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Estimation of antioxidant potential of caramelized products by DPPH assay

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ABSTRACTS

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 Dextrose showed the highest activity. CPs from glucose were found to exhibit to lowest activity, compared to CPs from other sugars. For CPs prepared under alkaline conditions, an exponential increase in DPPH radical scavenging activity was observed with increasing heating time. CPs from D-Glucose exerted greater DPPH radical scavenging activity compared to CPs from other sugars. DPPH radical scavenging activity was in the descending order: D-Glucose>glucose>Dextrose> xylose. 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.

Key words: Caramelization, DPPH

Introduction

Caramelization is the thermal degradation of sugars leading to the formation of volatiles (caramel aroma) and brown colored products (caramel colors). 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 ^{o}C at pH range of 3-12 (Davies and Labuza, 2005). 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 undergo regulations by food laws. There are four major classes as identified by JECFA on the basis of reactant used and intended use: (Class I): Plain caramel Class II: caustic sulphite Class III: amonia Class IV sulphite amonia. Daily Intake (ADI) of Class I Caramel coloring as "not specified"; that of Class II as 0-160 mg/kg body weight; that of Class III as 0-200 mg/kg body weight; and that of Class IV as 0-200 mg/kg body weight. (JECFA- Joint FAO/WHO Expert Committee on Food Additives, 1986).

The process of caramelization produces large mixture of many species, which make up flavor, fragrance and color (Adrian, 1987) but 5-Hydroxymethyl furfural (HMF) is the principal degradation product in caramel (Theander, 1985). Hydroxy methyl furfural (HMF) is an intermediate product in the Maillard reaction (Morales, Romero and JimeÂ nez-PeÂ rez, 2001) and is also formed from the caramelization of sugars at high temperatures (Kroh, 1994). Brown pigment formation is desirable during bread making. Furfural and Methyl furfural are also furanic compounds formed in non-enzymatic browning reaction during thermic treatments. They have been determined in fruit juices, wines beer (Hayase, 1996), coffee and infant formula.

Thermal treatment of sugar acids, reducing sugars and lactitol effects on caramelization via enolization, isomerization, dehydration and fragmentation of the substrates and their intermediates. Carboxylic acids and their salts, phosphates, metallic ions, nitrogenous substance and other auxochromes enhance the reaction process. Caramel has been prepared from cane sugar, molasses, malted-cereal/tuber syrups, goat milk but there is no report of caramel from toddy or palm saps. This paper describes work to optimize the preparation of caramel from E. guineensis and R. hookeri saps. Caramelization products (CPs) contain volatile and nonvolatile fractions of low and high molecular weights. CPs

have shown antioxidant activity as acetone extracts form the glucose reduced the peroxide values of soybean and that from the D-Glucose inhibit lipid oxidation (Benjakul *et al*,. 2005).

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. 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 (Miller *et al*., 2003).

It is well known that the caramelization influences the oxidative stability of food products. This process of oxidation can be initiated by enzyme catalysts, metal ion catalysis, or photochemical processes and thus result in off flavor, aroma, color, texture, nutritive value, and the formation of toxic compounds such as aldehydes and epoxides. These changes in food are significant to both food stability and food safety. Traditionally, lipid peroxidation has been prevented by the use of synthetic antioxidants such as BHA and BHT. Recently, however, the safety of these compounds in respect to human health has been questioned and, therefore, the discovery of safer natural alternatives is in order (Tosuna and Ustun, 2003).

Caramel color and flavor is also widely used in Pakistan but it is to be imported form 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 cast to be used as color, flavor and antioxidant additive. While reviewing the literature we come to know that all the study on antioxidant activity of caramelization products have been done using sugars of analytical grade (Benjakul *et al*,. 2005), which are quiet expensive and can not be used as commercial source of caramel color and flavor. Thus the present study was designed to produce caramel having high tinctorial strength and high antioxidant activity by utilizing local resources.

OBJECTIVE:

To develop caramelisation products (CPs) using local resources.

To estimate antioxidant activity of the caramelization products.

MATERIALS AND METHODS

Chemicals:

D- Glucose, 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 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-caped test tube and was subjected to heating for time duration range of 0-300 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 (Benjakul *et al*., 2005).

DPPH radical scavenging activity:

The radical scavenging activity was measured according to the method of Tsai, Wu, and Chang, 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 as follows (Singh and Rajini, 2004):

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

Where A_{sample} (517 nm) is the absorbance of sample and A_{control} (517 nm) is the absorbance of the control.

Note: 1 mM solution of DPPH was prepared by dissolving 0.00473g of DPPH chemical on dissolving in required quantity of methanol solvent.

Statistical Analysis:

Data was subjected to analysis of variance. Mean difference was determined by the least significant differences multiple range test. (Phonkanpai *et al., 2005)*.

Results and discussion

DPPH radical scavenging activity:

DPPH radical scavenging activity of CPs from sugars prepared by heating under neutral and alkaline condition is shown in Fig. 1. 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 Dextrose showed the highest activity (Fig. 1(a)). CPs from glucose were found to exhibit to lowest activity, compared to CPs from other sugars. For CPs prepared under alkaline conditions, an exponential increase in DPPH radical scavenging activity was observed with increasing heating time (Fig. 1(b)). CPs from D-Glucose exerted greater DPPH radical scavenging activity compared to CPs from other sugars. DPPH radical scavenging activity was in the descending order: D-Glucose>glucose>Dextrose> xylose. 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 possesses a proton free radical with a characteristic absorption, which decreases significantly on the exposure to proton radical scavengers (Yamaguchi, Takamura, Matoba, and Terao, 1998). It was found that CPs were able to reduce the DPPH radical to the yellow-colored 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, Kim, and Sung, 2003). Thus it was suggested that either intermediates or the final brown polymer could function as hydrogen donors. Kirigaya, Kato, and Fujimaki (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. Furthermore, the possible involvement of CPs in concert with MRPs as the antioxidant in high-temperature cooked foods, such as grilled or roasted products, can be proposed.

Table: 1.a: Evolution of antioxidant activity of CPs at **pH 3** by heating various sugars for different time durations by using DPPH assay

Time (min)	**% Scavenging activity ± SD**			
	Dextrose	**D- Glucose**	**L- Glucose**	**Sucrose**
30	36.3±1.58	39.6±0.99	29.3±0.99	32±0.95
60	38.5±2.20	45.6±1.73	35.3±1.73	38±2.37
90	40.3±0.94	46.3±4.78	36.3±4.78	38.9 ±0.62
120	45.6±1.36	49.6±1.81	39.3±1.81	42±0.74
150	46.2±0.97	53.3±0.87	43±0.87	45.7±0.78
180	48.6±2.02	54.7±0.88	44.4±0.88	47.1±0.68

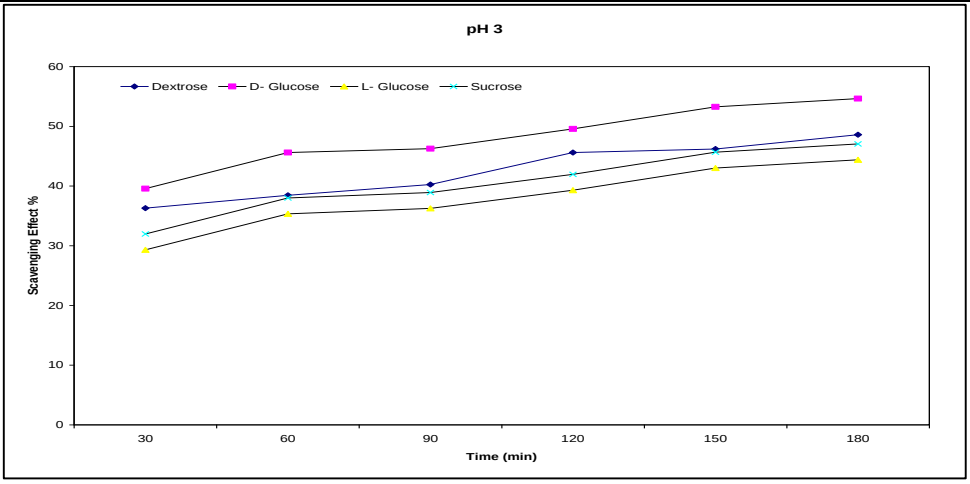

Figure: 1.a

Table 1(b) Evolution of antioxidant activity of CPs at **pH 7** by heating various sugars for different time durations by using DPPH assay

Time (min)	% Scavenging activity ± SD			
	Dextrose	**D- Glucose**	**L- Glucose**	**Sucrose**
30	40.5±0.87	35.05±0.35	34.6±065	37.3 ±0.65
60	45.6±0.96	37.43±0.37	40±0.89	42.7±2.25
90	49.8±1.25	58.48±1.29	43±1.23	45.7 ±0.78
120	50.2±1.36	70.96±2.58	50.1±1.58	52.7±1.02
150	60.6±1.58	81.60±2.65	53.2±2.65	55.9±1.15
180	69.6±2.56	93.84±2.78	63.6±2.54	66.2±1.32

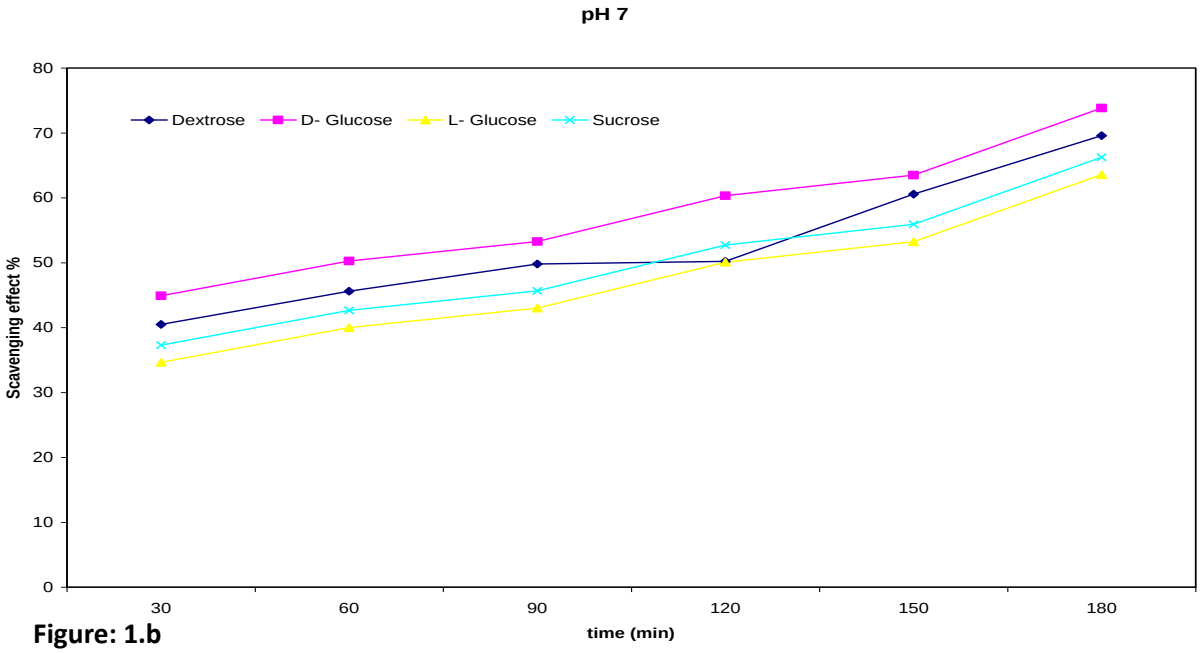

Figure: 1.b

Table 1(c) Evolution of antioxidant activity of CPs at **pH 10** by heating various sugars for different time durations by using DPPH assay

Time (min)	% Scavenging activity ± SD			
	Dextrose	**D- Glucose**	**L- Glucose**	**Sucrose**
30	50.6±1.58	54.8±0.99	44.6±0.99	47.2 ±0.95
60	57.9±2.20	62.1±1.73	51.9±1.73	54.5±2.37
90	64.9±0.94	68.8±4.78	58.6±4.78	61.3 ±0.62
120	69.5±1.36	73.8±1.81	63.3±1.81	63.3±0.74
150	72.5±0.97	76.7±0.87	66.4±0.87	65.5±0.78
180	82.4±2.02	81.3±0.88	69±0.88	66±0.68

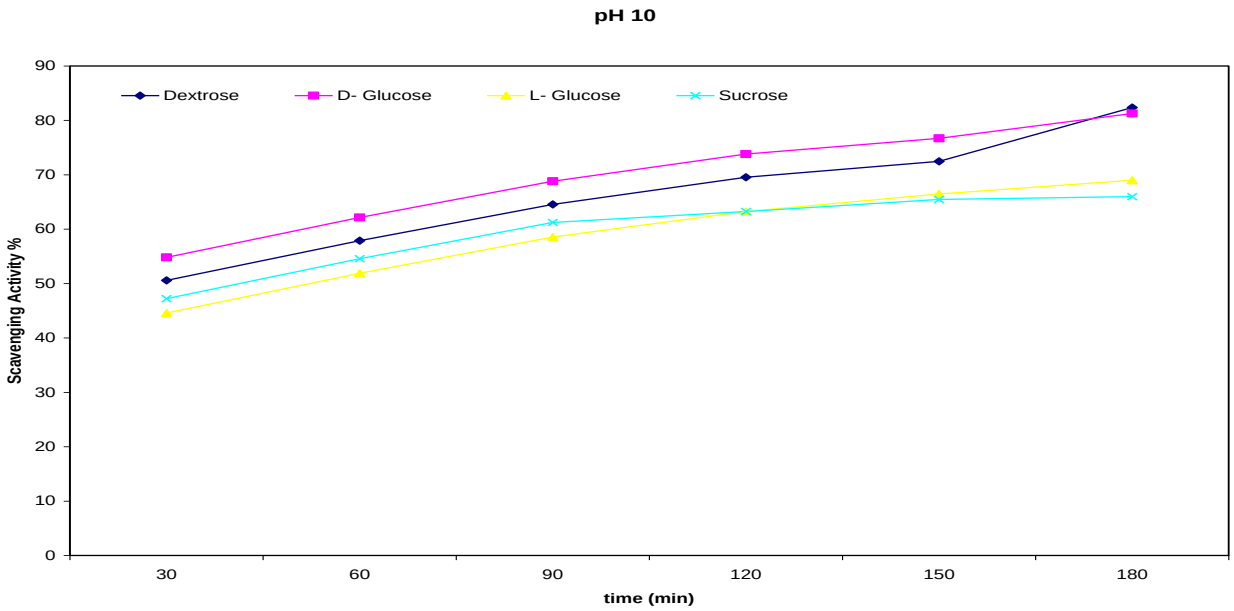

Figure 1.c

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