

## **COLOUR CHANGES DURING THE PROCESSING OF READY TO EAT CEREALS.**

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### **ABSTRACT**

Colour assays have been used to evaluate heat effects induced during the processing of ready to eat cereals. The cereal flakes were made of wheat flour, rice flour and yellow corn meal. Model systems comprising starch, glucose and amino acids were also included in this study. Glutamine, lysine, proline and asparagine were used in this study. The colour changes in the cereal flakes as well as in the model systems were followed up over a heating period of 180 minutes at 100°C. Three methods were used to detect colour changes being: spectrophotometric absorbance, tristimulus colorimetric and sensory evaluations. Tristimulus colorimetric evaluation was based on CIELAB uniform space  $L^*$ ,  $a^*$  and  $b^*$ , where  $L^*$  stands for "lightness",  $a^*$  for "redness" and  $b^*$  for "yellowness"

The results have indicated that the rate of browning of the model system containing lysine was much greater than that in case of systems containing glutamine, proline or asparagine. The rate of browning of the three cereal flours used in this study were increased in the following order; rice < wheat < corn.

The results have shown that the changes in the lightness  $L^*$  of the cereal flakes or the model systems with heating time follow first order kinetics. However, the change in the redness  $a^*$  and the browning index due to the development of the brown pigments follow zero order kinetics. The combination of the parameters ( $L^*a^*/b^*$ ) were used successfully to evaluate a total colour change with time and it followed zero-order kinetics.

**Keywords:** Breakfast cereals, Rice flour, Wheat flour, Yellow corn meal, Model systems, Reaction rates, kinetics, Modelling.

### **INTRODUCTION**

Ready to eat (RTE) cereals are processed grain formulations suitable for human consumption without further cooking. They are relatively shelf-stable, light weight and convenient to ship and store. They are made primarily from corn, wheat, oat or rice in about that order of the quantities produced, usually with added flavour and fortifying agents, Fast (1990). Cereal manufacturers in US have fortified sixteen of their cereals with 100% DV folic acid, vitamins B<sub>6</sub> and B<sub>12</sub>. However, vitamins A, C and E which act as antioxidants remain the most desirable components for general nutrient fortification; Sloan (2000). Since flaked cereals are usually consumed with milk, they should together approximate a good meal not only for Breakfast but also at other times, since milk is a good source of lysine. Therefore, Breakfast cereals are now considered one of the functional foods that are available in the world market, Goldberg (1994).

Regarding the nutritive value of Breakfast cereals, it is influenced very much by processing treatments which may cause some loss of nutrients and essential amino acids. However, these treatments may have a positive

effect towards inactivating protease inhibitors, thereby increasing the nutritional value. Flaked cereals are also advantageous over other types of cereals due to their lower fat, cholesterol and phytic acid contents; Kent and Evers (1993).

Processing of Ready-to-eat cereals involves several steps being; cereal cleaning and possibly pearling, cutting or grinding, addition of adjuncts such as salt, malt, sweeteners and flavouring materials, mixing with sufficient water to give a paste or dough of the required moisture content, cooking, cooling, partial drying, shaping into the desired form and toasting to maintain moisture content safe for packaging; Kent and Evers (1993).

One of the critical parameters affecting cereal quality is the extent of the Maillard Reactions between reducing sugars and amines occurring during processing and distribution; Fast (1990). These reactions cause changes in colour, flavour, functional properties and nutritional value; Kroh (1994). The rate of Maillard reaction is influenced by many factors such as temperature, pH, chemical composition and moisture content, Ames (1990). If the cereals are cooked with minimum amount of water or without water at high temperatures as in toasting, a non-enzymatic browning reactions may occur. Therefore, Breakfast cereals should be manufactured under controlled conditions to ensure products with adequate organoleptic and nutritional properties.

A good way to investigate the non-enzymatic browning reaction in heated foods is the use of model systems in which sugars and amino acids react under more simplified conditions. Starch / glucose / amino acid model systems were proved to be ideal for analyzing conditions favouring non-enzymatic reactions during the industrial processing of cereals; Sensidoni *et al.* (1999). Starch is used in these systems as a dispersion medium for sugar and amino acids, Lee *et al.* (1984) while glucose is used as a reducing sugar. It should be emphasized that in real process, reducing sugars arise from the changes in the conformation of starch and proteins, as well as their partial degradation due to mechanical stresses; Harper (1979).

Although, there are several studies on browning reactions in cereals such as those of Broyart *et al.* (1998) and Artigas *et al.* (1999), few data are available concerning the kinetics of colour formation during the processing of Breakfast cereals. This work was proposed to investigate colour changes induced by heating during the processing of Breakfast cereals as well as starch / glucose / amino acids model systems. Amino acids which are commonly present in cereal grains and which are highly reactive in Maillard reaction include Asparagine (Aspn), glutamine (Gln), proline (Pro) and lysine (Lys).

## **MATERIALS AND METHODS**

### **MATERIALS**

Pure standards of D-glucose, glutamine (Gln), lysine monohydrochloride (lys), proline (pro) and asparagine (aspn) were all products of Sigma Co., USA. All other reagents were of an analytical grade.

Lysine was selected because it is the limiting amino acid for the nutritional quality of protein from various cereal based foods. Asparagine, glutamine and proline were also used in this study because they are responsible for the formation of the prolamine fraction which is the main characteristics of the cereal proteins, Lásztify (1996).

Yellow corn meal and rice flour were obtained from king M Co. for food industries, Egypt. Wheat flour was obtained from flour land, Co., Giza, Egypt. They were all used for the preparation of Breakfast cereal flakes. Table (1) lists the composition of these raw materials according to the nutritional information labels provided by the manufacturing companies in addition to the colour parameters of these materials in terms of L\*, a\* and b\*.

**Table (1): Protein, carbohydrates and fat contents of the used raw materials per 100 grams and their colours.**

	Rice flour	Yellow corn meal	Wheat flour
Total protein	7.25	7.14	10.02
Total carbohydrates	79	78.57	72.10
Total fats	0.90	0	1.30
L <sub>0</sub> * (lightness)	93.42	84.69	91.89
a <sub>0</sub> * (Redness)	0.02	3.40	0.51
b <sub>0</sub> * (yellowness)	7.42	29.06	10.38

## **METHODS**

### **1- Preparation of the model systems:**

Homogenous mixtures of corn starch (970g), D-glucose (20g) and amino acids (10g) were prepared. Glutamine, lysine, proline and asparagine were used in this study. To each 1 kg of the mixture, 0.18-0.20 Kg water was added. The quantities of these ingredients were selected as to simulate the actual composition of cereals. The procedures of checking and adjusting the model systems were carried out as described by Clawson and Taylor (1993).

### **2- Preparation of cereal flakes:**

Cereal flakes were made from wheat flour, rice flour and yellow corn meal. The flow chart in Fig (1) shows the procedures followed to process Breakfast cereals.

For comparison, the model system containing starch / glucose / lysine was also processed using same procedure as cereal flakes. This model system was selected out of the four systems used in this study because lysine is highly reactive and because it is the limiting amino acid for the nutritional quality of protein from various cereal based foods, Dexter *et al.* (1984).

### **3- Browning studies:**

Brown pigment formation by heating effect was studied in case of each of the previously mentioned four model systems. Such pigment formation was also tested during the heating of each type of flour used as raw material in making Breakfast cereals (yellow corn meal, rice flour and wheat

flour). Samples of each of the flours and the model systems were sieved and two grams portions of each of the sieved fractions were introduced into 20 ml vials. The vials were then sealed to maintain control of moisture content; Sensidoni *et al.* (1999), and also to ensure a uniform heat distribution according to the recommendation of; Avila and Silva (1999). The sealed vials were then heated at 100°C for different periods ranging between 15 and 180 minutes.

**4- Analytical methods:**

**4-1- Moisture content of each sample was determined according to the AOAC methods;** AOAC (1980).

**4-2- Extraction of the coloured material:** Samples of the heated cereal flours and model systems (0.5g each) were finely ground and extracted with methanol. The extraction was carried out according to the method described by Fogliano *et al.* (1999).

Visible spectra of the supernatants were recorded over a wavelength range between 350 nm and 600 nm using LKB ultraspec. plus No 4054.

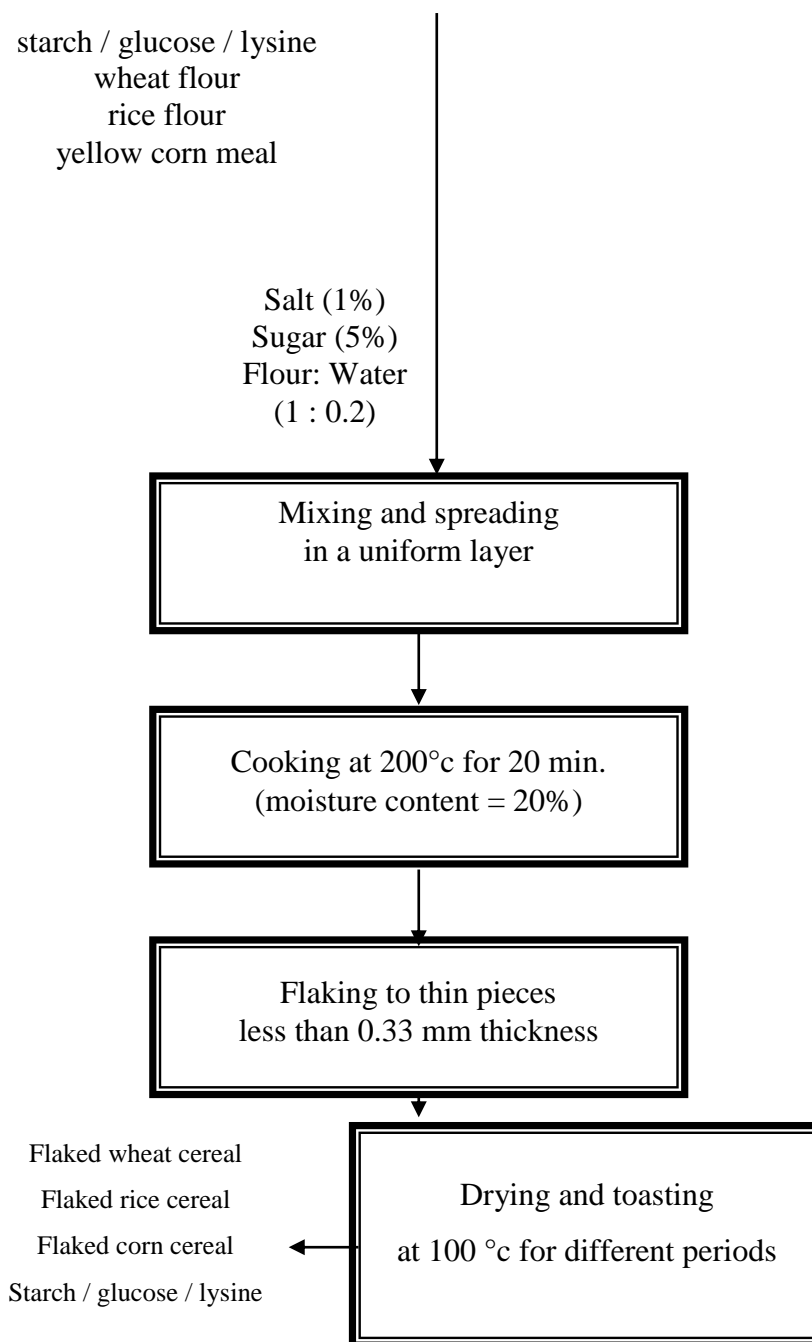
- 5- Measurement of the browning:** The absorbance readings of the methanol extracts were recorded at 420 nm and were corrected for turbidity by subtracting the absorbance readings at 550nm. The absorbance readings of the unheated samples (control) were subtracted from sample readings in each case.

The browning index was defined as described by Morales and Van Boekel (1999) as the optical density difference between that at 420 nm and that at 550 nm. The results were expressed as  $\Delta OD.g^{-1}$  after correction for dilution.

- 6- Measurement of the colour:** Changes in the colour of processed cereal flakes induced by their heating for different periods were followed up using a tristimulus colour analyzer; (hunter lab scan XE, Reston VA). The instrument was equipped with a sensor measuring head connected to a computer. The instrument was calibrated using a standard white tile ( $x= 77.26$ ,  $y = 81.94$  and  $z = 88.14$ ). The colour was measured in terms of redness;  $a^*$ , yellowness;  $b^*$  and lightness;  $L^*$ . The model system starch / glucose / lysine which was processed following same steps as cereal flakes was similarly tested for its colour changes by heating for different periods.

- 7- Sensory evaluation:**

Colour, taste, texture, aroma and overall acceptability of wheat, corn and rice flakes were subjectively determined by trained panelist using a nine points scale as described by Al Kahtami (1999) and Kwok *et al.* (1999).



**Fig. (1): Processing steps of cereal flakes**

## RESULTS AND DISCUSSION

### Brown colour formation of both model systems and cereal flours.

Figure (2) shows the rate of brown colour formation upon heating each of the four model systems used in the present study. The rate was assessed, as previously explained, according to the difference in the absorbance readings at 420 nm of the methanol extract of the heated sample and that of the unheated one. It can be seen that brown pigment formation follows zero order kinetics; a result which is supported by that of, Baisier and Labuza (1992). The estimated rates of Browning at 100°C are listed in Table (2) for the four model systems studied.

It is quite clear that the browning of the model system containing lysine was exceptionally fast and its rate was more than five times greater than that of the system containing asparagine. This indicates that lysine is more reactive than the other amino acids used in this study in connection with Maillard reaction. This is because lysine molecule has two amino groups;  $\alpha$  and  $\beta$  and the latter one is considered one of the most reactive groups in the Maillard reaction chemistry, Coultate (1989).

In general, it can be stated that the browning rates increase in the following order: starch / glucose / asparagine < starch / glucose / glutamine < starch / glucose / proline < starch / glucose / lysine. This order reflects the increasing order in the reactivity of amino acids from asparagine up to lysine. This result agrees well with the established fact that the reactivity of amino acids increases as the number of carbon atoms in the molecules increases; Candiano *et al.*, (1993).

The absorbance spectra of the methanol extracts of the starch / glucose/ lysine system after being heated for different periods at 100°C are represented in Fig. (3). It is clear that the absorbance of the methanol extract at all wavelengths ranging between 350 to 600 nm increases as the heating period increases. This increment is more remarkable at 350 nm during the early heating stages (up to 30 min). This is attributed to the early formation of pre-melanoidins, Fogliano *et al.*, (1999) and Morales and Van Boekel (1999). By prolonging the heating period, the formed pre-melanoidins are converted into high molecular weight melanoidins which absorbs at 420 nm; Wijewickreme *et al.* (1997).

The rate of brown colour formation during heating of yellow corn meal, wheat and rice flours are compared in Figure (4). The browning rate increases in the following order: rice flour < wheat flour < yellow corn meal. The high browning rate in case of yellow corn meal can be attributed to the interaction of some native pigments, Like zeaxanthin with the melanoids which are formed during heat treatment. The estimated values of the browning reaction rate are listed in Table (3). The data were highly significant ( $r^2 \geq 0.88$ ,  $p < 0.01$ ), in all the studied samples.

**Table (2): Zero order rate constant (Au. g<sup>-1</sup> min.<sup>-1</sup>) for browning index in model systems at 100°C.**

<b>Model system</b>	<b>K x 10<sup>3</sup>, Au. Min.<sup>-1</sup> g<sup>-1</sup></b>	<b>R<sup>2</sup></b>	<b>SE</b>
Starch / glucose / lysine	2.84	0.993**	0.017
Starch / glucose / glutamine	0.957	0.972**	0.012
Starch / glucose / proline	1.126	0.999**	0.002
Starch / glucose / asparagine	0.570	0.999**	0.0060

\*\* highly significant  $P \leq 0.01$

SE: standard error

**Fig. (2): Brown colour development in starch / glucose / amino acids model systems heated at 100°C as a function of heating time.**

**Fig3,4**



**Table (3): Zero order rate constant (Au. g<sup>-1</sup> min.<sup>-1</sup>) for browning index in different cereal flour at 100°C.**

System	K x 10 <sup>3</sup> Au. g <sup>-1</sup> min. <sup>-1</sup>	R <sup>2</sup>	SE
Wheat flour	0.653	0.885 <sup>**</sup>	0.013
Yellow corn meal	1.000	0.965 <sup>**</sup>	0.011
Rice flour	0.210	0.985 <sup>**</sup>	0.0014

<sup>\*\*</sup> highly significant P ≤ 0.01

SE: standard error

**Colour parameters L\*, a\*, b\* and L\* a\* / b\* of the flaked cereals and starch / glucose / lysine model system.**

As previously explained, the colour parameters measured were the lightness (L\*), yellowness (b\*) and the redness (a\*). These values are useful for complete characterization of the browning that took place during the processing of Breakfast cereals.

Figure (5) shows the relation between the natural logarithm of the lightness value, L\* of cereal flakes as well as the model system starch / glucose / lysine, (processed as cereal flakes) and the heating period. It is obvious that the fall in lightness with time follows first order kinetics. Such fall in lightness indicates that the samples were turning darker. Broyart *et al.* (1998); Lozano and Ibarz (1997) and Barreiro *et al.* (1997) have also proved this first order relationship between lightness and time. The estimated rate constants and corresponding r<sup>2</sup> values for the three types of flakes as well as for starch/ glucose / lysine model system are listed in table (4).

Figure (6) shows the relation between the change in the yellowness (Δb\*) of the different samples of cereal flakes as a function of heating time. (Δb\*) stands for the difference between the value of (b\*) of the heated and the unheated samples. It is obvious that (Δb\*) increases during the early heating stages and it starts to level - off or decrease during the late stages. This can be attributed to the fact that two different reactions may occur during processing, the non enzymatic browning reactions and pigment destruction. For example, some of the carotenoids that are naturally present in the cereal may be destroyed by heating causing colour loss; Ilo and Berghofer (1999).

Fig. (5): Changes in the L-value colour parameter of starch / glucose / lysine model system and wheat, corn and rice flakes as a function of heating time.

Table (4): First-order rate constant for the decrease of lightness L\* in case of starch / glucose / lysine model system and cereal flakes.

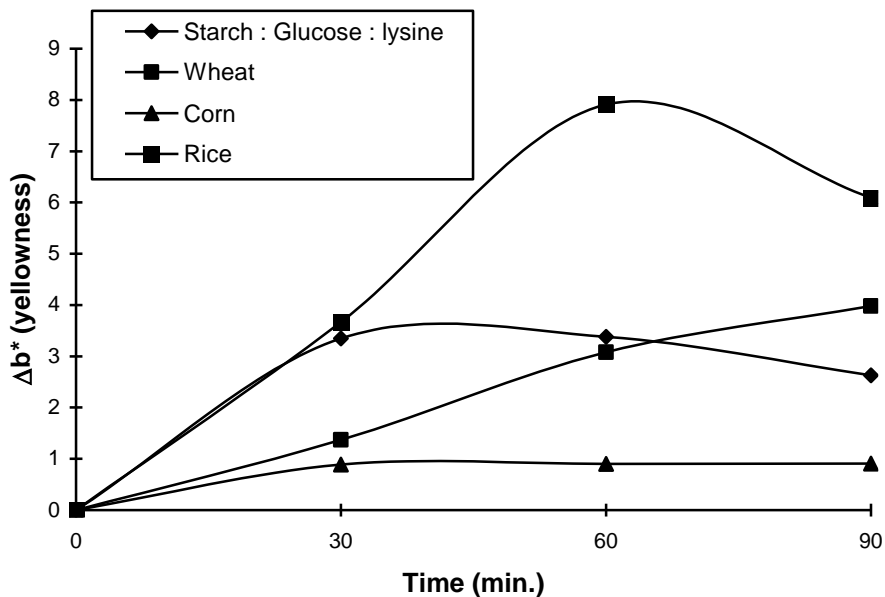
System	Rate constant, K x 10 <sup>3</sup>	R <sup>2</sup>	SE
Starch / glucose / lysine	6.33	0.954**	0.075
Wheat flakes	1.53	0.965**	0.016
Corn flakes	1.50	0.940**	0.020
Rice flakes	1.00	0.889**	0.019

\*\* highly significant P≤0.01

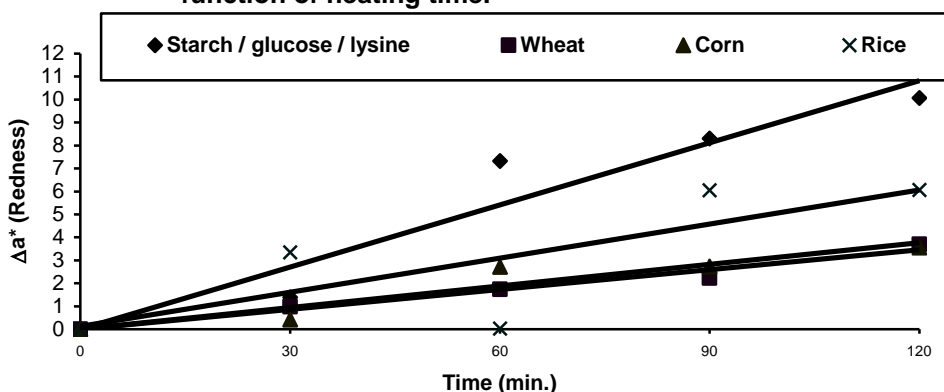
SE: standard error

The change in  $\Delta a^*$  (amount of redness) value with time (Fig 7) followed zero order reaction kinetics ( $r^2 \geq 0.770$ ,  $P < 0.0$ ) Table (5) lists the values of the rate constant for the change of the redness of the model system and that of the cereal flakes induced by heating effect.

Colour concentrations could be also successfully expressed by combining the three attributes  $L^*$ ,  $a^*$  and  $b^*$  as recommended by Shin and Bhowmik (1995) in terms of  $(L^* a^*/b^*)$ . The results are shown in Fig (8) where the data fitted zero order kinetics ( $r^2 \geq 0.880$ ,  $p < 0.05$ ). Table (6) shows the calculated values of the rate of change of  $L^* a^*/b^*$  with heating time in case of the model system as well as in the cereal flakes.



**Fig. (6):** Changes of chromaticity coordinate  $b^*$  of starch / glucose / lysine model system and wheat, corn and rice flakes as a function of heating time.



**Fig. (7):** Changes of chromaticity coordinate  $a^*$  of starch / glucose / lysine model system and wheat, corn and rice flakes as a function of heating time.

Table (5): Zero order rate constant for the change in redness ( $\Delta a^*$ ) values in case of starch / glucose / lysine model system and cereal flakes.

System	Rate constant, K (colour unit. min <sup>-1</sup> )	R <sup>2</sup>	SE
Starch / glucose / lysine	0.090	0.924**	1.415
Wheat flakes	0.029	0.973**	0.260
Corn flakes	0.031	0.898**	0.579
Rice flakes	0.049	0.770**	1.479

\*\* highly significant  $P \leq 0.01$

SE: standard error

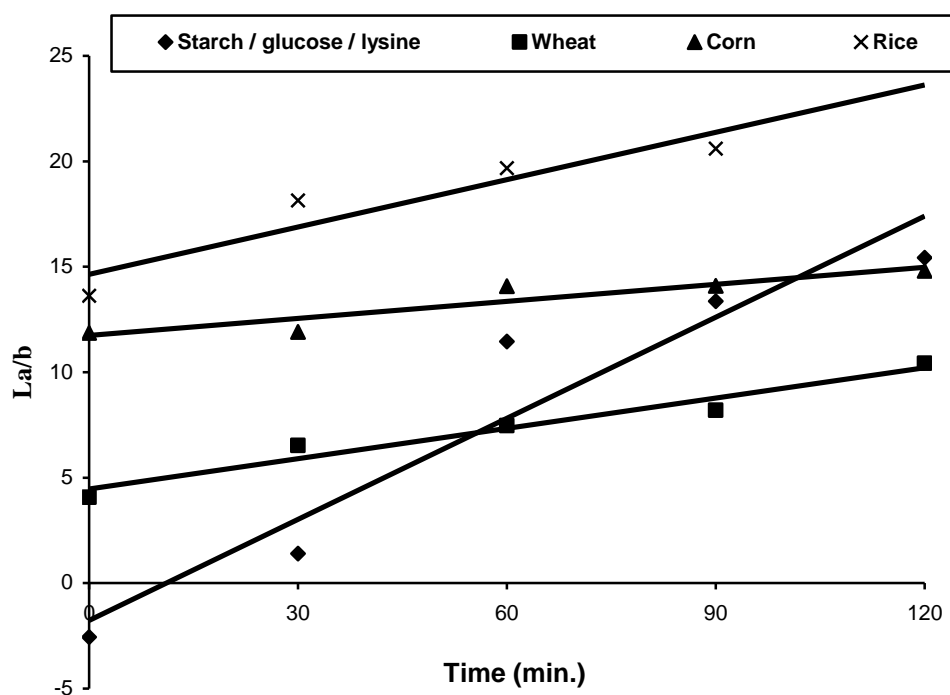


Fig. (8): Changes in the colour parameter,  $L^*a^*/b^*$  of starch / glucose / lysine model system and wheat, corn and rice flakes as a function of heating time.

**Table (6): Zero order rate constant for (L\*a\*/b\*) values in case of starch / glucose / lysine model system and different cereal flakes.**

System	Rate constant, K (colour unit. min <sup>-1</sup> )	R <sup>2</sup>	SE
Starch / glucose / lysine	0.160	0.916**	2.64
Wheat flakes	0.048	0.957**	0.558
Corn flakes	0.027	0.868**	0.575
Rice flakes	0.075	0.877**	1.325

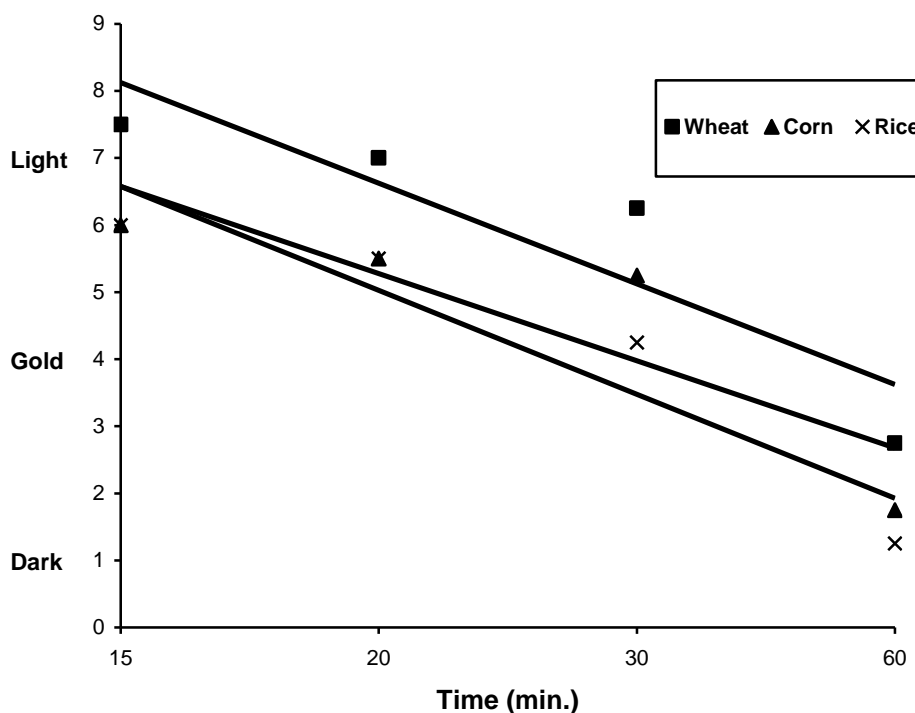
\*\* highly significant  $P \leq 0.01$

SE: standard error

**Sensory evaluation:**

The results of sensory evaluation showed insignificant ( $p > 0.05$ ) relationship between the toasting time and taste, texture, aroma and overall acceptability of the cereal flakes used in this study.

The mean panel score which represent the visual colour quality; showed an inverse relationship between colour and heating time (Fig 9). This relation seemed to obey zero order rate which agrees with that of kwok *et al.* (1999).



**Fig. (9): Effect of heating time on the panel score for the colour of cereal flakes.**

**CONCLUSION**

The results of this work indicated that the rate of brown pigment formation follows zero – order kinetics. The browning rates increase in the following order : starch / glucose / asparagine < starch / glucose / glutamine < starch / glucose / proline < starch / glucose / Lysine. Also the rate of browning of the three cereal flours were increased in the following order, Rice < Wheat < Corn .

Colour parameters presented in the forms of L\*, a\* and b\* values were measured in this study. It is quite clear that the L\* values (lightness) of both cereal flakes and the model systems are lowered as a result of heating time increment and followed first order kinetics.

The data also indicated that  $\Delta b^*$  (amount of yellowness ) increases during the early stages and starts to level-off during the later stages of heating time, so no reaction rates can be determined. The change in  $\Delta a^*$  (amount of redness )has a direct proportional relationship with time and it is proposed to follow zero order reaction rate. A total colour change were found to be successfully explained in the form of (L\*a\*/b\*) and followed zero –order kinetics.

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تغيرات فى لون الحبوب المجهزة للأكل خلال التصنيع  
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استخدمت تقديرات اللون لتقييم التأثيرات الحرارية التي حدثت خلال إعداد الحبوب المجهزة للأكل . وقد أعدت هذه الرقائق من دقيق القمح ودقيق الأرز ودقيق الذرة الأصفر. ولمعرفة هذه التأثيرات بشكل أوضح استخدمت في هذه الدراسة **Model systems** اشتملت على النشا والجلوكوز وبعض الأحماض الأمينية مثل الجلوتامين، الليسين ، البرولين وحامض الأسبارجين. التغييرات التي حدثت في اللون في كل من رقائق الحبوب وكذلك في **Model systems** قد تم تتبعها خلال فترة تسخين لمدة 180 دقيقة عند 100°م. استخدمت في هذه الدراسة ثلاثة طرق للتعرف على التغيير في اللون هي **Spectrophotometric absorbance** ، **Tristimulus colorimetric analysis** وطرق الاختبارات الحسية.

وقد أوضحت النتائج أن معدل تكوين اللون البني في **Model systems** المحتوية على الليسين كانت أعلا من الأخرى التي احتوت على الأحماض الأمينية الجلوتامين ، البرولين، الأسبارجين. في حين أن معدل تكوين اللون البني في أنواع الدقيق المختلفة في هذه الدراسة قد زاد بالترتيب التالي: دقيق الأرز، دقيق القمح ثم دقيق الذرة.

وقد أوضحت النتائج أن التغييرات التي حدثت في قيم  $L^*$  في كل من رقائق الحبوب المستخدمة أو في **Model systems** خلال فترات التسخين المختلفة يمكن أن تمثل بتفاعل من الدرجة الأولى. في حين أن التفاعلات التي حدثت في قيم  $a^*$  ومعدل تكوين اللون البني يمكن تمثيلها بتفاعل من الدرجة صفر. ودمج هذه القيم السابقة على الصورة  $(L^* a^* / b^*)$  وجد أنها تمثل بكفاءة تغير اللون الكلي خلال فترات التسخين المختلفة ويمكن تمثيلها بتفاعل من الدرجة صفر.