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INVESTIGATION OF CHANGES IN ANTIOXIDANT ACTIVITIES OF CARAMELIZATION PRODUCTS UNDER VARIOUS TIME REGIMES AND pH RANGES

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ABSTRACT

Caramelization is a process of heating sugars to produce brown color and typical caramel flavor which is most widely used in food industry as a natural food color, flavor and antioxidant agent. These properties of caramelization products (CPs) are heavily dependent on type of sugar, time of heating and pH range. A study was conducted to prepare CPs utilizing different type of sugars (dextrose; fructose; Liquid glucose; sucrose); and to investigate the changes in products characteristics at different time and pH reaction conditions using response surface methodology. The ranges of processing variables selected for this study were: time, 30-150 min and pH, 4-10. The experimental values of reducing sugars, browning intensity, reducing power and antioxidant activity showed that response variable was mainly dependent on increase in time of processing regardless of sugar type. Browning reactions occurred to a greater extent at alkaline pH while dextrose was more reactive to caramelization than other sugars at neutral pH. After 150 min, dextrose, fructose, L-glucose and sucrose were degraded to 46.9%, 34.9%, 23.4% and 39.7%, respectively. CPs from hexose sugars rendered the greater reducing power, compared with CPs from pentose. DPPH radical scavenging activity was observed in descending order such as: fructose>dextrose>sucrose>L-glucose ($p \leq 0.01$). The results of this study demonstrated that dextrose and fructose are a good source of natural antioxidant involving caramelization and can be potentially used as new food ingredients to enhance the shelf life of food products.

1.Introduction

Caramelization is the thermal degradation of sugars leading to the formation of volatiles and brown colored products. It is a type of non-enzymatic browning and may be carried out in the presence of acid, alkali and salt or without these at a temperature more than 80 oC and pH range of 3-12 (Davies and Labuza, 2005). Caramels are formed by heating low molecular weight carbohydrates, such as dextrose or starch hydrolysate under a variety

of reaction conditions. Caramels; dark brown to black viscous liquids with the characteristic odor of burnt sugar and a somewhat bitter taste; are among the oldest colorants known to be added to human food. Their use accounts for about 95% by weight of the permitted color additives used in food. Never the less, because of their complex composition, caramels color have remained chemically rather ill defined (Houben and Penninks, 1994).

Caramel colors have been in general use of more than 100 years, but only recently extensive effort has been made for characterize these color additives to specify better the materials being marketed today (Fadel and Farouk, 2002). Caramels are 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 their stabilizing, emulsifying, free radical scavenging, antimicrobial and antioxidative properties (Faraji and Lindsay, 2005; Tsai, 2009). Addition of fructose, glucose, maltose or citrate to the raw material increases contributions to volatile formation during baking and heating but Millard and caramelization reactions are also responsible for flavor formation in baked cereal products (Rehman *et al*., 2006).

The caramelization of carbohydrate polymers and their mixtures with low molecular weight sugars is of interest for food processing not only because of the caramel flavor and color, but also because the changes in sugar structure and the liberation of water during the caramelization reaction (Kroh, 1994). The exact reaction conditions and chemical reactants used are selected to give the caramel its desired characteristics. The influence of reaction conditions on the quality of caramels is continuously considered to be the problem of present interest. Composition of caramel from the qualitative point of view is independent of the sugar used but it is influenced by the method employed for its preparation. Several reports documented the effect on caramelization and its results of such parameters like temperature, mode of its application, time, pH, pressure, atmosphere and catalyst added (Sikora *et al*., 1989; Ajandouz and Puigserver, 1999).

The response surface methodology is a mathematical and statistical approach which has been widely used to evaluate the response of multivariate parameters during modeling of variable processing conditions for food production. The objective of this study was to investigate the influence of processing time and pH conditions for production of caramel products and their color and antioxidant characteristics using response surface approach

2. Materials and methods

2.1. Chemicals

Fructose, Potassium Ferricsyanide, Ferric Chloride, Di-Sodium Phosphate, Mono Sodium Phosphate and 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; while sucrose was supplied by Crescent Sugar Mills, Faisalabad, Pakistan.

2.2. Preparation of Caramelized Products (CPs)

Solutions of sugars were prepared by mixing with 0.05 M phosphate buffer of a pH range of 4-10. 10 mL of each sugar solution was transferred to a screw-caped test tube and was subjected to heating for time duration range of 30-150 minutes. At the heating time

designated the samples was taken out and cooled in ice water immediately and was stored at 4 ^{o}C for further analysis.

2.3. Browning Intensity Determination

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.

2.4. Determination of Reducing Sugars Concentration

Reducing sugars in CPs were measured according to the method of Benjakul et al. (2005) Fifty-fold dilution was made for all samples before analysis. Standard curves were prepared using the individual sugars. The changes in reducing sugar were expressed as the relative concentration (%) in comparison with the original content.

2.5. Determination of reducing power

The reducing power of Cps was measured as described by Benjakul et al. 2005 with slight modification. 0.5 mL of each sample was mixed with 0.5 mL of 0.2 M sodium phosphate buffer, pH 6.6 and 0.5 mL potassium ferric cyanide. The reaction mixture was incubated at 50 ^{o}C for 20 minutes and 0.5 mL of 10% (w/v) trichloroacetic acid (TCA) was added. Thereafter, 2 mL of distilled water and 400 µL of 0.1% (w/v) ferric chloride were added to mixture and then absorbance was measured.

2.6. DPPH Radical Scavenging Activity

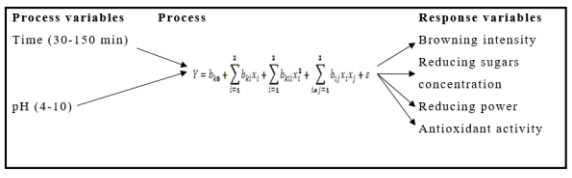
The radical scavenging activity was measured according to the method of Tsai et al. 2008. One millimeter of the freshly prepared 1 mM DPPH solution was added to the samples. The solution was than mixed vigorously and allowed to stand at 25 ^{o}C for 30 minutes. The absorbance of the mixtures was read at 517 nm using a UV-1601 spectrophotometer. The control was prepared in the same way, except that distilled water was used instead of CPs samples. For the blank, the assay was conducted in the same manner but distilled water was added instead of DPPH solution. The percentage of DPPH radical scavenging activity was calculated by following the method of Singh and Rajini (2004) as follows:

$$\text{Radical scavenging activity \%} = (1-(A_{\text{sample (517 nm)}} / A_{\text{control (517 nm)}})) \times 100 \quad (1)$$

2.7. Experimental Design and Statistical Analysis

Response surface modeling for quality changes involves multiple process control input parameters and selected product output properties. Response surface methodology was applied to determine the best combination of process variables for the production of caramelized products. This experimental study was carried out to determine the effects of independent processing variables on CPs using the faced central composite design (CCD). The effect of time (30–150 min) and pH (4–10) on the response values of CPs was examined. For better accuracy and simplification of result interpretation, the coded multiple regression coefficients were used and reconverted into original values at the end of experiment using MATLAB® (Ver. 7.9.0) software (Mathworks, Inc., Natick, USA). The coded coefficients at three levels used in this study were –1 (lowest level), 0 (medium level) and 1 (highest level), respectively (Table 1).

The following empirical ‘‘black box’’ modeling presents the relationships among process and response variables:



The expression inside the ''black box'' represents browning intensity, reducing sugars concentration; reducing power and antioxidant activity when the value of *i* is changed from 1 to 4; b_{ko}; b_{ki}; x_{kii} ; and b_{kij} represent the constant and coefficients of linear, quadratic and interactive effects, respectively; X_i ; X^2_i and X_iX_j represent the linear, quadratic and interactive effects of the independent variables, respectively, and ε is the random error primarily to account for the inability to determine the true model (Reyes-Moreno *et al*., 2003).

3.Results and discussions

3.1. Browning Intensity of CPs

The final stage of the browning reaction in CPs at pH (4, 7 and 10) and time (30, 90 and 150 min) was monitored by the increase in absorbance at 420 nm (Figure 1). The analysis of variance (ANOVA) for browning intensity of caramelized products from different sugars at time and pH reaction conditions has been presented in Table 2. Increase in browning was generally observed as the heating time increased. Regardless of sugar type, browning reactions occurred to a greater extent at alkaline pH (10), compared with at neutral pH (7). Browning at pH 7 increased continuously with increasing heating time; whereas browning occurred sharply within the first 30 min at pH 10. Subsequently, the browning was increased at a slower rate. The result is in agreement with Phongkanpai1 et al. (2006) which observed browning development of fructose at alkaline pH ranges. At pH 7, dextrose was more reactive to caramelization than the other sugars as indicated by the development of browning (Figure 1A). However, fructose was more likely to undergo browning via caramelization at pH 10 (Figure 1B). The differences in browning found among all sugars tested might be related to their different relative structural stability, including mutarotation, opening of the hemiacetal ring and enolization of the sugar. Browning development is influenced by the type of sugar and pH and the rate of color development decreased as the pH decreased. Buera et al. (1987) reported that rates of browning development of reducing sugars via caramelization processes were in the descending order: fructose > xylose > lactose > maltose > glucose. Thermolysis causes dehydration of sugar molecules with the introduction of double bonds or formation of anhydro rings. Introduction of double bonds leads to unsaturated rings and conjugated double bonds absorb light and produce color. Unsaturated rings will condense to polymers leading to the development of color (Benjakul *et al*., 2005).

3.2. The Loss of Sugars

The increased degradation of all sugars was observed as the heating time increased (Figure 2). The rate of sugar degradation was much greater under alkaline pH conditions, compared with a neutral pH. At pH 7, a slight decrease in sugar was found during the first 30 min of heating. Thereafter, sugars, especially fructose and sucrose, underwent more extensive degradation as evidenced by the marked decrease in reducing sugar content (Figure 2B; 2D). The analysis of variance (ANOVA) for reducing sugars concentration in caramelized products from different sugars at time and pH reaction conditions has been presented in Table 3. Fructose and sucrose 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 30 min of heating. Subsequently, sugars underwent degradation gradually up to 150 min. Among all of the sugars tested, dextrose was degraded to a smaller extent, compared to the other (Figure 2A). Higher levels of degradation of both fructose and dextrose occurred at 100 ºC under alkaline conditions ((Benjakul *et al*., 2005; Ajandouz and Puigserver, 1999; Ajandouz *et al*., 2001). After 150 min,

glucose, fructose, L-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.

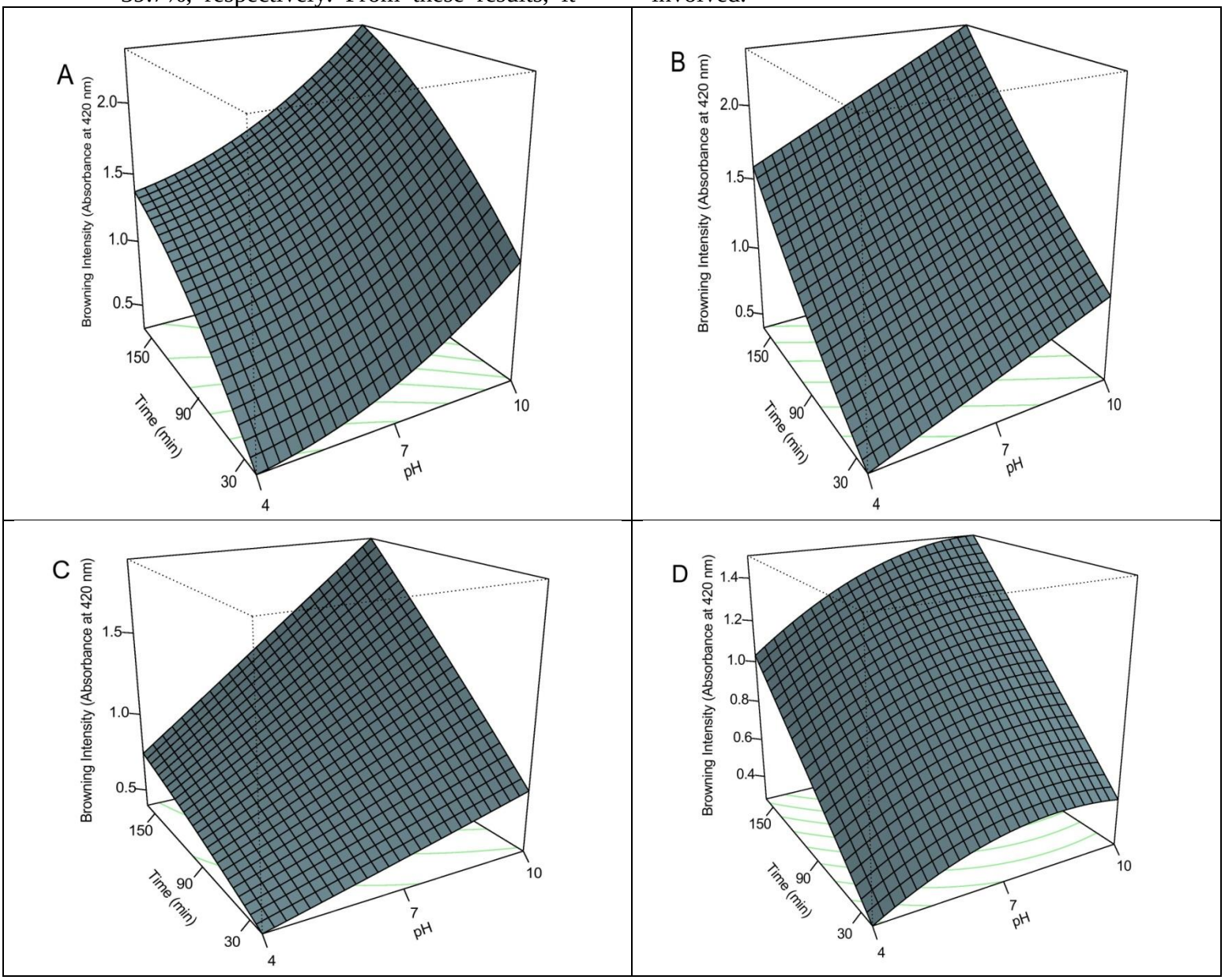


Figure 1. Mutual Interaction effect of time and pH reaction conditions on browning intensity of caramelized products for sugar type (A) Dextrose (B) Fructose (C) L- Glucose (D) Sucrose

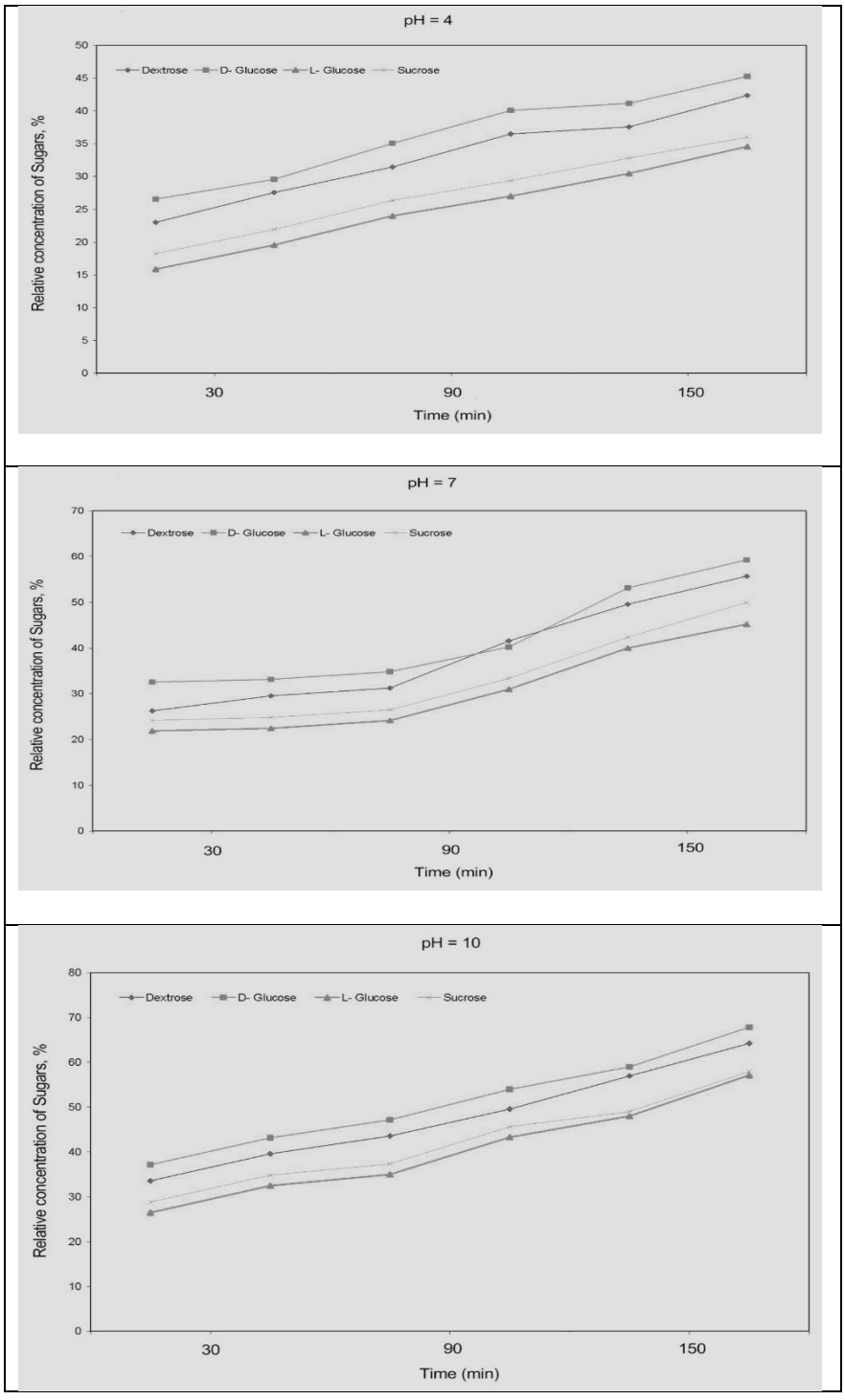


Figure 2. Effects of time and pH reaction conditions on relative concentration for sugar types in caramelized products

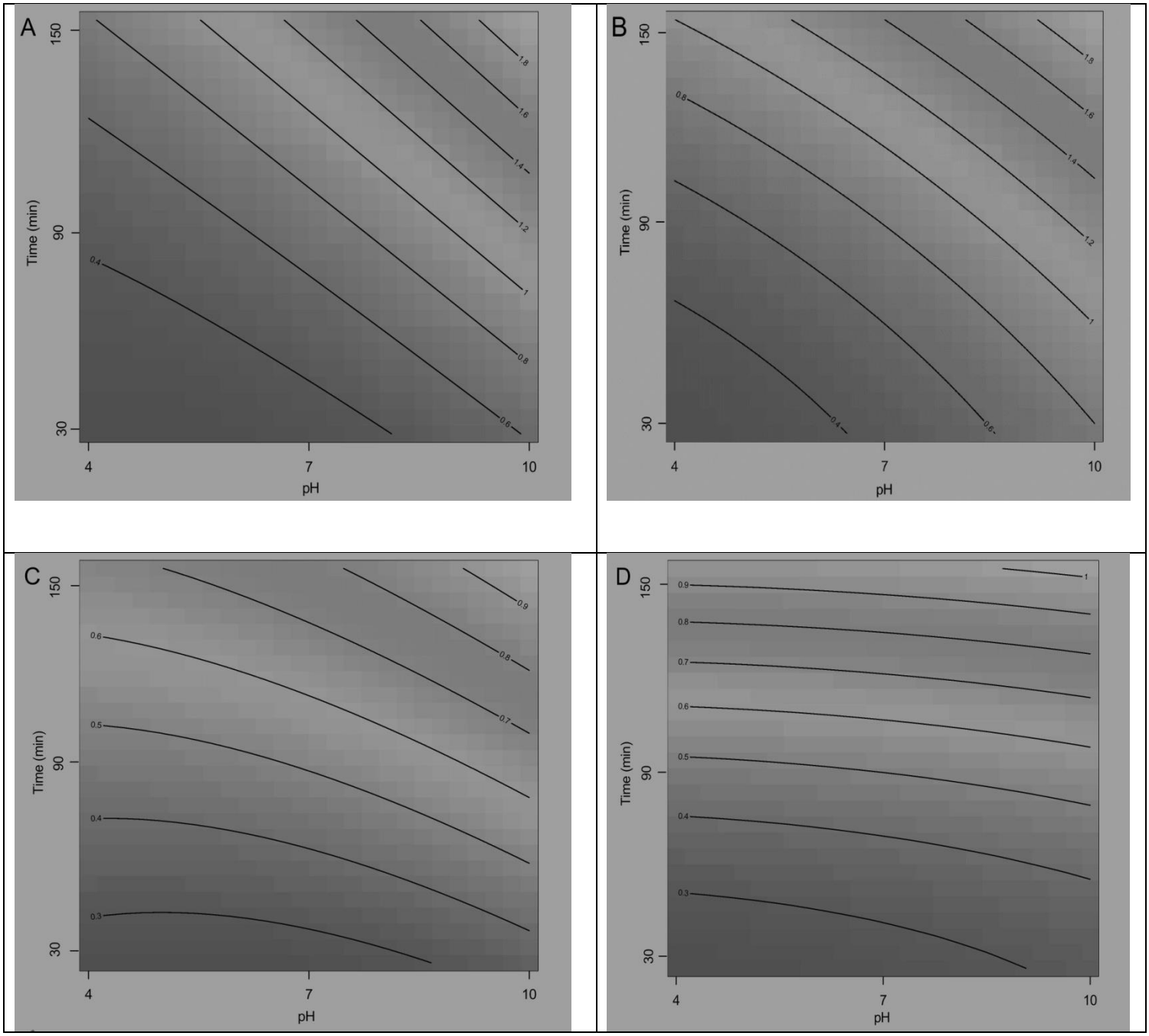


Figure 3. Mutual Interaction effect of time and pH reaction conditions on reducing power of caramelized products for sugar type (A) Dextrose (B) Fructose (C) L- Glucose (D) Sucrose

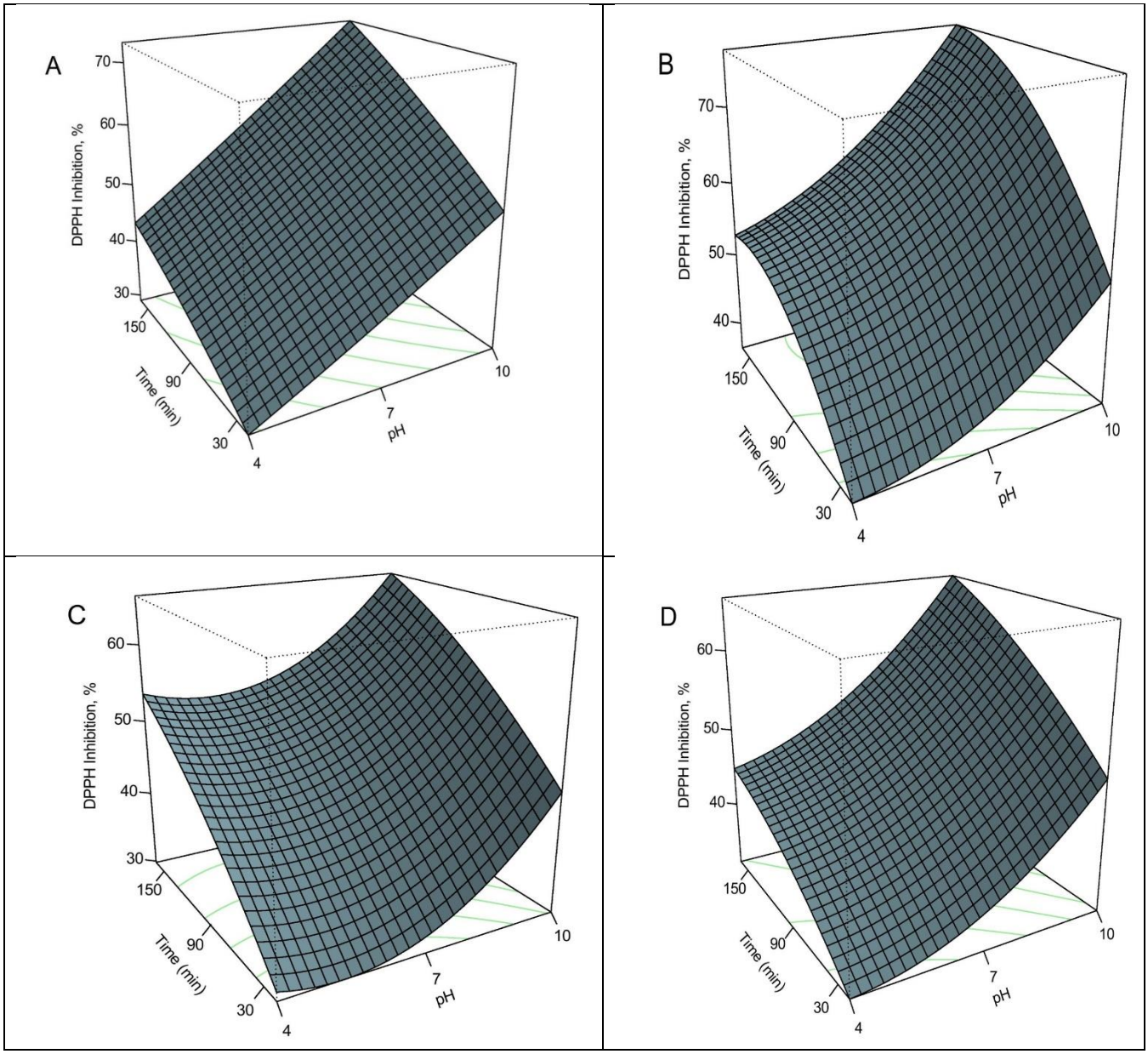


Figure 4. Mutual Interaction effect of time and pH reaction conditions on antioxidant activity of caramelized products for sugar type (A) Dextrose (B) Fructose (C) L- Glucose (D) Sucrose

3.3. Reducing Power of CPs

Reducing power of CPs from different sugars prepared by heating at pH 3, 7 and 10 for different times is depicted in Figure 3. Under neutral conditions, the reducing power of CPs, as indicated by the increase in absorbance at 700 nm, increased linearly as the heating time increased (Figure 3). Fructose CPs showed the highest reducing power, compared with CPs from other sugars (Figure 3B). The analysis of variance (ANOVA) for reducing power of caramelized products from different sugars at time and pH reaction conditions has been presented in Table 4. For CPs prepared under alkaline conditions, reducing power

increased exponentially with increasing heating time. A sharp increase in reducing power was observed when heating was conducted for up to 30 min. Heating for a longer time did not result in increased reducing power. Generally, CPs from fructose exhibited the highest reducing power (Figure 3B) and CPs from dextrose (Figure 3A) showed higher reducing power than those from sucrose and L-glucose (Figure 3C; 3D). Thus, it can be concluded that CPs from hexose sugars rendered the greater reducing power, compared with CPs from pentose. During heating of sugar solutions, especially under alkaline conditions, reducing compounds might be formed and these could exhibit antioxidative activity. Antioxidative activity of Maillard reaction products was associated with reducing power (Yen and Hsieh, 1995). The reducing power of CPs might be due to hydrogen-donating ability (Shimada *et al*., 1992; Benjakul *et al*., 2005;).

3.4. DPPH radical scavenging activity

DPPH radical scavenging activity of CPs from sugars prepared by heating under neutral and alkaline condition is shown in Figure 4. The analysis of variance (ANOVA) for antioxidant activity of caramelized products from different sugars at time and pH reaction conditions has been presented in Table 5. DPPH radical scavenging activity of CPs prepared under neutral conditions increased linearly as the heating time increased. Among CPs from all sugars tested, those from sucrose showed the highest activity (Figure 4D). CPs from dextrose were found to exhibit to lowest activity, compared to CPs from other sugars (Figure 4A). For CPs prepared under alkaline conditions, an exponential increase in DPPH radical scavenging activity was observed with increasing heating time. CPs from fructose exerted greater DPPH radical scavenging activity compared to CPs from other sugars (Figure 4B). DPPH radical scavenging activity was in the descending order: fructose>dextrose>sucrose> L-glucose ($p \leq 0.01$). From the result, it was noted that DPPH radical scavenging activity of CPs prepared under alkaline conditions was approximately five-fold greater than that of CPs prepared under neutral conditions. The higher radical scavenging activity of CPs prepared at pH 10 was coincidental with the higher reducing power, browning and intermediate formation. DPPH is one of compounds that possess a proton free radical with a characteristic absorption, which decreases significantly on the exposure to proton radical scavengers (Yamaguchi *et al*., 1998). It was found that CPs was able to reduce the DPPH radical to the yellow-coloured diphenylpicrylhydrazine. The reduction of alcoholic DPPH solution in the presence of a hydrogen- donating antioxidant is due to the formation of the non-radical form, DPPH-H (Shon *et al*., 2003). Thus it was suggested that either intermediates or the final brown polymer could function as hydrogen donors. Kirigaya and fellows (1968) found that antioxidant activity increased with increasing color intensity. However, Rhee and Kim (1975) reported that effective antioxidant compounds were formed at an earlier stage of browning reactions. Therefore, CPs, especially those from caramelization under alkaline conditions, exhibited antioxidant activity.

Table 1. Coded and Actual Levels of Independent Variables Used for Production of Caramelized Products (CPs) as Determined by The Central Composite Design (CCD).

Independent variable	Coded levels		
	-1	0	1
Time, min	30	90	150
pH	4	7	10

Table 2. Mean Sum of Squares for Browning Intensity of Caramelized Products from Different Sugars at Time and pH Reaction Conditions.

Source of variation	df	Dextrose	Fructose	L- Glucose	Sucrose
Intercept	5	0.73**	0.63**	0.39**	0.32**
pH(A)	1	1.27**	0.7**	0.9**	0.18*
Time(B)	1	2.34**	2.44**	0.86**	1.33**
A×B	1	5.476^{NS}	0.011^{NS}	0.16**	0.015^{NS}
A^2	1	8.103^{NS}	4.253^{NS}	5.344^{NS}	0.06^{NS}
B^2	1	6.572^{NS}	5.265^{NS}	1.107^{NS}	3.523^{NS}
Residual	5	0.018	2.058	7.689	0.011

**Significant at 0.001 level
*Significant at 0.01 level
^{NS} Non-significant

Table 3. Mean Sum of Squares for Relative Sugar Concentration in Caramelized Products from Different Sugars at time and pH Reaction Conditions

Source of variation	df	Dextrose	Fructose	L- Glucose	Sucrose
Intercept	5	210.53**	274.50^{NS}	157.99**	148.16**
pH(A)	1	315.81**	276.62^{NS}	253.5**	240.67**
Time(B)	1	676.28**	529.78^{NS}	479.54**	459.2**
A×B	1	13.9^{NS}	13.18^{NS}	16^{NS}	6.25^{NS}
A^2	1	17.19^{NS}	181.52^{NS}	7.44^{NS}	5.28^{NS}
B^2	1	21.70^{NS}	280.8^{NS}	27.54^{NS}	24.64^{NS}
Residual	5	8.60	247.9	6.79	4.49

**Significant at 0.001 level
^{NS} Non-significant

Table 4. Mean Sum of Squares for Reducing Power of Caramelized Products from Different Sugars at Time and pH Reaction Conditions.

Source of variation	df	Dextrose	Fructose	L- Glucose	Sucrose
Intercept	5	0.51**	0.42**	0.096**	0.16**
pH(A)	1	0.91**	0.82**	0.056**	0.01^{NS}
Time(B)	1	1.41**	1.15**	0.41**	0.75**
A×B	1	0.18**	0.054*	9.312*	1.6^{NS}
A^2	1	0.029**	0.059*	4.253^{NS}	4.947^{NS}
B^2	1	0.025**	0.012^{NS}	3.942^{NS}	0.031*
Residual	5	1.231	6.167	8.642	4.387

**Significant at 0.001 level

*Significant at 0.01 level

NS Non-significant

Table 5. Mean Sum of Squares for Antioxidant Activity of Caramelized Products from Different Sugars at Time and pH Reaction Conditions.

Source of variation	df	Dextrose	Fructose	L- Glucose	Sucrose
Intercept	5	315.19**	354.71^{NS}	212.88**	197.37**
pH(A)	1	1027.83**	622.2*	276.08**	543.4**
Time(B)	1	533.36**	1124.77*	691.23**	421.68**
A×B	1	13.43^{NS}	16.81^{NS}	1.21^{NS}	6.0^{NS}
A^2	1	0.5^{NS}	6.47^{NS}	92.36**	15.09^{NS}
B^2	1	0.61^{NS}	1.85^{NS}	11.9^{NS}	2.14^{NS}
Residual	5	2.21	69.98	4.34	4.44

**Significant at 0.001 level

*Significant at 0.01 level

NS Non-significant

4. Conclusions

The results of this study demonstrated that the caramelized products can be produced with good quality to be used as color, flavor and antioxidant additive. Among the experimental conditions used in this study, time (~150 min) and alkaline pH was found most significant for browning intensity, reducing power and antioxidant activity. Based on the results of this study, it can be concluded that caramelized products can be successfully produced having high tinctorial strength and antioxidant activity by utilizing local resources. Our results suggest that dextrose and D- glucose are a good source of natural antioxidant involving carameliation and can be potentially used as new food ingredients to enhance shelf life of food.

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