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Nonenzymatic Browning of Tomato Products

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ABSTRACT

The effects of ascorbic, malic, galacturonic, citric and glutamic acids on nonenzymatic heat-induced browning were studied with special reference to formation of HMF and degradation of reducing sugars occurring in tomato (fructose and glucose).

Galacturonic acid was found to slow down browning reactions, which allows the behaviour of tomato products prepared by the hot-break technique to be differentiated from that of cold-break products with regard to nonenzymatic browning.

INTRODUCTION

Colour of fruit may change during processing and preservation due to the development of brown substances (Weast & Mackinney, 1941). Browning may be caused either by enzymes present in the fruit or by reactions of certain components of the juice.

In the case of a thermally-stabilized product, in which enzymatic activity has been almost completely destroyed by heat, particular attention should be given to nonenzymatic browning.

Knowledge of the chemical mechanism underlying this reaction is still rather limited; detrimental changes in colour are influenced by a series of variables, such as humidity, oxygen and the use of inhibitors (SO_2, etc.) that are closely correlated and some of which may be controlled by means of suitable processing, production and handling methods.

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The variables that cannot be directly controlled during industrial processing are those involved in the composition of the product. The browning process is strictly linked to the concentration of reducing sugars, nitrogenous compounds, acids and metallic ions in fruit. The intermediate 5-(hydroxymethyl)-2-furfural (HMF), produced in the early stages of nonenzymatic browning (Maillard), after condensation of reducing sugars and the amino compounds, is the first sign of degradation of foods (Joslyn & Marsh, 1935).

In this work, the interference of the main components of tomato juice in nonenzymatic browning reactions, and in particular in the formation of HMF and degradation of reducing sugars (fructose and glucose), is investigated.

MATERIALS AND METHODS

Listed below are the components used and their approximate concentrations in tomato (Davies, 1957; Goose & Binstead, 1964; Davies & Kempton, 1975; Geigy, 1980): fructose (1·5%), glucose (1·0%), citric acid (0·5%), malic acid (0·04%), ascorbic acid (0·2%) (all from Carlo Erba Analyticals, Milan, Italy), L-glutamic acid (0·2%) (E. Merck, Darmstadt, FRG) (all with a purity spectrophotometrically checked >99%). In addition galacturonic acid was also used (0·2%) (Aldrich-Chemie, Steinheim, FRG).

Galacturonic acid is normally present in traces in tomato; it is formed as a result of processing and its absence is a sign of total enzymatic inactivation undergone by crushed tomatoes before subsequent processing (Kertesz, 1951; Doesburg, 1965). Of the various amino acids, L-glutamic acid was chosen because, besides occurring in greater concentration in tomato, it is the most reactive amino acid in the condensation reactions with sugars (Eichner & Ciner-Doruk, 1981).

A preliminary study showed that the percentage variations of these components were small during the reactions and it is therefore impossible to investigate browning spectrophotometrically because of imperceptible variations in absorbance, especially after only a few hours' heating. Model solutions were therefore prepared in which, keeping the same ratio between the components, concentrations were increased; in particular, individual percentages were multiplied by a factor of 5·3.

The model solutions were adjusted to pH = 4·5 with NaOH 1 N in 100 ml calibrated flasks and heated for different periods (2, 4, 6, 12, 18 and 24 h) at 85°C in a thermostat with ventilation. This temperature was chosen to simulate technological conditions of enzymatic inactivation and sterilization, as well as to accelerate degradation reactions occurring during storage; in

fact preliminary tests had shown that, compared with normal storage temperature (30°C), heating at 85°C accelerates these reactions about 12 times (from 10–13 times according to the different compounds examined).

The observations by other authors (Kato *et al.*, 1981; Johnson *et al.*, 1983) that metal ions (in particular Ca^{2+}, Na^{+} and K^{+}) occurring in higher levels in tomatoes, accelerate nonenzymatic browning reactions were also confirmed. The objective of this work was mainly to study the behaviour of those components with comparatively little variability, which can therefore more easily be simulated by model solutions. However, metal ions, in particular K^{+} and Na^{+}, are markedly affected by tomato growing conditions, especially fertilization, and this makes their variability ranges very wide. Oxygen is present during some processing steps and remains, though at reduced pressure, in the finished packaged product.

In the model solutions used, very little residual oxygen remained, owing to the small headspace and the sealing of the containers immediately after heating; in any case, considering preparation and testing conditions, oxygen was nearly constant in all systems.

Water activity of a 22–29° Brix tomato concentrate, to which the model solutions refer, is 0·97–0·99 (Chirife *et al.*, 1981); therefore any deviations of the solutions from these values could not be regarded as significant sources of error.

HMF and sugar determinations were carried out using an HPLC system consisting of a Model 712 automatic sample injection module with a 20 μl injection loop (Waters Associates, Milford, MA, USA). In particular, HMF was determined by a method previously reported (Porretta *et al.*, 1989), with a Radial-Pack C-18 column (250 mm × 4 mm i.d.) having a mean particle diameter of 10 μm (E. Merck, Darmstadt, FRG), a Model 480 LC spectrophotometer (Waters Associates, Milford, MA, USA) set at 285 nm as detector and water–methanol (90:10, v/v) as eluent.

For sugar determinations a NH_2 column with the same particle diameter as the previous one (E. Merck, Darmstadt, FRG), acetonitrile–water (80:20, v/v) as eluent and a Model 410 differential refractometer (Waters Associates, Milford, MA, USA) as detector were used. In both cases flow rate was 1·5 ml/min and injection volume 10 μl.

Peak heights and areas were calculated with a Model C-R3A Cromatopac integrator (Shimadzu, Japan).

HPLC grade organic solvents and water were purchased from Carlo Erba Analyticals (Milan, Italy) and Baker (Deventer, Holland), respectively.

The experimental data were statistically processed to ascertain differences between the various model systems at the different times (concentrations by time and samples), as well as the existence of interactions between the solutions and the different reaction times. Replicates of all results were

subjected to two way ANOVA using the SPSS-PC (SPSS-PC, 1975) with a 99% confidence limit.

RESULTS AND DISCUSSION

No significant variation in pH ($>0{\cdot}1$) was recorded during the heating reactions in any of the model solutions investigated.

Formation of HMF

HMF formation was facilitated in particular by the low pH value of the system and by the presence of organic acids and their salts (Ramchander & Feather, 1975; Lee & Nagy, 1988).

Figure 1 shows that, at 24 h, the solution that produced the greatest amount of HMF (250 ppm) was the one in which only galacturonic acid was absent, followed by the solution with all the components (175 ppm) and by the one without ascorbic acid (154 ppm). After the first 6 hours' heating a change in the pattern of the reaction was observed, the solution without galacturonic acid needing more than 6 h to become the first in producing HMF.

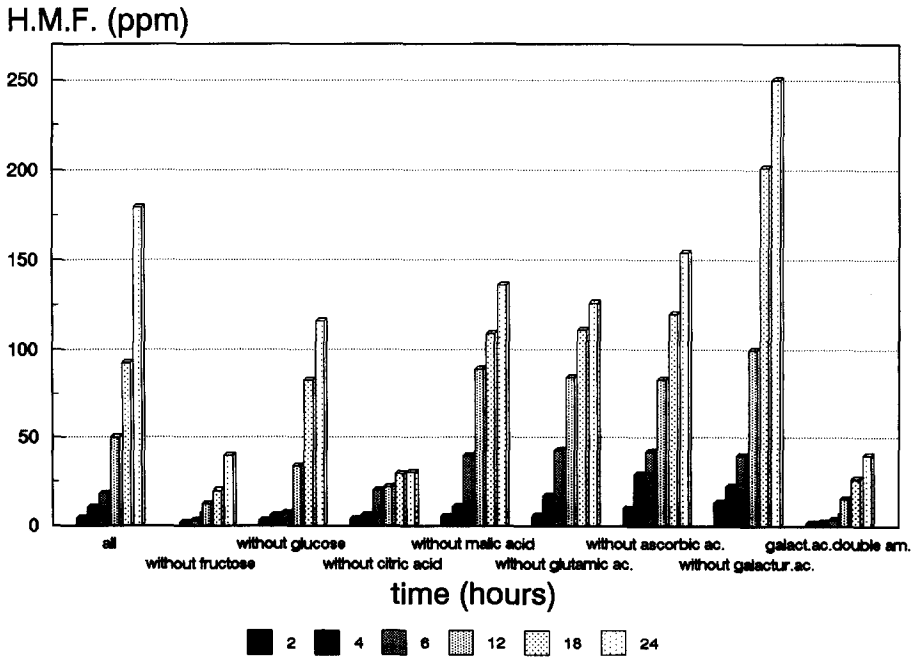

Fig. 1. HMF production for the different model solutions at different heating times.

At 24 h the following results were observed about the reactivity of the various solutions investigated: solution without galacturonic acid (250 ppm HMF) > with all the components (179 ppm) > without ascorbic acid (154 ppm) > without malic acid (136 ppm) > without glutamic acid (126 ppm) > without glucose (116 ppm) > without fructose (39·4 ppm) > without citric acid (30·2 ppm). On the basis of these results, we investigated the behaviour of galacturonic acid and, in particular, whether a greater quantity of this acid could slow down formation of HMF.

Thus, model solutions were prepared with all the components under investigation and with double the amount of galacturonic acid; in this case the production of HMF was considerably smaller as compared with that recorded when it was absent (approx. 45 ppm versus approx. 250), viz. galacturonic acid slows down the formation of HMF.

The behaviour of galacturonic acid could be extremely important for a better storage of tomato products: cold-break products, which contain a certain amount of galacturonic acid because of incomplete enzyme inactivation, would have a longer shelf-life thanks to a lower HMF formation and consequently less browning, as compared with hot-break ones which contain, on the other hand, only traces of galacturonic acid because of the complete enzymatic inactivation.

In order to confirm that the slowing-down effect of galacturonic acid on the nonenzymatic browning reaction was due to galacturonic acid rather than to the milder heat treatment applied to cold-break products, the time-course of HMF formation in some model solutions heated at 60°C was monitored.

Figure 2 shows the results obtained, which allow the conclusion that the time-courses of reaction at the two temperatures (85°C and 60°C) are quite similar. This was not the case with a cold-break tomato paste, for it is impossible to prepare such a product free from galacturonic acid and therefore to ascertain whether the effect observed is due to the temperature or to the acid.

The data obtained from the test with model solutions, especially those regarding HMF production, were confirmed on a tomato paste of the same composition as that of the model solutions. To this end, two tomato pastes of 25° Brix, one hot-break, the other cold-break, were prepared. The results show that a very good agreement exists between model solutions and tomato pastes (Fig. 3).

Fructose degradation

It was confirmed that fructose is the most reactive reducing sugar in nonenzymatic browning (McWeeney, 1973).

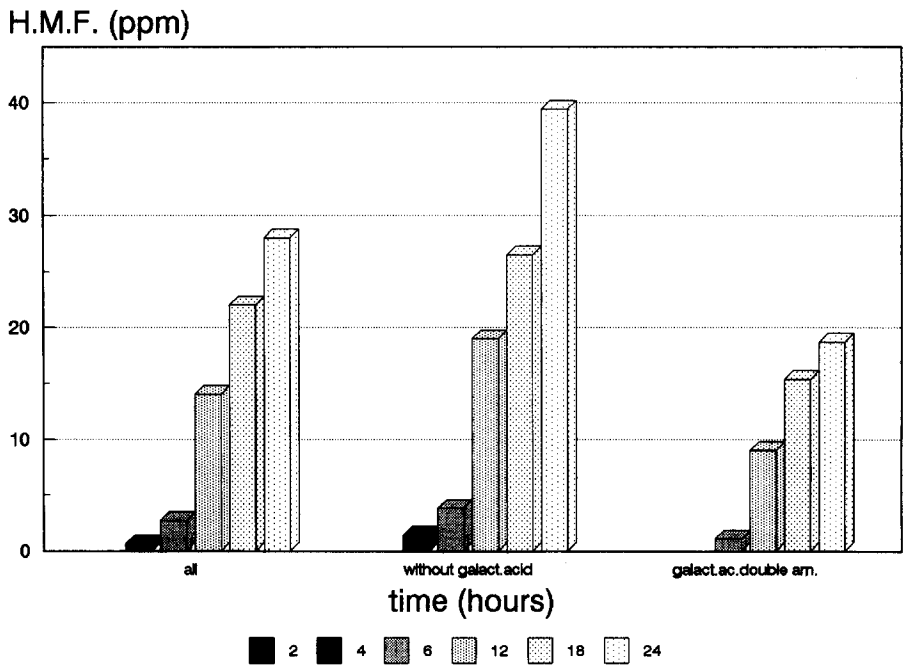

Fig. 2. HMF production for three particular model solutions at different times heated at 60°C.

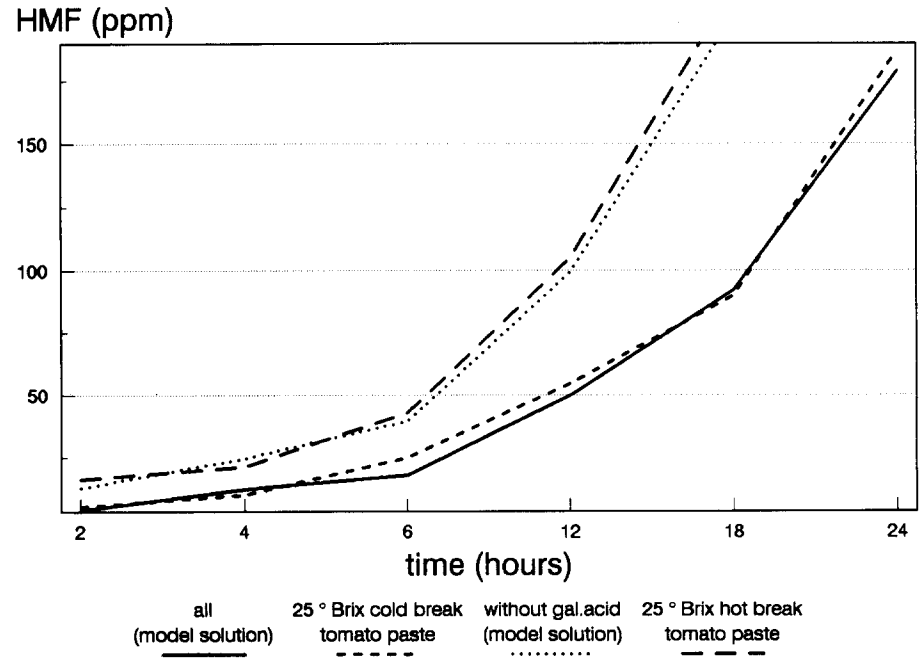

Fig. 3. Comparison between model solutions and tomato pastes.

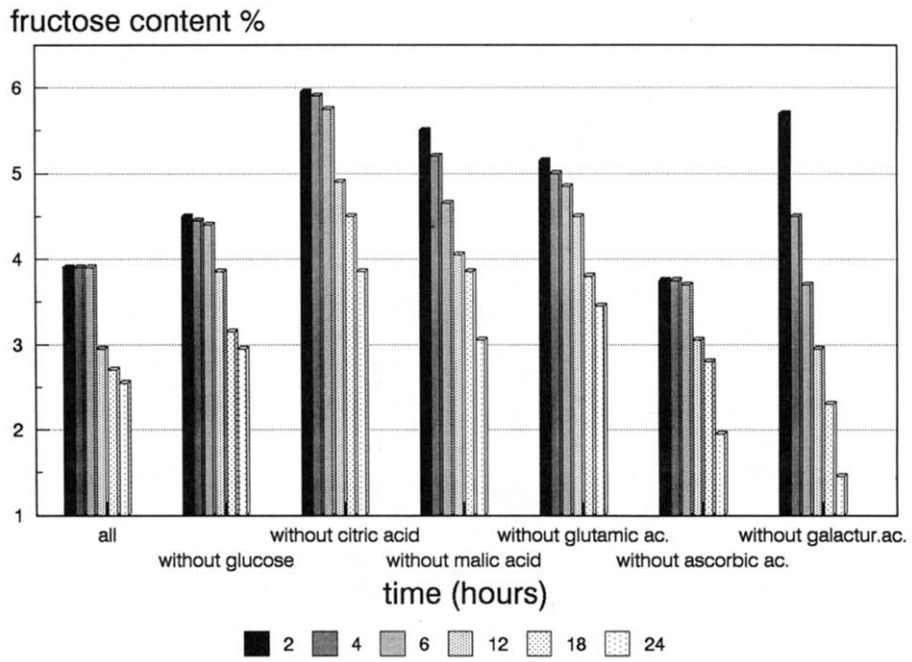

Fig. 4. Fructose content for the different model solutions at different heating times.

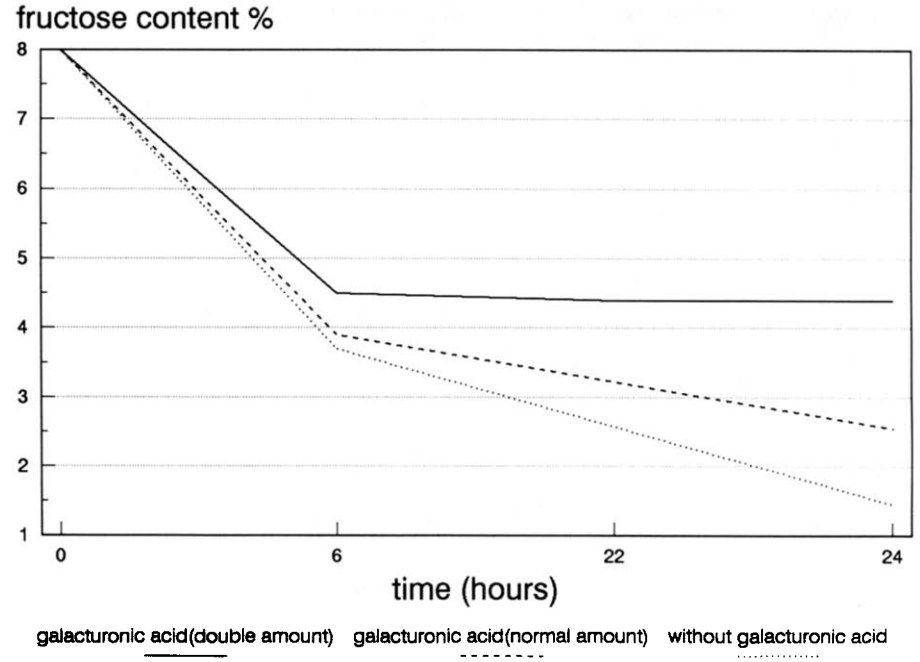

Fig. 5. Fructose content for the different model solutions containing different galacturonic acid amounts.

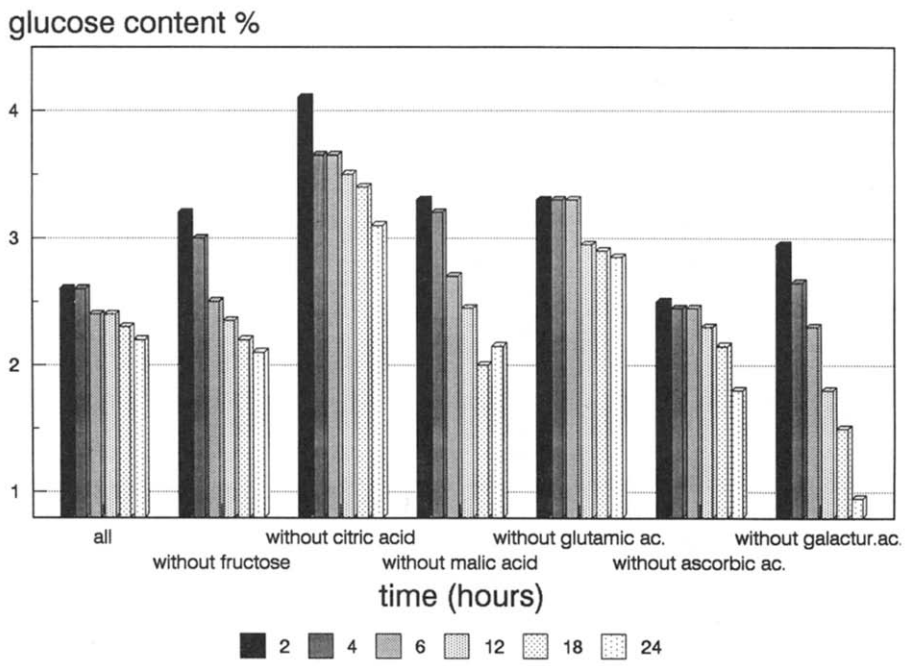

Fig. 6. Glucose content for the different model solutions at different heating times.

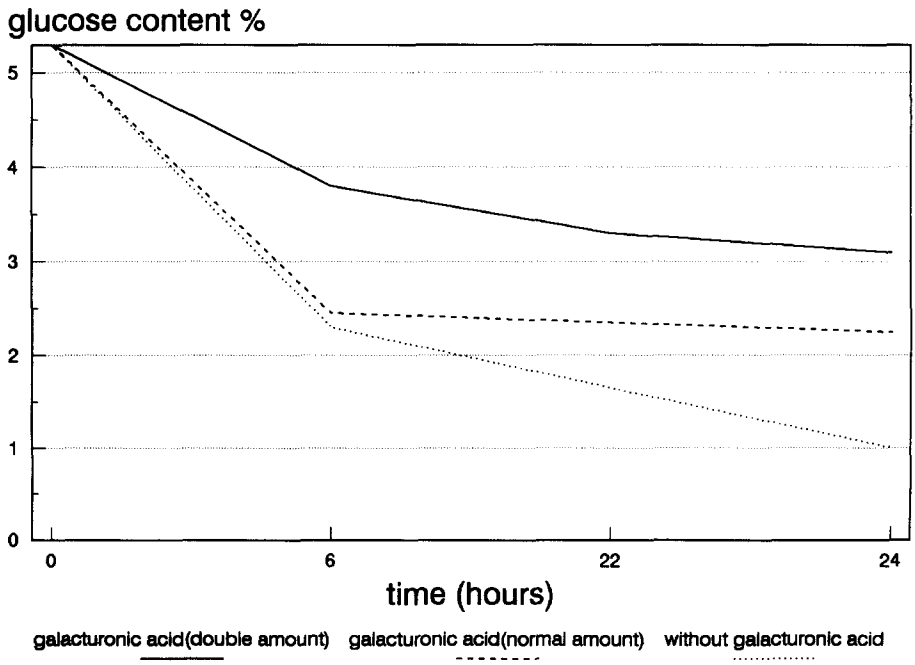

Fig. 7. Glucose content for the different model solutions containing different galacturonic acid amounts.

In the first 6 hours' heating at 85°C, the solution showing the least fructose degradation was the one without citric acid (Fig. 4) which, as has already been pointed out, favours nonenzymatic browning.

In the case of tomato products, it should be noted that citric acid is added, in particular to crushed tomatoes and peeled tomatoes, in order to lower the pH so as to allow their treatment by milder processing, with improved preservation of their sensory properties as a result.

The model solution with greatest fructose degradation was the one without galacturonic acid. This seems to confirm, as has already been observed in the production of HMF, that galacturonic acid would have a protective action on reducing sugars.

With regard to the other components, the following order of fructose degradation in 24 hours' heating was observed (the percentage of degraded sugar is given in brackets): without citric acid (52·5%) < without glutamic acid (57·5%) < without malic acid (62·5%) < without glucose (63·5%) < with all the components (68·7%) < without ascorbic acid (73·5%) < without galacturonic acid (82·5%).

In 24 hours' heating fructose degradation was 46·3% in the presence of double the amount of galacturonic acid (Fig. 5).

Glucose degradation

Figure 6 shows the trend for the different model solutions with respect to glucose degradation in the first 24 h. The results are similar to those for fructose: without citric acid (41·5%) < without glutamic acid (46·2%) < with all the components (57·6%) < without malic acid (60·3%) < without fructose (61·3%) < without ascorbic acid (66·0%) < without galacturonic acid (81·1%).

Figure 7 shows the different trends for the solutions containing different amounts of galacturonic acid.

Two-way analysis of variance

Tables 1–5 show the results of 2-way ANOVA.

For all model solutions the results of the 2-way analysis of variance are significant, i.e. at each sampling time the models are different. In particular there exist interactions between models and time: the heating treatments give results that are dependent on time.

For the model solutions heated at 60°C the results of the 2-way ANOVA are significant (Table 6).

Finally, Table 7 shows the results of the 2-way ANOVA for the two tomato products compared with the corresponding model solutions for

TABLE 1

2-Way Analysis of Variance for the Different Model Solutions as Regards the Production of HMF

Source of variation	*Sum of squares*	*DF*	*Mean square*	*F*	*Significance of F*
Times	192 536	5	38 507	10 306	0·000
Samples	74 781	7	10 683	2 859	0·000
2-way interactions times × samples	62 324	35	1 780	476	0·000
Explained	330305	47	7 027	1 881	0·000
Residual	175	47	3·736		
Total	330 480	94	3 515		

TABLE 2

2-Way Analysis of Variance for the Different Model Solutions as Regards Fructose Degradation

Source of variation	*Sum of squares*	*DF*	*Mean square*	*F*	*Significance of F*
Times	48·424	5	9·685	314·1	0·000
Samples	36·486	6	6·081	197·2	0·000
2-way interactions times × samples	8·275	30	0·276	8·9	0·000
Explained	93·185	41	2·273	73·713	0·000
Residual	1·295	42	0·031		
Total	94·480	83	1·138		

TABLE 3

2-Way Analysis of Variance for the Different Model Solutions as Regards Glucose Degradation

Source of variation	*Sum of squares*	*DF*	*Mean square*	*F*	*Significance of F*
Times	9·678 3	5	1·936	140·6	0·000
Samples	19·225	6	3·204	171·4	0·000
2-way interactions times × samples	3·376	30	0·113	6·0	0·000
Explained	32·279	41	0·787	42·1	0·000
Residual	0·785	42	0·019		
Total	33·064	83	0·398		

TABLE 4

2-Way Analysis of Variance as Regards Fructose Degradation of the Model Solutions having Respectively Double Content, Normal Content and Absence of Galacturonic Acid

Source of variation	*Sum of squares*	*DF*	*Mean square*	*F*	*Significance of F*
Times	4·750	2	2·375	78·2	0·000
Samples	7·565	2	3·783	124·6	0·000
2-way interactions times × samples	2·165	2	1·083	35·6	0·000
Explained	16·356	6	2·726	89·8	0·000
Residual	0·212	7	0·030		
Total	16·569	13	1·275		

TABLE 5

2-Way Analysis of Variance as Regards Glucose Degradation of the Model Solutions having Respectively Double Content, Normal Content and Absence of Galacturonic Acid

Source of variation	*Sum of squares*	*DF*	*Mean square*	*F*	*Significance of F*
Times	1·735	2	0·868	37·67	0·000
Samples	6·880	2	3·440	149·34	0·000
2-way interactions times × samples	0·605	2	0·303	13·141	0·004
Explained	10·612	6	1·769	76·78	0·004
Residual	0·161	7	0·023		
Total	10·774	13	0·829		

TABLE 6

2-Way Analysis of Variance as Regards the Production of HMF for the Model Solutions Heated at 60°C

Source of variation	*Sum of squares*	*DF*	*Mean square*	*F*	*Significance of F*
Times	4 574·2	5	914·836	1 784·1	0·000
Samples	369·744	2	184·872	360·5	0·000
2-way interactions times × samples	328·203	10	32·820	64·01	0·000
Explained	5 272·1	17	310·125	604·8	0·000
Residual	9·230	18	0·513		
Total	5 281·4	35	150·896		

TABLE 7
2-Way Analysis of Variance as Regards the Production of HMF for the Model Solutions and the Tomato Pastes

Source of variation	*Sum of squares*	*DF*	*Mean square*	*F*	*Significance of F*
Times	29 361	5	5 872·3	2·024	0·111
Samples	9 473·5	3	3 157·8	1·673	0·373
2-way interactions times × samples	22 918	15	1 527·9	0·527	0·900
Explained	61 753	23	2 684·9	0·925	
Residual	69 633	24	2 901·4		
Total	131 386	47	2 795·5		

HMF formation. The results show no significant differences nor does any interaction exist between samples and times.

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