

## INFLUENCE OF pH AND TEMPERATURE ON NONENZYMATIC BROWNING REACTION BETWEEN AFFINED C SUGAR AND AMINO ACIDS

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**ABSTRACT:** Affined C sugar is a by-product which is obtained in three-stage crystallization process. It is characterized with the highest content of coloured matter formed during nonenzymatic browning reaction which occurs during crystallization process.

In thermally treated (pH 7, 70 °C, 1200 min) affined C sugar solution, alkaline degradation of invert sugars mainly occurred and the formation of coloured matter was determined.

With the aim to investigate the rate of formation of coloured matter in the Maillard reaction sugar model system (the affined C sugar solution with glutamine, lysine, valine, methionine, and tryptophan, respectively,  $c = 0.01$  mol/L), it was thermally treated (pH 7, 70 °C, 1200 min and pH 10, 80 °C, 1200 min) and the formation of coloured matter over the thermal treatment was monitored.

It was established that the effectiveness of amino acids in the formation of coloured matter in investigated model system was as follows: tryptophan > lysine > valine > methionine > glutamine.

The increase in pH and temperature in the Maillard reaction sugar model system was followed with the increasing content of Maillard reaction products.

**Key words:** *affined C sugar, amino acids, Maillard reaction products, colour*

## INTRODUCTION

During the whole procedure of sugar production a wide range of coloured compounds is formed as a result of sugar degradation reactions, pH changes, thermal effects and reaction between amino compounds and carbohydrates. The coloured matter adsorbs on the surface of sugar crystals and occludes within them affecting their quality (Coca et al, 2004). It is known that beet coloured matter is mainly produced during processing from alkaline degradation of invert sugars and melanoidin formation (Godshall, Vercollotti, Triche, 2002). The melanoidins represent the third-stage products of Maillard reaction that occurs between the reactive carbonyl group of the sugar and the amino group of the amino acids (Cämmerer and Kroh, 1995; Clarke et al., 1997).

Sugar beet processing involves a series of consecutive processes, such as extraction, purification, concentration and three-stage crystallization. During all these processes the mechanisms of the degradation of reducing sugars are very complex and result in many intermediate products. Therefore, the difficulties in characterization of colour composition of process samples are overcome by using model colourant systems (Kelly and Brown, 1978/1979) which are created to get information about the browning reaction that has occurred under specific conditions.

White sugar, B sugar, and C sugar are obtained from the crystallization process, where the affined C sugar is a by-product that is characterized with the highest content of coloured matter. Coca et al. (2008) assumed that the arising of coloured matter of affined C sugar was addressed, among other colourants, to the formation of melanoidins due to the relatively high N-compounds content in sugar beet.

The affined C sugar is directed in the first crystallization stage and strongly influences the quality of white sugar contributing to the increase of its coloured matter content (Gyura et al., 2005; Šereš et al., 2004). Since some process parameters, i.e. pH value and temperature influence the formation of coloured matter in sugar beet processing, the objective of this work was to determine the influence of amino acids as well as the changes in pH and temperature on the formation of coloured matter in the Maillard reaction sugar model system (affined C sugar solution with amino acids).

## MATERIAL AND METHODS

### ***Materials***

The affined C sugar was obtained at the third stage of crystallization from the process of sugar production in Sugar factory “Šajkaška”, Žabalj, Serbia. The basic characteristics of the affined C sugar were determined according to the methods specified by Reinefeld and Schneider (1978).

### ***Thermally induced nonenzymatic browning reaction of affined C sugar***

Affined C sugar was dissolved in distilled water and diluted to 40 °Bx dry matter. The mentioned dilution was chosen based on the literature data that indicated that the most efficient formation of coloured matter was in C affined sugar solution with the concentration of 40 °Bx (Gyura, 1992).

50 g of affined C sugar solution was thermally treated at 70 °C and pH=7 during 1200 min and at 80 °C and pH=10 during 1200, respectively.

### ***Thermally induced nonenzymatic browning reaction of affined C sugar and amino acids***

The influence of the addition of amino acids (0,01 mol/l) in affined C sugar solution (40 °Bx) on the formation of coloured matter content at different pH values (pH 7 and 10) and temperatures (70 °C and 80 °C) was measured spectrophotometrically at 420 nm and 560 nm using diluted solutions (5 °Bx) and expressed in IU.

### ***Determination of coloured matter***

The content of coloured matter ( $B_{\lambda}$ ) was determined spectrophotometrically at 420 nm and 560 nm using diluted solutions (5 °Bx) and expressed in IU.

The changes of coloured matter content after the thermal treatment ( $\Delta B_\lambda$ ) were calculated according to:

$$\Delta B_\lambda = (B_\lambda^i - B_\lambda^0)$$

where  $B_\lambda^i$  is the coloured matter content after the thermal treatment and  $B_\lambda^0$  is the coloured matter content prior to thermal treatment.

The content of coloured matter formed in min (IJ/min) is calculated according to:

$$B_{v,\lambda} = \Delta B_\lambda / t$$

where  $t$  is the duration of thermal treatment.

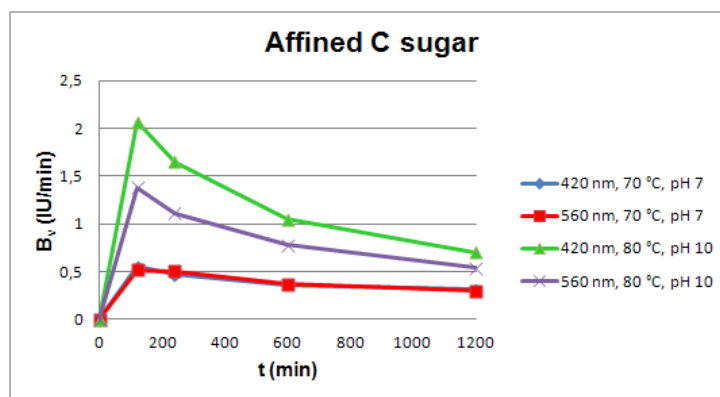
## RESULTS AND DISCUSSION

Basic quality parameters of affined C sugar are presented in Table 1. The obtained values were expected for that type of an intermediate product.

Table 1. Basic quality parameters of affined C sugar

Parameter	Affined C sugar
Dry matter content (%)	98.75
Sucrose content (°S)	94.5
Invert sugar content (%DM)	0.46
pH value	5.97
Coloured matter content 420 nm (IJ/DM)	7384
Coloured matter content 560 nm (IJ/DM)	1769

The nonenzymatic browning reaction in the affined C sugar solution (pH 7, 70 °C) mainly involves alkaline degradation of invert sugars and results in formation of coloured matter (Heitz, 1995). The content of coloured matter in the affined C sugar solution measured at 420 nm and 560 nm increased during the thermal treatment, but the rate of their formation depended on the time of thermal treatment (Fig 1). At the beginning of the treatment the amount of coloured matter formed per min was the highest and decreased over the time (Fig 1).



**Figure 1.** Formation of coloured matter ( $B_v$ ) in affined C sugar solution over the thermal treatment (0-1200 min) at 70 °C and pH 7

With the aim to investigate the rate of formation of coloured matter in Maillard reaction sugar model system, the affined C sugar solution with amino acids (glutamine, lysine, valine, methionine, tryptophan) was thermally treated (pH 7, 70 °C and pH 10, 80 °C) and the formation of coloured matter over the thermal treatment was monitored. The glutamine was chosen together with other amino acids because glutamic acid represents the major amino acid in sugar beet regarding its content (van der Poel et al., 1998). In water solution both glutamine and glutamic acid are converted into pyrrolidone carbonic acid which is decomposed to produce glutamic acid in alkaline media (Nelson and Cox, 2012).

The addition of amino acids into the affined C sugar solution influenced the degree of browning (Fig 2), i.e. the contribution of Maillard reaction products to the overall browning of the investigated system can be seen from the Figure 2 compared to the Figure 1.

As in the case of thermal treatment of the affined C sugar solution (pH 7, 70 °C), nonenzymatic browning in Maillard reaction sugar model system was the most intensive at the beginning of the treatment and became less intensive at the end of the examined period (0-1200 min), probably due to the decrease of the reactants.

The presence of glutamine in the model system did not influence the formation of coloured matter, while other examined amino acids increased their production. The effectiveness of amino acids in the formation of coloured matter was as follows: tryptophan > lysine > valine > methionine > glutamine.

pH and temperature influence the rate and extent of nonenzymatic browning reaction which occurred in in the Maillard reaction sugar model system (Fig 2). It is in line with previously existing statement that the Maillard reaction is influenced by increasing pH and temperature (Kim and Lee, 2008; Martins et al., 2001; Wang et al., 2011).

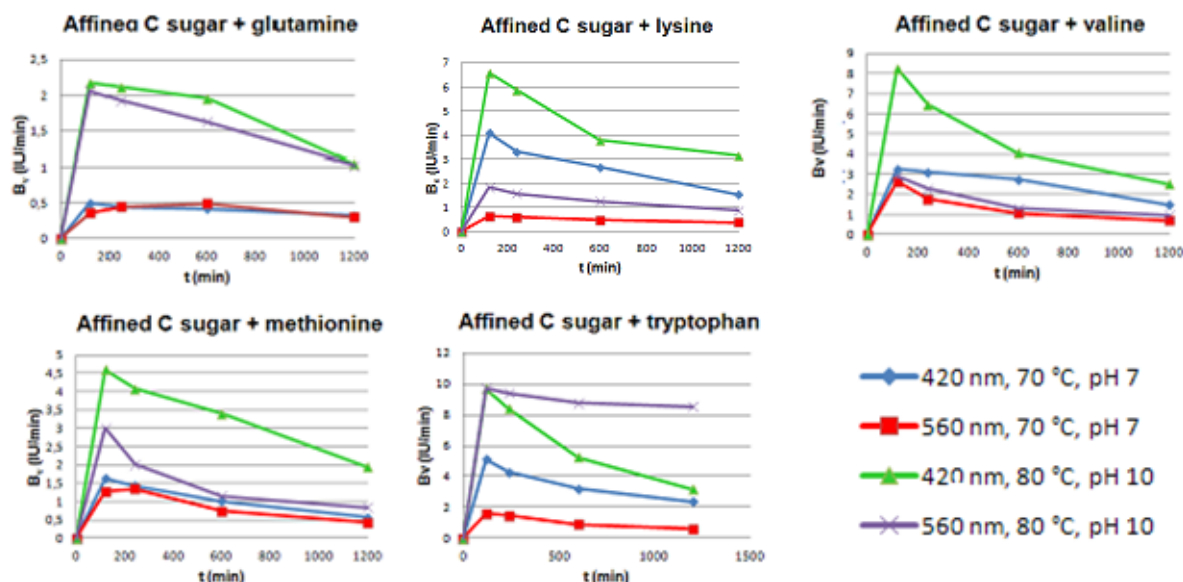
## CONCLUSIONS

It was found that the addition of amino acids in affined C sugar solution (Maillard reaction sugar model system) increase the formation of coloured matter compared to their production in affined C sugar solution. The effectiveness of amino acids in the formation of coloured matter was as follows: tryptophan > lysine > valine > methionine > glutamine.

pH and temperature strongly influenced the formation of coloured matter in investigated Maillard reaction sugar model system.

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**Figure 2.** Formation of coloured matter (Bv) in affined C sugar solution with amino acids (glutamine, lysine, valine, methionine, tryptophan) over the thermal treatment (0-1200 min) at 70 °C and pH 7 and 80 °C and pH 10

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## **УТИЦАЈ рН И ТЕМПЕРАТУРЕ НА РЕАКЦИЈУ НЕЕНЗИМСКОГ ТАМЊЕЊА ИЗМЕЂУ АФИНИСАНОГ Ц ШЕЋЕРА И АМИНОКИСЕЛИНА**

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**САЖЕТАК:** Афинисани Ц шећер је споредни производ добијен у тростепеном поступку кристализације шећера. Њега карактерише највећа количина бојених материја које настају током реакције неензимског тамњења, која прати фазу кристализације у производњи шећера.

У термички третираном (pH 7, 70 °C, 1200 min) раствору афинисаног Ц шећера бојене материје углавном настају као резултат алкалне деградације шећера.

Формирање бојених материја у модел систему за праћење Maillard-ове реакције (раствор афинисаног Ц шећера са појединачним додацима глутамин, лизина, валина, метионина и триптофана, c = 0.01 mol/L) вршено је термичким третирањем (pH 7, 70 °C, 1200 min и pH 10, 80 °C, 1200 min) и мерењем количине насталих бојених материја током термичког третмана.

Ефикасност испитиваних аминокиселина у формирању бојених материја у дефинисаном модел систему била је следећа: триптофан > лизин > валин > метионин > глутамин.

Пораст pH и температуре у модел систему за праћење Maillard-ове реакције пратио је пораст количине реакционих производа.

**Key words:** афинисани Ц шећер, аминокиселине, производи Maillard-ове реакције, боја

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