A NOTE ON THE RELATIVE RATES OF REACTION OF SEVERAL REDUCING SUGARS AND SUGAR DERIVATIVES WITH CASEIN

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Results previously reported for the casein-reducing sugar reaction[1] have been obtained using glucose as the reacting carbohydrate. In the present communication, data showing the comparative rates of reaction of several sugars and sugar derivatives with casein are presented.

METHODS

The methods used were essentially those already described[1]. In all cases 1.5 equivalents of the carbohydrate were added for each free amino group of the casein, the solutions (at p_H 6.3) were freeze-dried and the solid materials, after adjustment to the required moisture content, were held at 70% relative humidity and 25 or 37° C. Measurements of free amino-N were made by the VAN SLYKE method, and of colour with the LOVIBOND Tintometer.

RESULTS

Losses in the free amino-N content of the casein resulting from reaction with the various carbohydrates at 25° C are summarized in Table I, the figures being read off from curves drawn through a larger number of experimental points. Initial rates of reaction (as % drop in amino-N per day) obtained by drawing tangents to the curves of

TABLE I

RELATIVE RATES OF REACTION OF CASEIN WITH REDUCING CARBOHYDRATES

Carbohydrate	Loss of amino-N after days at 25° C (mg/g total N)			Colour after days at 37° C (Lovibond Y+R units)		
	4	8	16	4	8	16
Xylose.	23.2	27.7	30.5	1.8	2.4	2.7
Arabinose	15.9	22.6	24.3	1.7	2.2	2.5
Glucose	8.2	12.0	16.9	0.1	0.2	0.3
Galacturonic acid	8.5	13.0	19.1	0.5	0.8	1.2
Lactose	6.8	9.2	10.6	0.0	0.0	0.0
Maltose	5.7	8.4	10.7	0.0	0.0	0.0
Glucuronic acid	4.1	7.0	11.0	1.9	2.5	2.9
Fructose	0.9	1.5	2.4	0.0	0.0	0.0

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best fit at their points of origin were as follows, xylose, 19.7; arabinose, 14.6; glucose, 5.3; galacturonic acid, 4.2; lactose, 4.1; maltose, 3.6; glucuronic acid, 2.4; and fructose, 0.4.

The development of a brown colour in the casein-sugar mixtures at 37° C is also shown in Table I. Even at this temperature no detectable change occurred in the samples containing fructose, lactose and maltose during 20 days.

DISCUSSION

Amino-N

The aldo-pentoses xylose and arabinose reacted far more rapidly with the free amino groups of the protein than did the aldo-hexose glucose which, in turn, reacted more rapidly than the aldo-disaccharides lactose and maltose. The keto-hexose fructose reacted extremely slowly, but the fall in amino-N progressed steadily during at least 37 days and there was no indication that the observed reaction was due to impurities in the sugar: glucose was shown to be absent.

There was little difference between the *initial* rates of reaction of glucose and of the disaccharides, but the reaction with the disaccharides subsequently slowed down much more rapidly than did that with glucose, possibly for reasons associated with the larger size of the disaccharide molecule. A comparison of the data for the two uronic acids is also of interest. The rapid reaction of galacturonic as compared with glucuronic acid is probably due to the existence of this substance in the dry state predominantly in the acid form, whereas glucuronic acid exists mainly as the lactone with a double ring configuration.

Colour

The observations of colour development show an even greater difference between the sugars than do those of reaction rate between the reducing group and the amino group. Fructose, which is one of the most readily caramellized of the sugars, failed to produce any detectable colour in admixture with casein, even after 37 days at 37° C. Lactose and maltose had produced no colour after 20 days, when the amino-aldehyde reaction had virtually ceased. Colour production with the pentoses was far more rapid in relation to the amino groups destroyed than was the case with glucose, and with glucuronic acid colour development was most rapid of all the compounds tested despite the relatively slow rate of reaction with the amino group shown by this substance.

These observations confirm and extend those previously made with glucose or lactose and crude milk protein[2]. Whether the explanation of the variable production of colour lies simply in a different degree of stability of the protein-sugar complexes to the degradation producing browning, whether the protein-sugar complex in decomposing produces substances such as furfurals which are able to catalyse the caramelization of free sugar, or whether browning can result in this system (as it can in aqueous systems at higher temperatures[3]) from reactions independent of the amino group cannot at present be decided. No case of the production of discoloration under the mild conditions of these experiments has, however, been encountered to date without some accompanying loss of free amino-N.

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SUMMARY

1. The rates of loss of free amino-N and of browning in mixtures of casein with various reducing sugars at p_H 6.3, 70% relative humidity and 25 or 37° C have been studied.

2. The pentoses xylose and arabinose reacted most rapidly with the protein amino groups, followed by glucose, galacturonic acid, lactose, maltose and glucuronic acid. Fructose reacted extremely slowly.

3. Browning was most rapid with glucuronic acid, followed by the pentoses, galacturonic acid and glucose. Lactose, maltose and fructose produced no discoloration within the period of the experiments.

RÉSUMÉ

1. Nous avons étudié les pertes de l'amino-N libre et le brunissement des mixtures de la caséine avec des sucres réduisants variés à p_H 6.3, au 70% d'humidité relative et à 25° ou à 37° C.

2. Les pentoses xylose et arabinose étaient les plus rapides à réagir avec les groupes amino protéiques. Ils furent suivis du glucose, de l'acide galacturonique, du lactose, du maltose et de l'acide glycuronique. Le fructose était le plus lent à réagir.

3. Le brunissement le plus rapide fut observé avec l'acide glycuronique, suivi des pentoses, de l'acide galacturonique et du glucose. Le lactose, le maltose et le fructose ne produisirent aucune décoloration dans l'espace de temps reservé aux expériences.

ZUSAMMENFASSUNG

1. Der Grad des Verlustes an freiem Amino-N und der Bräunung wurde in Mischungen von Kasein mit verschiedenen reduzierenden Zuckern untersucht. p_H war 6.3, relative Feuchtigkeit 70% und Temperatur 25° oder 37° C.

2. Die Pentosen Xylose und Arabinose reagierten am schnellsten mit den Protein Aminogruppen; dann folgten Glukose, Galakturonsäure, Laktose, Maltose und Glukuronsäure. Fruktose reagierte am langsamsten.

3. Bräunung fand am schnellsten mit Glukuronsäure statt; dann folgten die Pentosen, Galakturonsäure und Glukose. Laktose, Maltose und Fruktose verursachten keine Verfärbung während der Zeit des Versuchsverlaufes.

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