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INFLUENCE OF SUCHAROSE ON CHANGES IN AMYLOLYTIC ACTIVITY OF SALIVA AND PROTEOLITIC ACTIVITY OF GASTRIC JUICE

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In addition to polysaccharides, it was found that sucrose from the group of oligosaccharides, which is of great importance in human nutrition, can affect the activity of various soluble enzymes, which manifests itself in a reversible decrease in activity, linearly related to the concentration of sucrose [7].

It was found that the activity of α-chymotrypsin increased with an increase in the low concentration of sucrose, and then returned to its original activity with a greater increase in sucrose. At the same time, the binding of sucrose to the enzyme causes local changes in the micro environment. Tryptophan remains in close proximity. Molecular dynamics modeling demonstrates that sucrose acts as a stabilizer. Studies also indicate that sucrose is absorbed onto the surface of the enzyme. It has been found that hydrogen bonding and van der Waals interactions predominate over electrostatic interactions. The effect of sucrose on increasing the stability of α-chymotrypsin and the ability of sucrose to protect its native structural conformation was found. It has been shown that hydrogen bonds play an important role in the stabilization of the complex due to higher H-bond formation and lower surface hydrophobicity after modification with sucrose. [6].

In addition, it was found that high concentrations of sucrose have a multidirectional effector effect on the activity of individual gastrointestinal enzymes: negatively on amylase, activates lipase, increasing the intensity of fat digestion by the body; this leads to the induction of the activity and amount of the intestinal sucrose -isomaltase complex, which enhances the hyperglycemic effect of sucrose. Considering that salivary ά-amylase and pancreatic amylase have similar substrate specificity and mechanism of action, the data obtained with the participation of salivary ά-amylase can be extrapolated to pancreatic amylase and conclude that, with the simultaneous use of foods, they contain starch and sucrose , the intensity of starch digestion in the intestine decreases [2].

In addition, the influence of the interaction of sucrose with proteins and the effect on their activity was found. Thus, at pH 7.0, in the case of the interaction of ovalbumin with sucrose, an increase in the hydrophilicity of the protein is noted, followed by a decrease in the surface activity of the protein, and in the case of Na-casein ate, an increase in the hydrophobicity of the protein and an increase in the surface activity of the protein. A decrease in pH to 5.5, accompanied by increased competition between less charged proteins and sucrose for water molecules, causes an increase in the hydrophobic aggregation of proteins. The features of the latter process mainly determine the change in the surface activity of proteins under the influence of sucrose [4].

In addition, the interaction of sucrose with starch has been established. The interaction of components in the sucrose-starch-water system with the inclusion of gelatinized starch was studied. It was found that more sucrose interacts with gelatinized starch than with untreated starch [five].

Purpose of the study: explore the effect of sucrose on changes in the activity of salivary amylase and proteolytic activity of gastric juice.

Material and methods. In vitro work studied the effect of sucrose on the hydrolysis of starch by salivary amylase and casein proteins, egg albumin (albumin) and hemoglobin by gastric juice. The activity of salivary amylase [1] was studied using starch together with sucrose as a substrate after preliminary 30 minutes of their joint incubation. Different ratios of starch and sucrose were used: only starch without sucrose, 1 part starch and 1 part sucrose, 1 part starch and 5 parts sucrose, 1 part starch and 10 parts sucrose. Amylolytic activity was studied after 30 minutes of saliva exposure to a mixture of starch and sucrose at various pH values from 2 to 7. At the same time, a

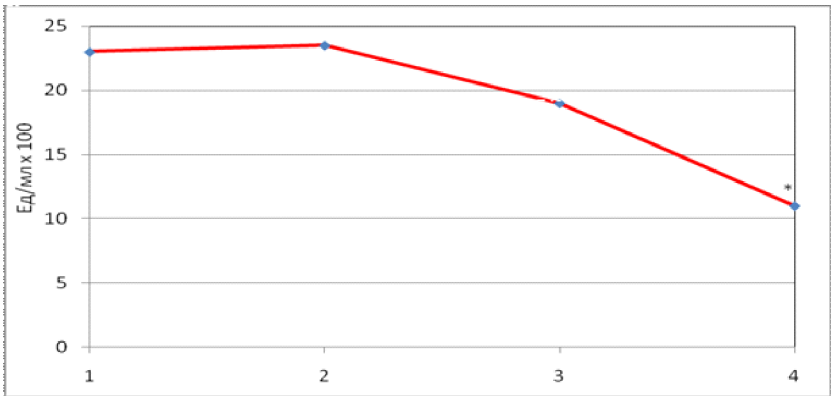
study was made of changes in amylase activity in absolute U/ml at pH 7, as well as in percent at pH from 2 to 7, by identifying the amount of starch cleaved by amylase, according to the change in the intensity of the blue color of starch in the presence of iodine. At the same time, the index of hydrolyzed starch was calculated as a percentage by the difference in the result of the intensity of starch staining without the presence of salivary amylase and the intensity of starch staining in the presence of salivary amylase in relation to the result of the intensity of starch staining without the presence of salivary amylase.

In addition, the activity of the total proteolytic activity (OPA) of gastric juice [3] was studied using casein proteins, egg albumin and hemoglobin as a substrate, together with sucrose after a preliminary 30-minute joint incubation. A different ratio of proteins and sucrose was used: only protein without sucrose, 1 part of protein and 1 part of sucrose, 1 part of protein and 5 parts of sucrose, 1 part of protein and 10 parts of sucrose

Statistical processing was carried out by the method of variational statistics with the calculation of average values and their average errors, determination of the coefficient of reliability of the Student-Fisher difference (t). Differences were considered statistically significant at $p<0.05$ or less.

Results. As a result of the studies of the effect of a mixture of starch and sucrose on the amylolytic activity of saliva. It was found that when using only starch as a substrate, the activity of salivary amylase was 23 ± 1.7 U / ml x 100. At the same time, using starch and sucrose as a substrate in a ratio of 1: 1, significant changes in amylolytic activity in relation to the use of only starch was not observed and it was equal to 24±1.6 U/ml x 100. The use of the same substrate in a ratio of 1:5 did not cause a significant decrease in the amylase activity index to 19±1.3 U/ml x 100, but the use of the same substrate in a ratio of 1:10 caused a significant decrease in amylolytic activity to 11±0.7 U/ml x 100 (Fig. 1).

Also, the results of these studies showed that with the use of only starch, the greatest the manifestation of salivary amylase activity in relation to the maximum indicator of 100% at a pH of 6.5 was noted at a pH value of 7 (92±7.8%), as well as pH 6 (95±8.3%). At the same time, at a pH value of 5.5 (84±7.2%) and 5 (77±6.4%), a pronounced but not significant decrease in amylase activity was noted in relation to its maximum value. However, when the pH values



Picture 1. Investigation of changes in salivary amylase activity when using starch - 1 as a substrate, as well as starch and saccharose in a ratio of 1:1 - 2, 1:5 - 3, 1:10 - 4.

* - significantly different values in relation to the use of only starch as a substrate.

were equal to 4.5 (62±5.3%) and 4 (39±2.8%), a further significant decrease in amylase activity was observed in relation to its maximum value. At pH 3.5 (21 ± 1.6%), amylolytic activity remained significantly at a low level, and at pH (5 ± 0.3%) it was not significantly pronounced, at pH 2.5 and 2 it was completely absent (Fig. 2).

Based on the results of using starch and sucrose together as a substrate in a ratio of 1:1, a similar dynamics of changes in salivary amylase activity at various pH values from 2 to 7 was revealed. At the same time, all these results of amylase activity at various pH values did not differ significantly from similar indicators using only starch as a substrate (Fig. 2).

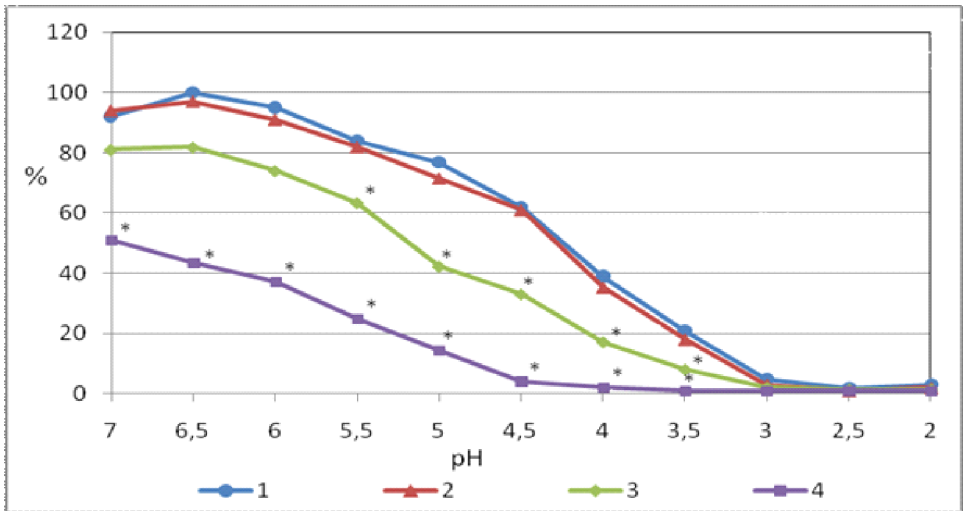


Figure 2. Investigation of changes in salivary amylase activity when using starch - 1 as a substrate, as well as starch and saccharose in a ratio of 1:1 - 2, 1:5 - 3, 1:10 - 4, at various pH values from 2 to 7.

* - significantly different values in relation to similar indicators using only starch as a substrate.

However, with application as a substrate together with starch and sucrose in a ratio of 1:5, there was a close dynamics of changes in the activity of salivary amylase at different pH values, however, all indicators were lower than similar results of changes in the dynamics of amylase activity at different pH values using

only starch as a substrate. Where in greatest the manifestation of salivary amylase activity in relation to the maximum indicator of 100% using only starch as a substrate was noted at pH 7 (81±7.2%), and at pH 6 (74±6.1%) an insignificant decrease in activity was noted amylase in relation to that using only starch. At the same time, at a pH value of 5.5 (63 ± 5.4%) and 5 (42 ± 6.4%), a pronounced and significant decrease in amylase activity was noted in relation to that with the use of only starch. At the same time, at pH values of 4.5 (33±2.5%), as well as 4 (17±1.5%) and 3.5 (8±0.5%), a further significant decrease in amylase activity was observed in relation to to a similar one using only starch. And at pH 3, 2.5, 2 there was no activity of salivary amylase (Fig. 2).

FROM application as a substrate, together starch and sucrose in a ratio of 1:10, there were significantly lower amylase activity in relation to similar results using only starch. At the same time, the dynamics of the decline in amylase activity was more pronounced. There was a significant and significant decrease in the activity of amylase at pH 7 (51±4.3%) in relation to the same result using only starch as a substrate. This result was maximal when using the substrate starch and sucrose in a ratio of 1:10. Subsequently, with a decrease in pH to 4, a gradual significant decrease in the activity of salivary amylase was noted in relation to similar results using only starch. And when the pH decreased from 4 to 2, there was no amylolytic activity (Fig. 2).

From the results of the study of the effect of sucrose on the TSA of gastric juice using casein, albumin and hemoglobin proteins as a substrate, it was found that using only casein as a substrate, the TSA was 91 ± 8.7 U / ml. And with the use of a substrate of casein and sucrose in a ratio of 1:1, this figure was 88 ± 7.9 U / ml, which was not significantly lower than the result with the use of casein alone. When casein and sucrose were used as a substrate in a ratio of 1:5, the OPA was 84±7.5 U/ml, and in a ratio of 1:10 it was 79±7.1 U/ml, which was not significantly less than the indicator using only casein (Fig. 3).

Similar dynamics of changes in TSA was observed using albumin and sucrose as substrates. At the same time, with the use of only albumin as a substrate, the OPA was not significantly lower than the result with the use of casein alone and amounted to 87±7.6 U/ml. With the use of albumin and sucrose in a ratio of 1:1, the OPA was 84 ± 7.3 U / ml, which was not significantly lower than the result with the use of only albumin, and not significantly less than the same

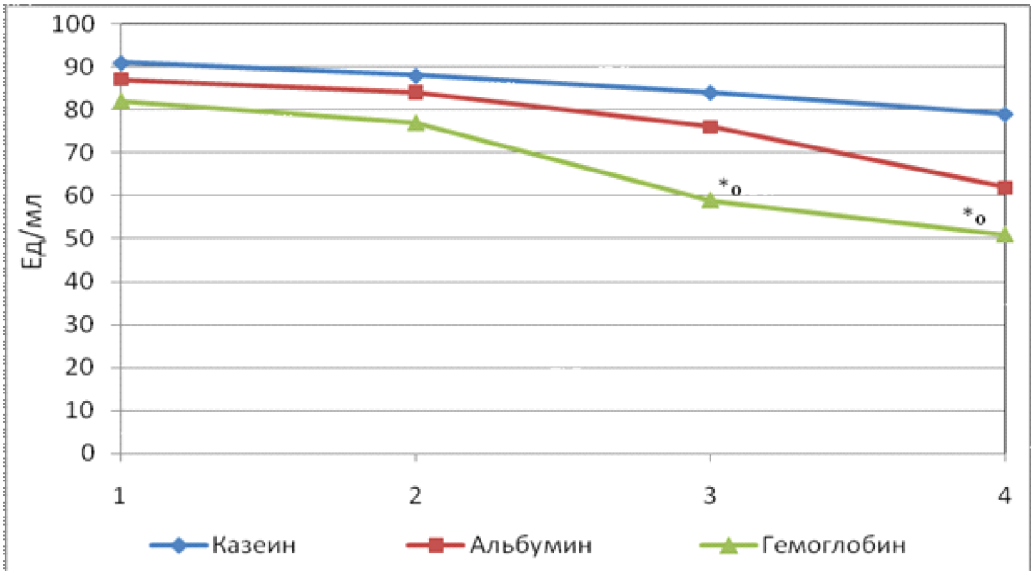


Figure 3 Investigation of the change in OPA when using casein, albumin, hemoglobin proteins as a substrate at various ratios: 1 - only protein, 2 - protein and saccharose in a ratio of 1: 1, 2 - protein and saccharose in a ratio of 1: 5, 2 - protein and saccharose in the ratio 1:10.

* - Significantly different values in relation to the indicators of the use of only protein as a substrate.

ABOUT-significantly different values in relation to similar indicators of the use of casein and sucrose as a substrate.

indicator using casein and sucrose in a ratio of 1:1. The use of albumin and sucrose in a ratio of 1:5 as a substrate caused an insignificant decrease in the OPA in relation to both the results of using only albumin and the same indicator with the use of casein and sucrose in a ratio of 1:5 and was equal to 77 ± 6.8 U /ml, and in a ratio of 1:10 it was 62±5.4 U/ml, which was not significantly less than the indicator with the use of only albumin, as well as a similar indicator with the use of casein and sucrose in a ratio of 1:10 (Fig. 3) .

Also, a similar direction of changes in the OPA was observed with the use of hemoglobin and sucrose as a substrate. When using only hemoglobin as a substrate, the OPA was not significantly lower than using only casein and was 82±7.8 U/ml. With the use of hemoglobin and sucrose in a ratio of 1:1, the OPA was 77 ± 6.9 U / ml, which was not significantly lower than the result with the use of only hemoglobin, and not significantly less than the same indicator with the use of hemoglobin and sucrose in a ratio of 1:1. The use of hemoglobin and sucrose in a ratio of 1:5 as a substrate caused a significant decrease in OPA to 59 ± 5.2 U / ml, both in relation to the use of only hemoglobin, and in relation to the result using casein and sucrose in a ratio of 1:5. It was also revealed

The discussion of the results. From the obtained research results, it was found that the activity of salivary amylase depends on the interaction of starch with sucrose. This is confirmed by the data we obtained, where it was found that with use as a substrate starch and sucrose in a ratio of 1:1 in relation to the use of only starch, no significant changes in amylolytic activity were noted. The use of the same substrate in a ratio of 1:5 did not cause a significant decrease in the amylase activity, and the use of the same substrate in a ratio of 1:10 relative to the use of only starch caused a significant decrease in amylolytic activity. Based on these data, it can be argued that sucrose at high concentrations can reduce the activity of salivary amylase using starch as a substrate.

In addition, studies have shown that with the use of only starch as a substrate, the greatest manifestation of salivary amylase activity was observed at pH 7, as well as 6.5 and 6. At the same time, at pH 5.5 and 5, there was a pronounced, but not significant decrease in amylase activity in relation to its maximum value. At the same time, when the pH values were around 4.5 and 4, a further significant decrease in amylase activity was observed in relation to its maximum value. At pH 3.5, amylolytic activity remained significantly at a low level, and at pH 3 it was not significantly pronounced, at pH 2.5 and 2 it was completely absent. All this indicates that amylolytic activity in the stomach can be maintained in the pH range from 7 to 3.

With the use of starch and sucrose as a substrate in the ratio1:1, a similar direction of change in amylase activity was observed at various pH values from 2 to 7. Also, all these results of amylase activity at various pH values did not significantly differ from the same results using only starch as a substrate. What indicates the absence of the influence of sucrose at a ratio of 1: 1starch and sucrose on changes in salivary amylase activity at different pH values.

According to the results of the application as a substrate together with starch and sucrose in a ratio of 1:5, there was a close dynamics of changes in the activity of salivary amylase at different pH values. However, all indicators were lower than similar results of changes in the dynamics of amylase activity at various pH values using only starch as a substrate. In the same time at pH values from 7until 6there was not a significant decrease in amylase activity in relation to that with the use of only starch. However, at pH 5.5 and 5.0there was a significant and significant decrease in amylase activity in relation to a similar result using only starch. A similar and significant decrease in amylase activity relative to that with the use of only starch was noted at pH values from 4.5 to 3.5, and pH 3, 2.5, 2 there was no activity of salivary amylase. These results demonstrate that at the ratio of starch and sucrose1:5, a significant decrease in the activity of salivary amylase under the influence of sucrose is observed at lower pH values from 5 to 3.5.

The use of both starch and sucrose as a substrate in a ratio of 1:10 caused significantly lower results of amylase activity in relation to similar results using only starch. At the same time, the level of decrease in amylase activity at different pH values was more significant than in the ratio of 1:5. There was a significant and significant decrease in amylase activity at pH 7 to 4 relative to similar results using starch alone. And when the pH decreased from 4 to 2, the activity of salivary amylase was absent. This result shows that sucrose at high concentrations reduces amylase activity at all pH values.

Also, the results showed that OPA of gastric juice using casein and sucrose as a substrate at ratios1:1, 1:5, 1:10 did not change significantly in relation to use only casein as a substrate. Although it had a tendency to decrease with increasing ratio of casein and sucrose. At the same time, the use of albumin and sucrose as a substrate at ratios1:1, 1:5, 1:10 also did not change significantly in relation to use albumin as a substrate. At the same time, all these indicators were not significantly lower in relation to the use of casein as a substrate and significantly lower, but not significantly lower in the ratio1:10.

Using as a substrate for hemoglobin and sucrose at the ratio of hemoglobin and sucrose1:1there was a non-significant decrease in OPA compared with the use of hemoglobin alone. Also, this indicator is not significantly less than the same result with the use of casein and sucrose in the ratio1:1. At the same time, using hemoglobin and sucrose substrate at the ratio of hemoglobin and sucrose1:5 and 1:10there was a pronounced and significant decrease in OPA, both in relation to using only hemoglobin, and in relation to those indicators using casein and sucrose. Thus, according to the assessment of the effect of sucrose on the gastric juice OPA of the proteins used, it can be assumed that sucrose does not so much affect the activity of the gastric juice OPA, but to a greater extent on the properties of proteins, changing their hydrolyze ability under the influence of gastric juice proteases.

Conclusions: When using a starch substrate with sucrose, it was shown that sucrose at high concentrations can reduce the activity of salivary amylase. It was also found that sucrose at high concentrations can significantly and significantly reduce the activity of salivary amylase at all pH values. In addition, it was found that with the use of various proteins and sucrose as a substrate especially at high concentrations of sucrose, it does not so much affect the activity of gastric juice OPA, but to a greater extent on the properties of proteins, changing their hydrolyze ability under the influence of gastric juice proteases.

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