FOOD-3030: Food Chemistry I

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Lab #: 2

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Abstract

The objective of this lab was to investigate the principle of the Maillard reaction and how it is affected by pH and the use of sulphites, to identify why sugars are reducing sugars using the Benedicts test and lastly to determine which preservation method could best help tp prevent enzymatic browning in avocados.

The formation of a red precipitate indicated that the carbonyl group of sugar reacted with the copper (II) ions and reduced them leading to the loss of the clear blue color. It was seen that glucose, lactose and fructose were all reducing sugars and therefore would participate in the Maillard reaction to form a glycosylamine.

The presence of sulphites was seen to prevent both and decrease the rate of enzymatic and non-enzymatic browning, by inhibiting the action of polyphenol oxidase and act as a stronger reducing agent. Using foods high in sulphites such as onions and garlic or antioxidants can help to reduce the extent of browning in susceptible foods.

Introduction

Browning reactions play a critical role in food chemistry and are responsible for the development of flavors, aromas, and pigments are commonly seen grilled meats, coffee, and caramel products. Browning reactions can either be classified as enzymatic or non-enzymatic (including caramelization and the Maillard reaction. The principles of caramelization are based on heating which releases volatile chemicals responsible for the aromas and dehydration of the sugars to produce four different classes of compounds (Bohrer, 2019). The Maillard reaction involves the formation of a glycosylamine via a condensation reaction between an amino acid and the carbonyl group (Hemmler et al., 2018). While both reactions produce brown pigments and alter flavors, the Maillard reaction involves the amine group of the sugar while caramelization does not.

If a sugar has a hydroxyl group bonded to the anomeric carbon and a free ketone or aldehyde, then it can be classified as a reducing sugar, all monosaccharides are reducing sugars. (Foist, n.d). When reducing sugars are heated in the presence of an alkali solution they form an enedoil that reacts with copper (II) ions reducing them and forming a brick-red precipitate. In experiment 2.1 a Benedict's test will be carried out to determine whether glucose and fructose, both monosaccharides, sucrose and lactose, both disaccharides and sorbitol, a sugar alcohol are reducing sugars or not (Ahern et al., 2019) For experiment 2.1 it is hypothesized that fructose, glucose, lactose will form a red precipitate indicating they are reducing sugars while sorbitol and sucrose will not.

It is hypothesized that the rate of the Maillard reaction is negatively impacted by low pH, as in acidic conditions the amine group becomes protonated and less nucleophilic and less reactive (Lersch, 2008). Experiment 2.2 will use phosphate and carbonic buffers to test the effect that acidic and alkali pH has on the rate of non-enzymatic browning in French onion soup. Non-enzymatic browning can be inhibited by the presence of sulfites which act as a stronger nucleophile in the Maillard reaction (Wedzicha et al., 1991). In experiment 2.3 we can assume that the set of test tubes that contain sodium bisulfites will not form a brown color as they will not participate in the Maillard reaction due to the presence of sulphites.

Enzymatic browning is mainly due to the oxidation of the phenols found in foods by polyphenol oxidase into quinones which then polymerize into melanins, that are responsible for the brown pigments (Wageningen University, 2017). Spoilage due to enzymatic browning in fruits and vegetables is a major concern for food processors as this contributes to food wastage, according to statistics the American household throws out nearly $1,600 USD of spoilt produce annually which equates to one-third of all the food grown (Chandler, 2016). Certain things can be done to reduce enzymatic browning by reducing the activity of polyphenol oxidase, such as lowering the pH, cooking, refrigerating and treating with antioxidants.

Methods & materials

All procedures were followed as per what was listed in Food Chemistry 1 : Laboratory outline 2. For part one of the experiment 2 sets off test tubes with the appropriate sugar solutions and Benedicts reagent were prepared, set A was submerged into boiling water for 10 minutes while the other set was left at room temperature.

Experiment 2 involved heating four mixtures of yellow onions and phosphate buffers at different pH levels (ph 4, 9 and 11) and noting the colour changes. A mixture was submerged in deionized water which acted as the control observation.

For the third experiment two sets of identical sugar and glycine (pH 9) mixtures were prepared. To set A, sodium bisulfites was added and both sets of test tubes were boiled for 20-30 minutes. It was noted if a brown colour developed in each test tube.

For the last experiment involved using avocados, onions and lemons. The avocados were immersed in olive oil, water, covered in plastic wrap and foil, treated with lime juice and onions, left with the pit in and one was left out in the open as a control, then they were all refrigerated for 24 hours. The colour was measured with a Minolta colorimeter after 24 hours and physical changes were noted. The avocado treated with lime showed the least extent of enzymatic browning, while the avocado treated with onions showed the most.

Small deviations that we're not noted in the methodology included using two solutions of sucrose - one being the regular sucrose solution and one dissolved in hydrochloric acid.

Results

EXPERIMENT 2.1

Table 1 : Time taken for solution set A and B to change from blue to orange

	Time taken for solution to change colour	
Sugar solutions	**SET A : Room temperature**	**SET B : Boiled**
Sucrose in water	No change	No change
Sucrose in HCL	No change	00 : 30 : 15
Lactose	No change	00: 01: 50
Glucose	No change	00 :01:11
Fructose	Turbid green solution / dirty brown colour observed at 01:14:23	00 :00 : 59
Sorbitol	No change	No change

The colour change observed in all of the above reactions was from clear blue to a bright orange unless otherwise noted

Figure 1 : Color changes for SET A : At room temperature

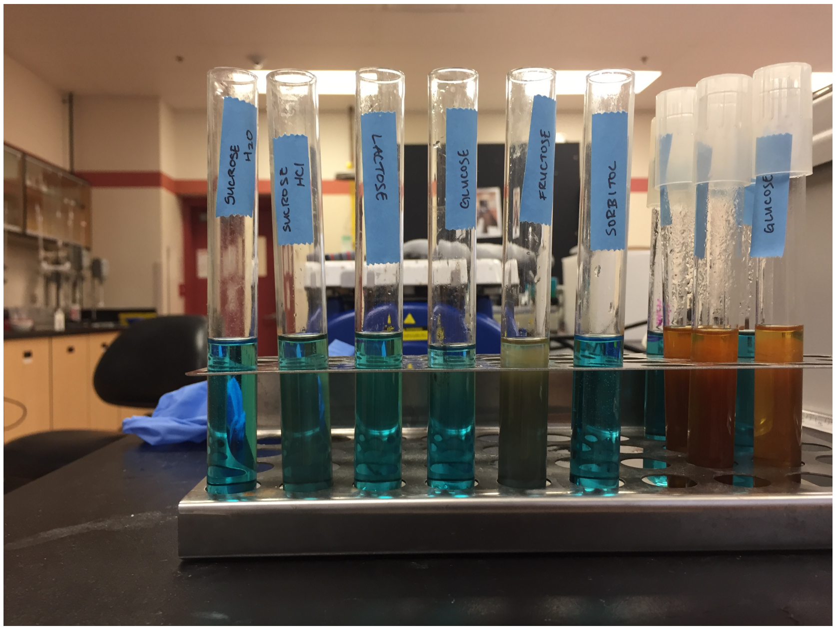
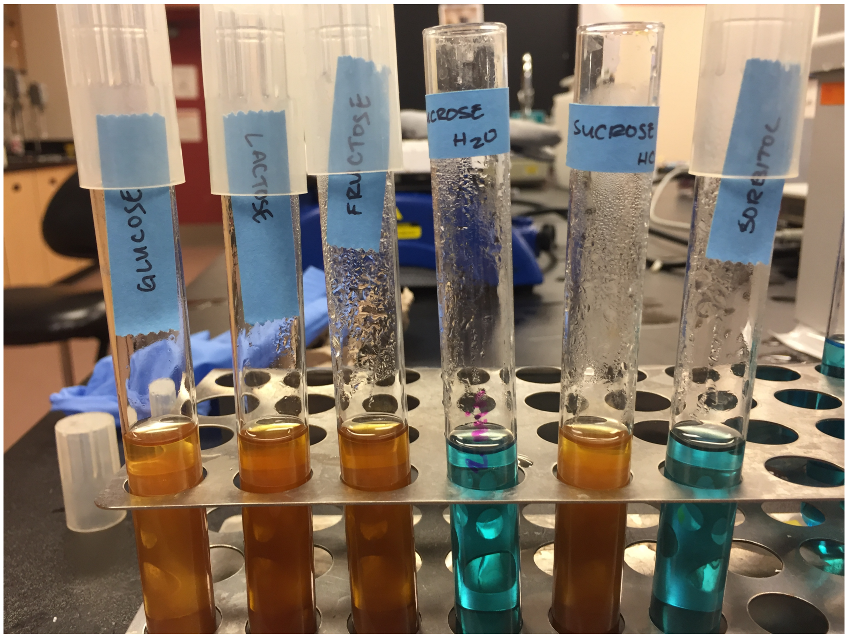


Figure 2 : Color changes for SET B : boiled



EXPERIMENT 2.2

Table 2 : Color changes and corresponding times for yellow onions in different pH solutions.

pH	Result (colour change)	Yellow	Yellow —> Orange	Orange —> Brown	Brown
dH2O	No change - remained white	x	x	x	x
4	No change - remained white	x	x	x	x
9	Changed to light yellow	00: 06 :40	x	x	x
11	Full colour change to brown	00 : 05 :20	00: 08 : 15	00: 10: 10	00: 15: 30

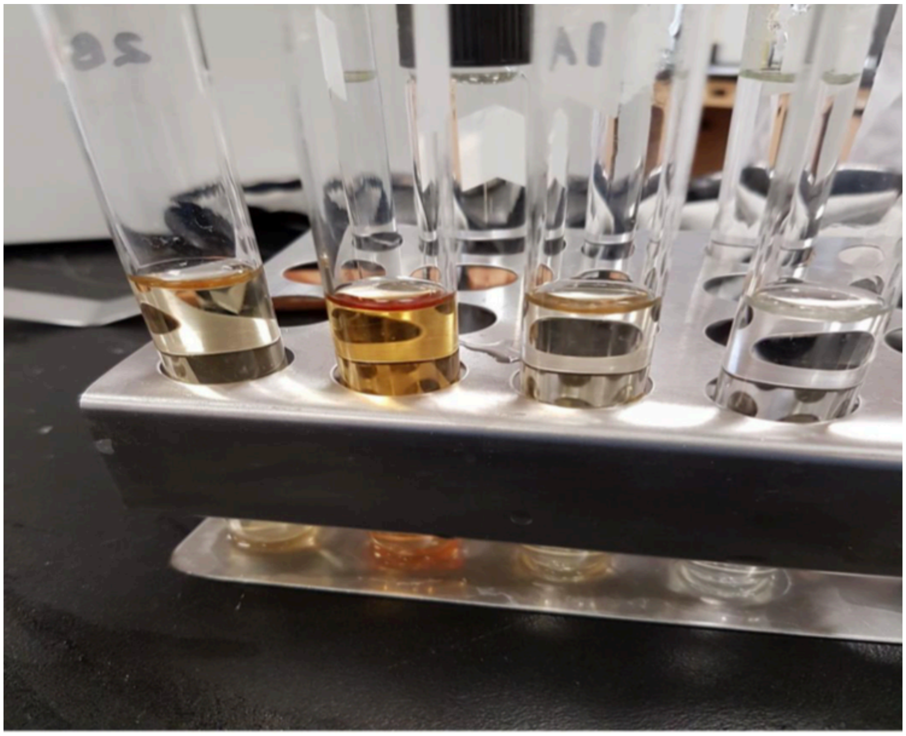
Figure 3 : Color changes in yellow onions in different pH solutions (4, 9, 11 & dH2O)

EXPERIMENT 2.3

Table 3 : Color change in set A (with sodium bisulphate) and the control set (without sodium bisulphate)

	Color change	
	Mixture set A : sodium bisulfite	**Mixture set B**
Glucose + Glycine pH 9	Brown (2nd darkest)	No change
Fructose + glycine pH 9	Brown (darkest)	No change
Lactose + glycine pH 9	Brown (3rd darkest)	No change
Sucrose + glycine pH 9	White	No change
Sorbitol + glycine pH 9	White	No change

Figure 4 : Color changes in set A (with sodium bisulphate) and the control set



EXPERIMENT 2.4

Figure 5 : Physical appearance of avocados after 24 hours under different conditions

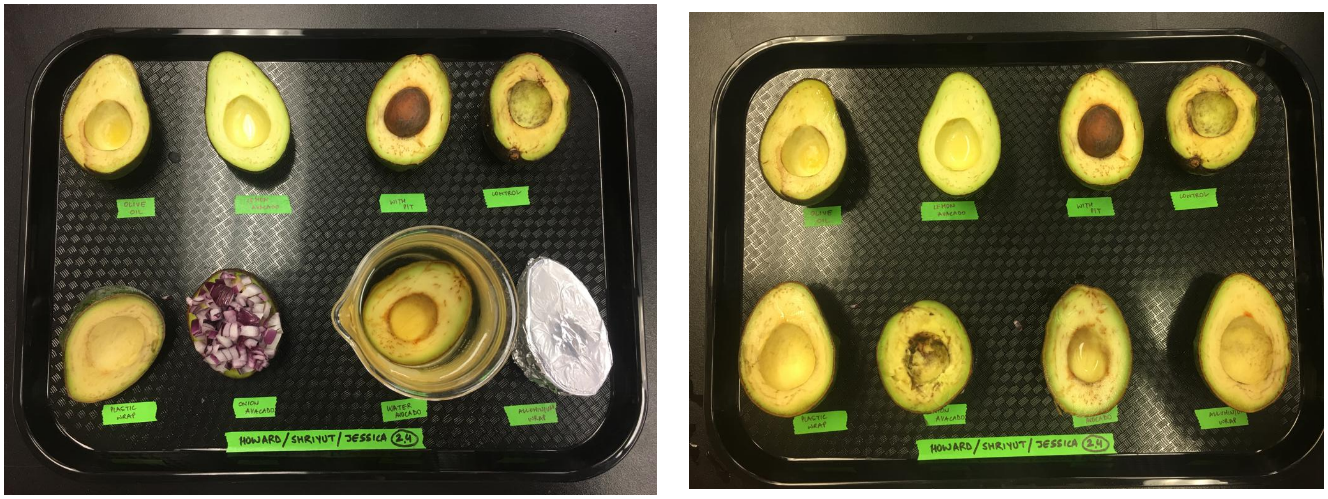


Table 4 : Minolta colorimeter reading values after 24 hours

Trail	L (D65)	A (D65)	B (D65)
Control	69.76	-2.26	34.83
Pit	73.64	-1.47	31.5
Lemon	79.4	-8.68	34.38
Oil	75.7	-7.1	33.48
Plastic wrapped	74.71	-2.19	31.93
Onion	69.72	-6.63	32.93
Water	67.61	-4.23	26.03
Foil	78.59	-2.84	34.21

Discussion

The results from table 1 show that except for fructose no other sugar solution at room temperature changed from the initial blue colour to form a reddish-brown precipitate. The fructose solution held at room temperature changed from clear blue to form a greenish turbid solution. The second set of test tubes (Set B) that was boiled for 10 minutes, showed that all of the sugar solutions, except for sucrose in water and sorbitol, formed a precipitate. In order for the energy barrier to be overcome and the initial bonds to be broken for the oxidizing reaction to take place between the sugar and the Benedicts solution, it must be heated. Benedicts test uses a solution made up of copper sulphite and sodium citrate. When heated in the presence of a reducing sugar, the carbonyl group will react with the Cu(II) ions and reduce them, thus forming a reddish - brown precipitate of copper (I) oxide and an enediol.

Therefore it is justified why there was no formation of a precipitate in set A as no reaction took place due to the absence of heat. The formation of a green precipitate in fructose test tube (Set A) indicates the presence of a trace amounts of reducing sugars. From experiment 2.1, it can be said that only sorbitol and sucrose (in water) did not act as reducing agents.

Sucrose does not have a free aldehyde and is bonded in a α-1,β-2-glycosidic (head-to-head) linkage, therefore the anomeric carbon is not free to react in redox reactions and hence it is not considered a reducing sugar when dissolved in water. However when sucrose is dissolved in a solution of hydrochloric acid, the glycosidic bond is broken via hydrolysis and producers the monosaccharides that make up sucrose ; glucose and fructose in solution. Glucose is a reducing sugar and therefore is responsible for the formation of a precipitate in the test-tube containing sucrose and HCL. Sorbitol was the other sugar that tested negative in the Benedicts test as it is a sugar alcohol, also known as glucitol. Sorbitol is produced upon the reduction of glucose during which the aldehyde group is converted to an alcohol group (https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/sugar-alcohols). Alcohols are not easily reduced and therefore sorbitol does not act as a reducing sugar in the Benedicts test and does not produce a reddish-brown precipitate as nothing reacts with the copper ions.

Fructose is a monosaccharide that is considered a non-reducing sugar despite being a monosaccharide. The carbonyl group in fructose is located in-between carbons making it a ketohexose. Aldehyde groups, that are located at the terminal ends of sugars tend to be easier to oxidized compared to ketones. Fructose therefore can be reduced by a stronger oxidizing agent and it undergoes a process known as tautomerism, in which it alternates between from a ketoses to an aldose, compared to glucose that can directly react with an oxidizing agent when present. Under the basic conditions of the Benedicts test, D-fructose isomerizes into D-glucose or D-mannose both of which are reducing sugars and are responsible for the green precipitate seen, making fructose test positive for reducing sugars in the Benedicts test(Raymond, 2009).

In experiment #2.2, the onions held in the higher pH solutions (pH 9 and 11) showed a significant colour change from their initial white colour to a dark brown. This indicates that they underwent Maillard browning. Maillard browning is the process that occurs when the amine group reacts with the carbonyl group to form a glycosylamine via a condensation reaction producing melanoidins, which are responsible for the brown pigmentation (Hemmler et al., 2018).

The Maillard reaction is dependent on pH as each amino acids is pH sensitive and has a set pKa value. At low pH’s the amino acids can become protonated due to the high concentration of hydrogen ions, this makes them less nucelophillic and less reactive. At higher alkaline pH's the nitrogen of the amino acid becomes deprotonated by loosing a proton, and in turn becomes more nucleophilic thus increasing the rate of Maillard browning. Each amino acid has its own pKa value, if the of the solution is above the pKa then the amino acid becomes protonated. The solutions with a higher pH showed development of brown pigments faster compared to those at a lower pH, indicating that the Maillard reaction was occurring at a faster rate.

The results from both experiment 2.1 and 2.2 have shown useful implications that can be used in food processing to ensure increase the shelf life and quality of products that are susceptible to non-enzymatic browning. In some products such as ultrahigh temperature milk, foods with high protein content and pilsner beers, development of compounds from the Maillard reaction such as melanoidins and strecker aldehydes are considered as flavour taints. Milk contains a high concentration of lysine, which is an amino acid with a pKa of 2.18 and is destroyed by the Maillard reaction, therefore lowering the pH to below its pKa and decreasing temperature can be used to control the extent of the non-enzymatic browning.

On the other hand, in products that browning is important for flavour development such as bread, coffee, chocolate and darker beers, the rate of the Maillard reaction can be increased to increase the browning and flavour development (Lund & Ray, 2017). The development of products associated with the Maillard reaction such as heterocyclic amine (HCA) which is a carcinogen found in meats cooked at a high temperature, can be reduced by using the methods discussed above (Mahmood & Tamanna, 2014.)

Having a very high ph of 9-11 can effect the quality and properties of the foods. Lower pH is known to inhibit the growth of most neutrophilic spoilage bacteria in food. At higher pH's the Maillard reaction progresses at a faster rate and therefore products that are susceptible to non-enzymatic browning will deteriorate faster. There are also associated costs with maintaining a high pH during food processing including corrosion to equipment such as caustic embrittlement due to the accumulation of alkali soda (Saha, 2016)

Minor errors may have included measurement errors while weighing the the onions in the various trails. A constant temperature may not have been maintained in the water bath as the lid was often opened and shut to permit other groups to use the water bath, thus leading to temperature fluctuations.

Sulphites are responsible for inhibiting Maillard browning due to the nucleophilic properties of the sulphite ion. Sulfites have antioxidant properties and help by reacting as a reducing agent and react with ortho-quinones that are produced in the Maillard reaction cascade, converting them back into diphenols that are colourless (Grotheer et al, 2005). In other substances that undergo enzymatic browning, sulphites inhibit the activity of polyphenol oxidase, which is responsible for the reaction. It was seen that sulphites bonded to polyphenoloxidase enzyme which caused a modification of the protein structure and therefore a loss of its properties (Sayavedra-Soto & Montogomery, 1986).

Alternatives to inhibit or slow down the Maillard reaction include using oxoreductase enzymes to catalyze the oxidation of sugars and prevent them from going down the Maillard reaction pathway. The use of fructosamine oxidase to degrade the amadori products produced during the reaction can help to limit the degree of non-enzymatic browning. Ohmic heating uses electrical resistance to heat the food evenly and can limit the Maillard reaction and inactivate peroxides faster than other thermal processing methods. Pulsed electrical fields inactivate the microbial cells by passing short bursts of high electric fields through the cell membrane. Lastly high pressure processing does not use heat and therefore helps to reduce the browning due to caramelization and the rate of the Maillard reaction (Marianne & Ray, 2017)

For experiment #2.4 based on physical observation, the avocado treated with lime juice showed the least extent of enzymatic browning followed by the plastic wrap, then aluminium wrap. Using lime or lemon juice can aid in slowing down the browning process as citric acid present is a strong antioxidant that will help reducing the rate of enzymatic browning. The citric acid prevents the oxidation of phenols to quinones by sacrificing itself, therefore preventing the development of brown pigments. While the lime juice did prevent browning, it altered the physical and sensory attributes of the avocado making them slightly slimy and altering the flavour.

Covering the avocado with plastic wrap or aluminum foil can help to minimize the contact with air and oxygen and prevent the oxidation reaction from taking place. In order to completely prevent any enzymatic browning the avocado should have been very tightly sealed, or preferably vacuum sealed in a pouch with no oxygen.

Keeping in pit in the avocado prevented enzymatic browning around the edges as it minimized the surface area that was in contact with oxygen. The area underneath the pit the avocado showed a lesser development of the brown pigments indicating a slower rate of browning within that portion.

Immersing the avocado in olive oil showed that there was a very minute degree of browning as it prevented the avocado from coming into contact with air, however it altered the sensory attributes and probably would leave a strong taste.

Placing the avocado in a water bath did not stop enzymatic browning as water contains oxygen and it altered the physical properties to the point where they avocado was considered very unappealing and uneatable due to the loss of firmness and texture (Butler, 2014).

These results are variant from what I hypothesized, which was that the avocado covered with onion pieces would show the least extent of browning. Avocados contain polyphenol oxidase which begins the process of enzymatic browning as soon as it comes into contact with air. Onions contain sulphur which is known to inhibit polyphenol oxidase and prevent the reduction of sugars during enzymatic browning (Yapi et al, 2015). However the results did not confirm my hypothesis as the avocado treated with onions showed the highest extent of enzymatic browning.

Using the results from the Minolta colorimeter taken at a length of 65nm, L refers to lightness, a refers to formation of red/green pigments and b refers to blue/yellow pigments. We can see that the avocado treated with lemon was the lightest, followed by foil, oil, plastic wrap, pit in, control, onion and water. Brown is a complex color made of a mixture of pigments including red, black and yellow. From the a values we can see that none of the samples had a strong development of red/green pigments as all the values were negative.

There are alternative methods to preventing an avocado from browning including blanching the avocados for a short period of time before cutting them, which helps to inactivate polyphenol oxidase enzyme and therefore prevent any form of enzymatic browning from occurring. Other methods include storing avocados with garlic, which also contains a high amount of sulphur.

Conclusion

Both enzymatic and Maillard browning play a key role in food processing and have significant impacts on foods sensory and physical attributes. Only reducing sugars can participate in the Maillard reaction and these can be identified using Benedict tests which produces a bright red precipitate in the presence of a reducing sugar. Our hypothesis stated that only fructose, glucose and lactose were reducing sugars, which was proven correct however sucrose in hydrochloric acid acted as a reducing sugar due to it decomposing into its monosaccharide components.

Methods such as using low pH, sulfites (found in onions or garlic), antioxidants and alternative processing methods such as high pressure processing, pulsed electrical fields and ohmic heating can be used to reduce the rate of the Maillard reaction. However these methods can have negative impacts on the quality of the food product as shown with the deterioration of firmness in the avocado treated with lime juice.

Our second hypothesis proved that the Maillard reaction was slowed down by lower pH's due to the deprotonation of the amino acids.

We can conclude that using sulphites to inhibit browning is the most promising method as demonstrated in experiment #2.4. It was also shown that using sulphites to inhibit Maillard browning only works for glucose, lactose and fructose as they undergo reducing reactions.

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