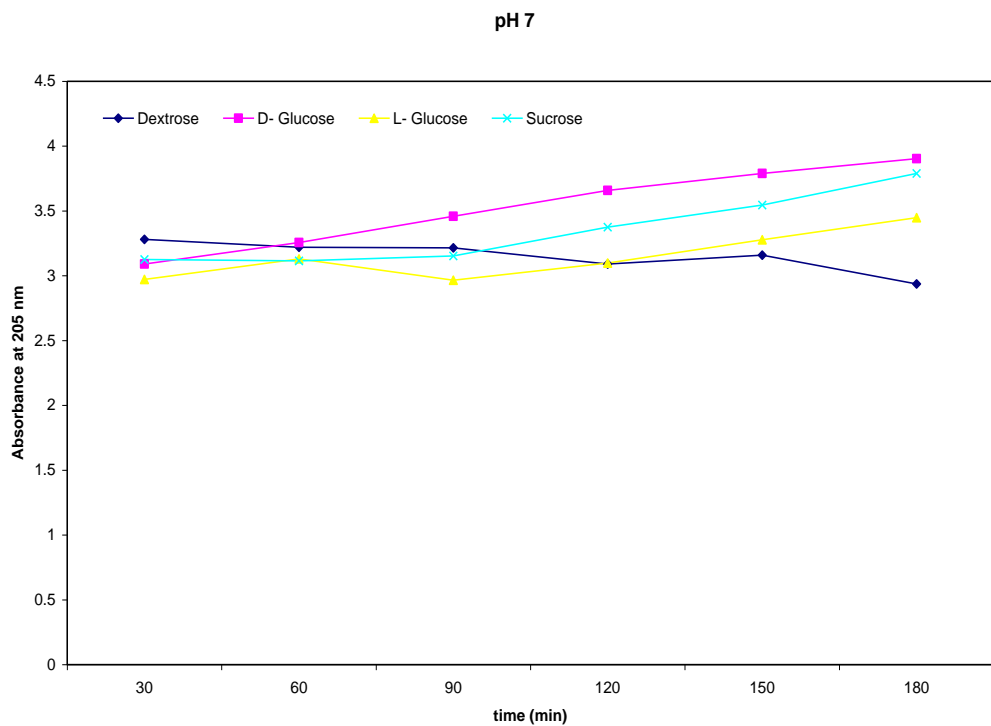


Figure (2.c)

Figure (2.c): Changes in UV-absorbing compounds in CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in A_{205} at **pH 7**

Table: 2.d: Changes in UV-absorbing compounds in CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in $A_{285\text{ nm}}$ at **pH 7**

Time (min)	UV-absorbance± SD			
	Dextrose	D- Glucose	L- Glucose	Sucrose
30	2.024±1.58	1.205±0.99	1.974±0.99	1.978±0.95
60	2.144±2.20	1.356±1.73	2.09±1.73	2.139±2.37
90	2.57±0.94	1.684±4.78	2.344±4.78	2.342±0.62
120	2.934±1.36	1.978±1.81	2.442±1.81	2.458±0.74
150	3.056±0.97	2.456±0.87	2.576±0.87	2.569±0.78
180	2.024±2.02	2.789±0.88	3.245±0.88	2.635±0.68

pH 7

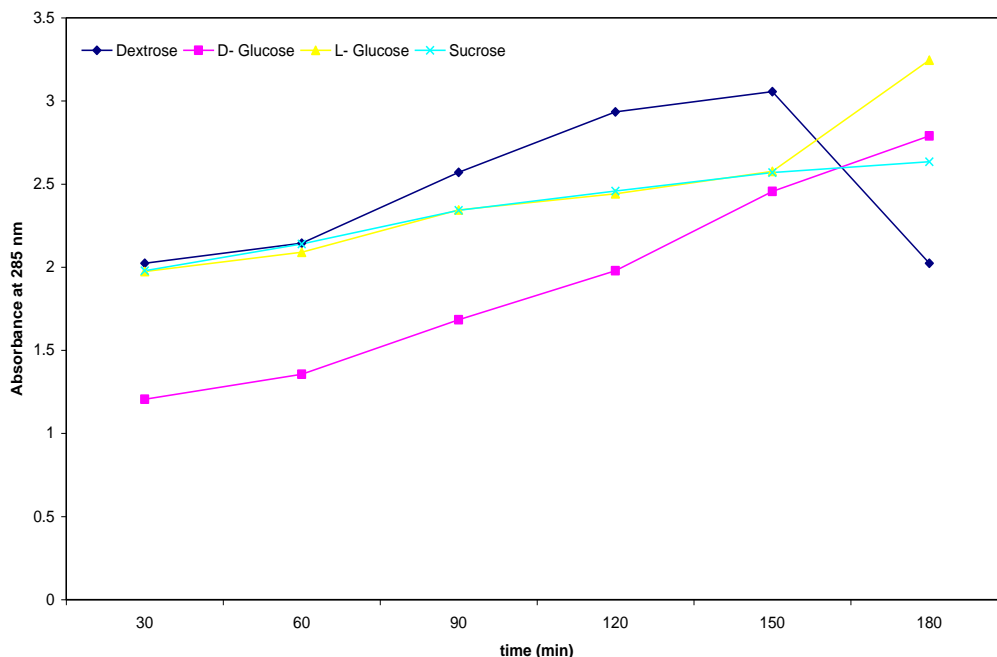


Figure (2.d)

Figure (2.d): Changes in UV-absorbing compounds in CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in A_{285} at pH 7

Table: 2.e: Changes in UV-absorbing compounds in CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in $A_{270\text{ nm}}$ at pH 10

Time (min)	UV-absorbance \pm SD			
	Dextrose	D- Glucose	L- Glucose	Sucrose
30	3.685 \pm 1.58	3.739 \pm 0.99	3.865 \pm 0.99	3.596 \pm 0.95
60	3.758 \pm 2.20	3.721 \pm 1.73	3.9 \pm 1.73	3.709 \pm 2.37
90	3.805 \pm 0.94	3.942 \pm 4.78	3.987 \pm 4.78	3.757 \pm 0.62
120	3.902 \pm 1.36	4.05 \pm 1.81	4.156 \pm 1.81	3.861 \pm 0.74
150	3.935 \pm 0.97	4.23 \pm 0.87	4.256 \pm 0.87	3.965 \pm 0.78
180	4.225 \pm 2.02	4.325 \pm 0.88	4.678 \pm 0.88	4.023 \pm 0.68

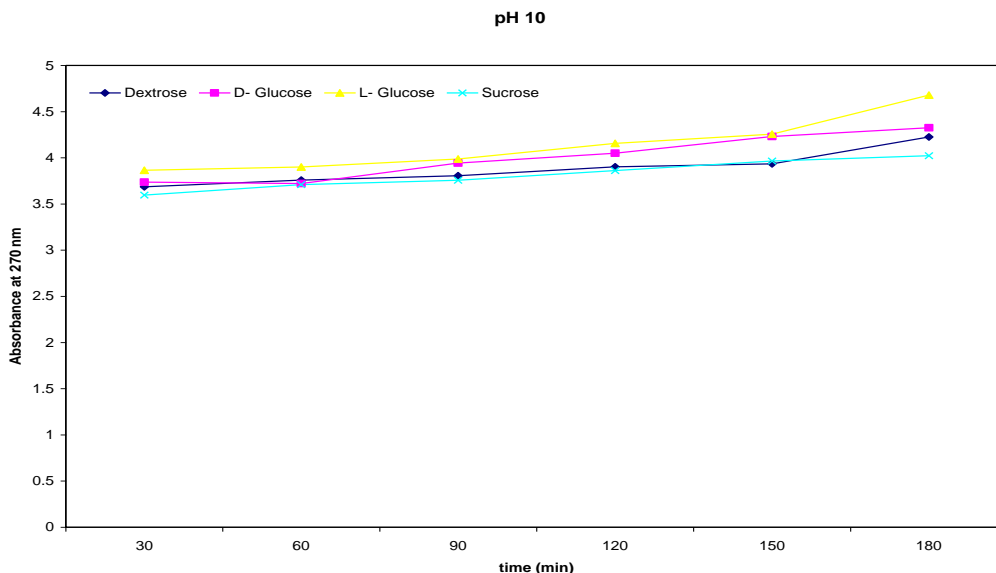


Figure (2.e)

Figure (2.e): Changes in UV-absorbing compounds in CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in A_{270} at pH 10

Table: 2.f: Changes in UV-absorbing compounds in CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in $A_{285\text{ nm}}$ at pH 10

Time (min)	UV-absorbance \pm SD			
	Dextrose	D- Glucose	L- Glucose	Sucrose
30	2.361 \pm 1.58	1.256 \pm 0.99	2.222 \pm 0.99	2.237 \pm 0.95
60	2.841 \pm 2.20	1.569 \pm 1.73	2.308 \pm 1.73	2.305 \pm 2.37
90	2.945 \pm 0.94	1.974 \pm 4.78	2.443 \pm 4.78	2.404 \pm 0.62
120	3.156 \pm 1.36	2.345 \pm 1.81	2.689 \pm 1.81	2.954 \pm 0.74
150	3.265 \pm 0.97	2.698 \pm 0.87	2.7 \pm 0.87	2.58 \pm 0.78
180	3.456 \pm 2.02	3.16 \pm 0.88	3.232 \pm 0.88	2.564 \pm 0.68

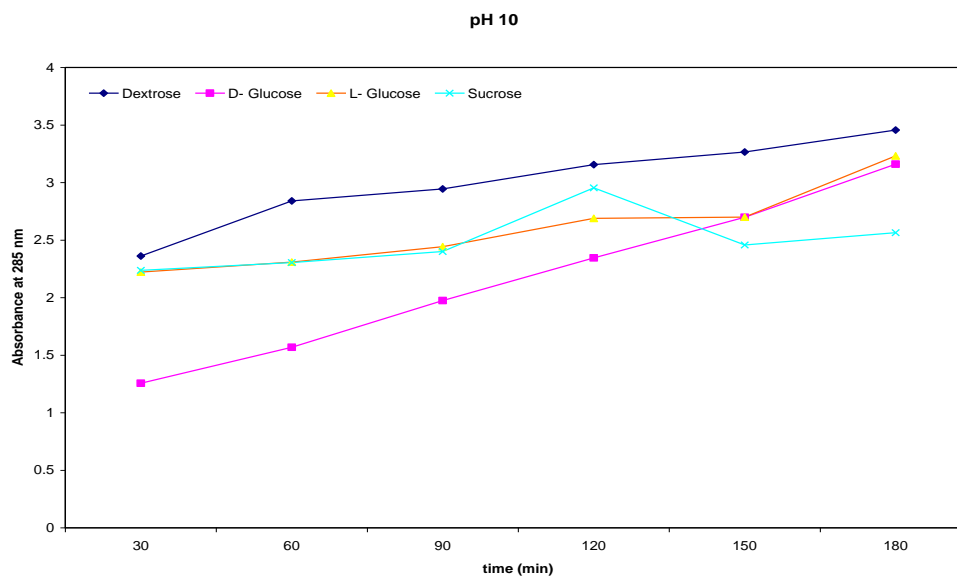


Figure (2.f)

Figure (2.f): Changes in UV-absorbing compounds in CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in A_{285} at pH 10

Table: **3.a:** Changes in browning intensity of CPs from sugars during heating for various times. Development of browning intensity was indicated by increase in $A_{420 \text{ nm}}$ at pH 3

Time (min)	Browning intensity \pm SD			
	Dextrose	D- Glucose	L- Glucose	Sucrose
30	0.356 \pm 1.58	0.359 \pm 0.99	0.321 \pm 0.99	0.252 \pm 0.95
60	0.659 \pm 2.20	0.773 \pm 1.73	0.453 \pm 1.73	0.631 \pm 2.37
90	0.897 \pm 0.94	0.986 \pm 4.78	0.681 \pm 4.78	0.726 \pm 0.62
120	1.265 \pm 1.36	1.321 \pm 1.81	0.987 \pm 1.81	0.884 \pm 0.74
150	1.402 \pm 0.97	1.564 \pm 0.87	1.135 \pm 0.87	0.987 \pm 0.78
180	1.789 \pm 2.02	1.945 \pm 0.88	1.564 \pm 0.88	1.156 \pm 0.68

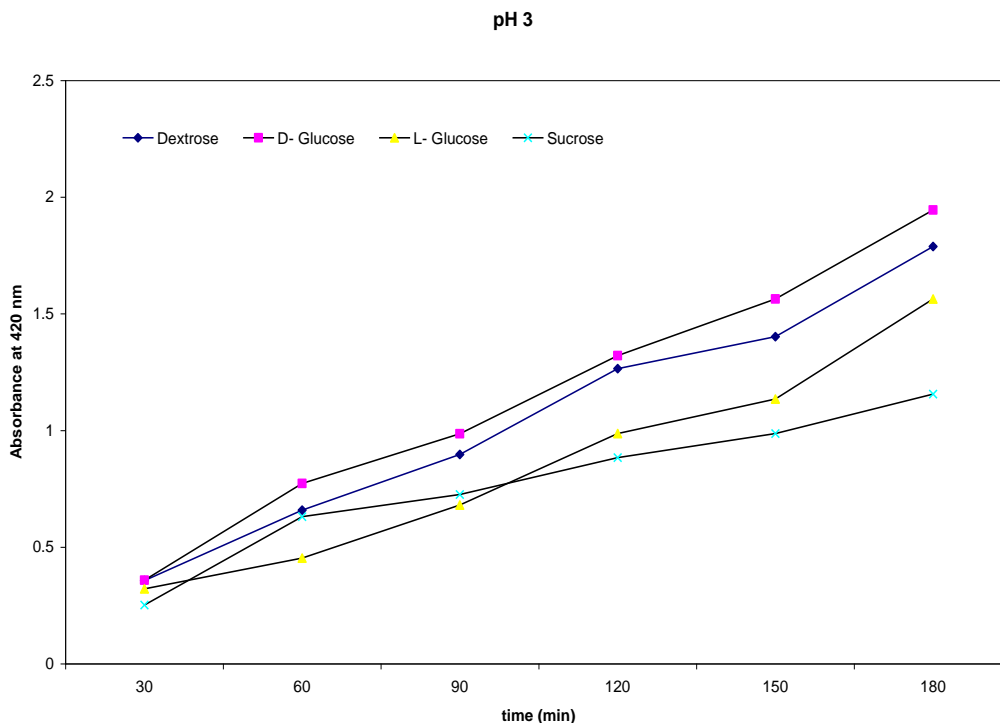


Figure (3.a)

Figure (3.a): Changes in browning intensity of CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in A_{420} at pH 3

Table: **3.b:** Changes in browning intensity of CPs from sugars during heating for various times. Development of browning intensity was indicated by increase in $A_{420 \text{ nm}}$ at pH 7

Time (min)	Browning intensity \pm SD			
	Dextrose	D- Glucose	L- Glucose	Sucrose

30	0.555±0.48	0.722±0.65	0.684±0.56	0.457 ±0.05
60	0.894±0.14	0.867±0.14	0.768±0.29	0.598±0.25
90	1.281±0.26	1.256±0.19	0.931±0.65	0.954±0.65
120	1.586±0.15	1.588±0.75	1.245±0.25	1.256±0.78
150	2.064±0.12	1.931±0.02	1.341±0.45	1.569±0.56
180	2.164±0.21	2.054±0.14	1.543±0.56	1.869±0.68

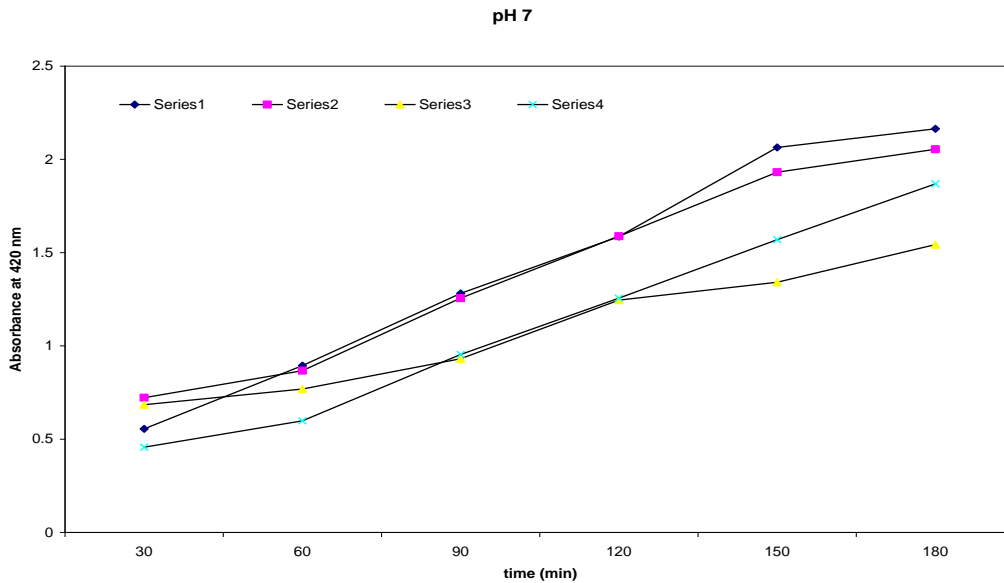


Figure (3.b)

Figure (3.b): Changes in browning intensity of CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in A_{420} at pH 7

Table: 3.c: Changes in browning intensity of CPs from sugars during heating for various times. Development of browning intensity was indicated by increase in $A_{420\text{ nm}}$ at pH 10

Time (min)	Browning intensity ± SD			
	Dextrose	D- Glucose	L- Glucose	Sucrose
30	1.14±0.25	0.976±0.47	0.731±0.125	0.564±0.45
60	1.451±0.14	1.27±0.145	0.923±0.24	0.793±0.24
90	1.946±0.25	1.599±0.25	1.378±0.14	0.901±0.14
120	2.045±0.1	2.125±1.14	1.646±0.19	1.246±0.06
150	2.334±0.25	2.389±0.24	1.946±0.24	1.546±0.04
180	2.456±0.24	2.654±0.25	2.234±0.26	1.894±0.02

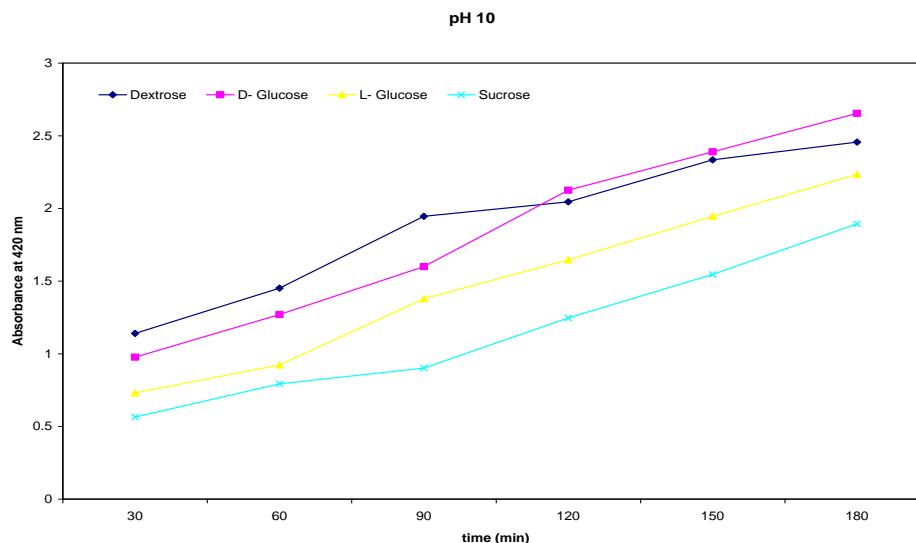


Figure (3.c Figure (3.a): Changes in browning intensity of CPs from sugars during heating for various times. Development of intermediate product was indicated by increase in A_{420} At pH 10

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