

Original Research Article

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## Influence of Saliva Amylase on Changes in Gastric Protein Hydrolysis

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### ABSTRACT

#### Keywords

Proteins, control, amylase, physicochemical, protein hydrolysis, starch

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Studied influence of salivary amylase on changes in gastric hydrolysis of proteins. The study was carried out in vitro, in saliva, gastric juice, solutions of starch, casein, albumin and hemoglobin were used in the work. It was concluded that the use of starch-protein mixtures helps to reduce the hydrolysis of protein by gastric juice, due to the formation of starch-protein complexes preventing the hydrolysis of proteins, and reducing the access of gastric proteases to proteins in the starch-protein complex. An increase in the ratio of starch and protein in the direction of increasing starch contributes to an additional decrease in protein hydrolysis, which may be an additional decrease in the access of gastric proteases to proteins, in addition to an obstacle to proteins in the starch-protein complex. In addition, salivary amylase helps to improve the digestibility of proteins by gastric juice, both due to a decrease in starch-protein complexes, and to increase the access of proteases to proteins by reducing the concentration of starch as a result of its digestion by salivary amylase.

### Introduction

Proteins and polysaccharides are present in many complex multi-phase food systems. They are key responsible for controlling food breakdown and nutrient absorption in the gastrointestinal tract. In addition, they play a role in providing key organoleptic characteristics (eg. textural characteristics and controlled flavor release) and phase stability of food products. Their physicochemical properties depend on the molecular parameters of individual biopolymers and the nature of the interaction between polymer molecules (Goh

*et al.*, 2020). The interaction between the protein and starch fractions has been shown, which can change the digestibility of the protein. The dependence of the degree of interaction between the polysaccharide and protein on the molecular weight of the polysaccharide has also been established (Kurchenko *et al.*, 2013).

An abundance of starch granules can reduce proteolysis by limiting the availability of proteolytic enzymes, especially when gelatinized during cooking. The uniqueness of the protein matrix and its interaction with starch affects the rate of starch

digestion. Taken together, these findings suggest that the properties of starch and protein may affect their mutual digestibility. Most of the published work focuses on protein digestibility and its effect on starch, but evidence to the contrary is scarce. To solve this problem, relevant facts are important, how protein and starch affect the breakdown of each other (Duodu *et al.*, 2002).

Starch digestion begins in the mouth (when saliva mixes with food particles) due to the action of human salivary  $\alpha$ -amylase (HSA), which cleaves amylose and amylopectin by cleaving their  $\alpha$ -1-4 glycosidic bonds (Bornhorst and Singh, 2012).

The role of starch digestion in the oral cavity is often considered of little significance due to the short duration of the oral phase, but this line of thought can be questioned after reviewing some of the bibliographic data. Indeed, bakery products are usually formed within 16-50 seconds, depending on the type of bread and the characteristics of the person (Jourdain *et al.*, 2016; Panouillé *et al.*, 2014).

However, HSA can continue to hydrolyze starch in the stomach (Bornhorst and Singh, 2012), until the pH falls below 4.0 and the enzyme is inactivated (Fried *et al.*, 1987). Since postprandial gastric acidification is a gradual process, it may take more than 45 minutes to reach this pH level (Malagelada *et al.*, 1979). Evidence from human studies showing that HSA can remain active in the stomach for a long time after a short oral treatment phase, and can even reach the small intestine without becoming inactive, indicates that this enzyme may be responsible for the hydrolysis of an important fraction of starch (Fried *et al.*, 1987). In addition, according to an interesting article from the early 20th century, which also reports the results of a human study, up to 76% of starch in puree and 59% in bread is hydrolyzed to maltose by HSA in the stomach (Bergeim, 1926). Since there is no other amylase, only an enzyme of salivary origin can be responsible for the enzymatic hydrolysis of starch during gastric digestion (Bornhorst and Singh, 2012). However, this work seems to have been

forgotten in the scientific community, given that the extent of HSA's contribution to starch digestion remains unclear (Butterworth *et al.*, 2011) and the final stage of digestion is often considered more important. The last stage of starch digestion occurs in the small intestine, where pancreatic  $\alpha$ -amylase completes amylase, and the end product of this process, glucose, is finally absorbed into the bloodstream (Gropper *et al.*, 2012; Freitas *et al.*, 2018).

### **Purpose of the study**

Explore influence of salivary amylase on changes in gastric hydrolysis of proteins.

### **Materials and Methods**

In vitro work studied the effect of salivary amylase on the change in total proteolytic activity (OPA) of gastric juice using polysaccharide-protein substrates of starch and casein proteins, egg albumin (albumin) and hemoglobin. The OPA of gastric juice (Kurchenko *et al.*, 2013) was studied using casein, albumin, and hemoglobin as a substrate during 30 min of incubation with gastric juice. Also, a substrate mixture of starch + casein, starch + albumin and starch + hemoglobin, with 30 min of incubation with gastric juice, a mixture of starch + casein, starch + albumin and starch + hemoglobin, with 60 min of incubation with gastric juice, a mixture of starch + casein, starch + albumin and starch + hemoglobin, at 30 min of preincubation with saliva and then 60 min of incubation with gastric juice.

Different ratios of starch and protein were used: 1-part starch and 5 parts protein, 5 parts starch and 1-part protein.

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 and Discussion

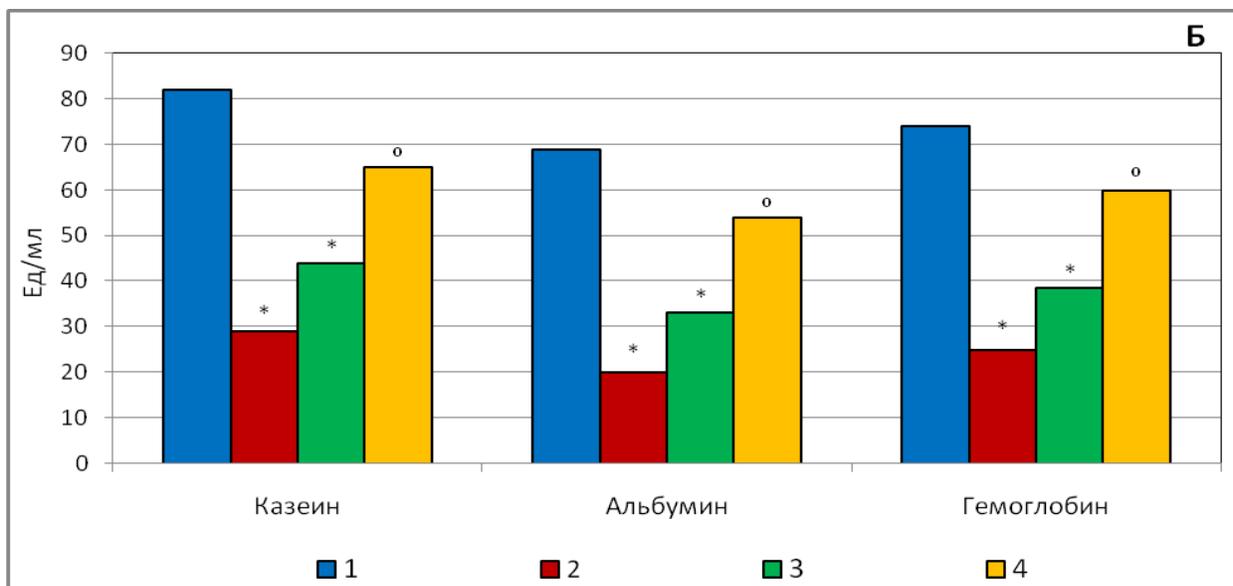
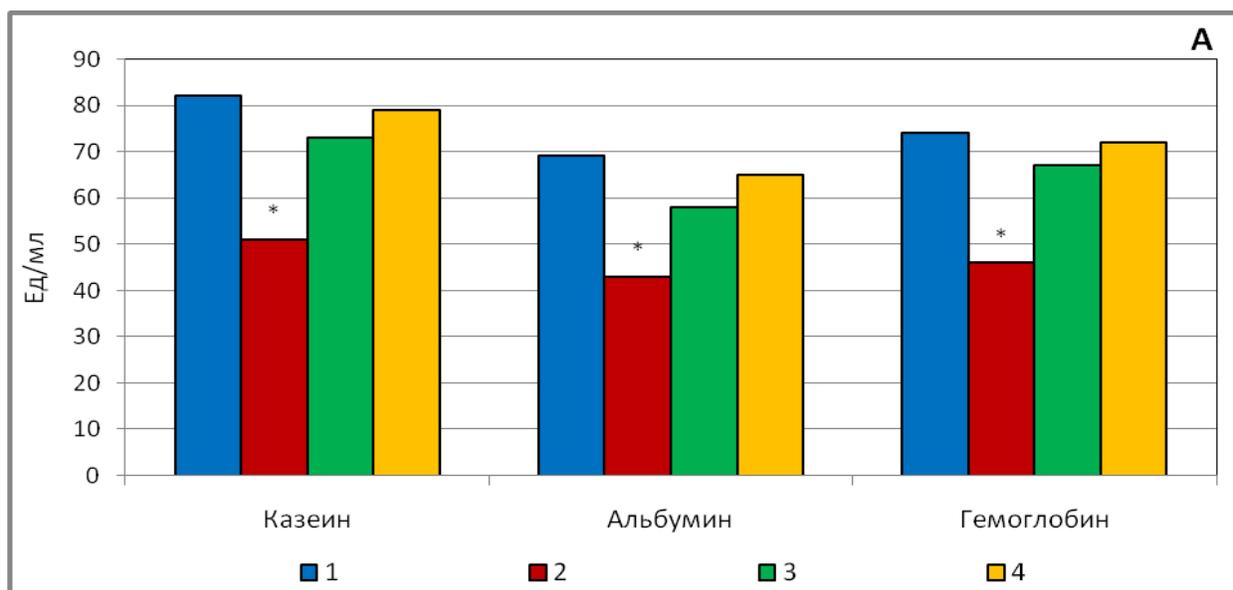
ATit was revealed that when only casein was used as a substrate and after 30 minutes of exposure to gastric juice, the OPA was  $82 \pm 7.1$  U/ml. Wherein with the use of a substrate of starch and casein in a ratio of 1:5 after 30 minutes of exposure to gastric juice, this figure was  $51 \pm 4.5$  U/ml, which was significantly lower than the similar result using only casein (Fig. A). In the same time the joint use of starch and casein as a substrate in a ratio of 5: 1, after 30 minutes of exposure to gastric juice, caused a change in the OPA, which was significantly lower similar result using only casein and amounted to  $29 \pm 2.7$  U/ml. This result was significantly lower than the similar value with the use of combined starch and casein in relation to 1:5 (Fig. B). It was also found that using a substrate from starch and casein in a ratio of 1:5 after 60 minutes of exposure to gastric juice, the OPA was equal to  $73 \pm 6.4$  U/mL, which was not significantly lower than with casein alone (Fig. A). At the same time, at change as a substrate starch and casein in a ratio of 5:1 after 60 minutes of exposure to gastric juice, the OPA was  $44 \pm 4.8$  U/ml, which was also significantly lower than the similar result of using starch and casein in relation to 1:5 (Fig. B). In addition to this, it was found that using a substrate starch and casein in a ratio of 1:5 after a preliminary 30 min incubation with saliva and a further 60 min incubation with gastric juice OPA was  $79 \pm 8.3$  U/ml, which was unreliably higher than the same indicator using starch and casein in a ratio of 1:5 after 60 minutes of exposure to gastric juice (Fig. A). Nonetheless use as a substrate starch and casein in a ratio of 5:1 after a preliminary 30 min exposure to saliva and a further 60 min exposure to gastric juice OPA was  $65 \pm 7.1$  U/ml, which was significantly higher than the similar indicator using starch and casein in a ratio of 5:1 after 60 minutes of exposure to gastric juice (Fig. B). The studies also found that when using only albumin as a substrate and after 30 minutes of exposure to gastric juice, the OPA was

$69 \pm 6.3$  U/ml. The same Index with the use of starch and albumin as a substrate in a ratio of 1:5 after 30 min of the influence of gastric juice, the OPA was significantly less similar result using only albumin and was at level  $43 \pm 3.9$  U/ml (Fig. A). The combined use of starch and albumin in a ratio of 5:1, after 30 minutes of exposure to gastric juice, caused a change in the VA, which was significantly lower.

Similar result using only albumin and amounted to  $20 \pm 1.7$  U/ml. This result was significantly lower than the similar result using starch and albumin in relation to 1:5 (Fig. B). Use as a substrate starch and albumin in a ratio of 1:5 after 60 minutes of exposure to gastric juice, the OPA was  $58 \pm 5.6$  U/mL, this result was not significantly lower than the similar indicator using only albumin (Fig. A). At the same time, in result at changes as a substrate starch and casein after 60 minutes of exposure to gastric juice, the OPA was  $44 \pm 4.8$  U/ml, which was significantly lower than the similar result of using starch and casein in relation to 1:5 (Fig. B). At the same time, use as a substrate starch and albumin in a ratio of 1:5 after a preliminary 30 min incubation with saliva and a further 60 min incubation with gastric juice OPA was equal to  $65 \pm 7.1$  U/ml, which was not significantly higher than the similar indicator using starch and casein in a ratio of 1:5 after 60 minutes of exposure to gastric juice (Fig. A). When used as a substrate starch and casein in a ratio of 5:1 after a preliminary 30 min exposure to saliva and a further 60 min exposure to gastric juice OPA was  $65 \pm 7.1$  U/ml, which was also significantly higher than the similar indicator using starch and casein in a ratio of 1:5 after 60 minutes of exposure to gastric juice (Fig. B).

A similar focus of the results of the study OPA of gastric juice was identified when used as a substrate starch and hemoglobin in a ratio of 1:5. At the same time, the OPA using only hemoglobin as a substrate after 30 minutes of exposure to gastric juice was  $74 \pm 6.8$  U/ml.

**Picture.1** Changes in gastric juice OPA under the influence of salivary amylase when using as a substrate a mixture of starch with proteins casein, albumin and hemoglobin in the ratio of starch and protein: A - 1:5, B - 5:1. 1 - proteins after 30 min of incubation with gastric juice. 2 - the use of a mixture: starch with proteins, with 30 minutes of incubation with gastric juice. 3 - use of a mixture: starch with proteins at 60 min of incubation with gastric juice. 4 - use of a mixture: starch with proteins at 30 min preincubation with saliva and then 60 min of incubation with gastric juice.



\* - Significantly different values in relation to the indicators of the use of proteins at 30 min of incubation.

o - significantly different values in relation to the indicators of the use of the mixture: starch + casein, starch + albumin and starch + hemoglobin, at 30 min of incubation.

Using both starch and hemoglobin as a substrate after 30 minutes of exposure to gastric juice the same index was significantly less the same result using only hemoglobin and was equal to  $46 \pm 4.1$  U/mL (Fig. A). It was also found that when using a mixture of starch and hemoglobin in a ratio of 5: 1, after 30 minutes of exposure to gastric juice OPA was significantly lower the same indicator only hemoglobin and amounted to  $25 \pm 2.2$  U/ml. This result OPA was significantly less than the same indicator using the substrate mixture of starch and hemoglobin in relation to 1:5 (Fig. B). Usage starch and hemoglobin in a ratio of 1:5 after 60 minutes of exposure to gastric juice, the OPA corresponded to a level of  $67 \pm 6.3$  U/ml, which was not significantly less than the same result using only hemoglobin (Fig. A). At the same time, using starch and hemoglobin in a ratio of 5:1 after 60 minutes of exposure to gastric juice, the OPA was  $39 \pm 3.5$  U/mL, which was significantly lower than the similar result using only hemoglobin, and significantly lower than the same indicator of the use of starch and hemoglobin in relation to 1:5 (Fig. B). However, use as a substrate starch and hemoglobin in a ratio of 1:5 after a preliminary 30 min exposure to saliva and a further 60 min exposure to gastric juice OPA was  $72 \pm 7.6$  U/ml, which was also not significantly higher than the similar indicator using starch and casein in a ratio of 1:5 after 60 minutes of exposure to gastric juice (Fig. A). In the same time use as a substrate starch and hemoglobin in a ratio of 5:1 after a preliminary 30 min exposure to saliva and a further 60 min exposure to gastric juice OPA was  $60 \pm 5.8$  U/ml, which was also significantly higher than the similar indicator using starch and casein in a ratio of 5:1 after 60 minutes of exposure to gastric juice (Fig. B).

The results of these studies showed that OPA depends on the interaction of starch with proteins due to the formation of starch-protein complexes. This is confirmed by the data we obtained, where it was found that with use as a substrate starch together with casein in the ratio 1:5 and 5:1, OPA was significantly lower than when using only casein as a substrate under similar conditions. Also, OPA

depends on the concentration of starch in the substrate mixture. This is confirmed by the results, where it was found that when use as a substrate starch together with casein in the ratio 5:1, OPA was significantly lower than when used under similar conditions as a starch substrate together with casein in the ratio 1:5. A similar direction of changes in the OPA, under the same conditions, was also noted when using as a substrate starch together with albumin, as well as starch together with hemoglobin. In addition, studies have shown that salivary amylase improves the digestibility of proteins by gastric juice. This is supported by the results obtained, in which it was found that use as a substrate starch and casein in a ratio of 5:1, after a preliminary 30 min exposure to saliva and a further 60 min exposure to gastric juice, OPA was significantly higher than the similar indicator using starch and casein after 60 minutes of exposure to gastric juice. A similar direction of changes in the OPA, under the same conditions, was noted when using as a substrate starch together with albumin, as well as starch together with hemoglobin.

The obtained results of the study show that the use of a mixture of starch with proteins helps to reduce protein hydrolysis by gastric juice. This may be due to the formation of starch-protein complexes that prevent the hydrolysis of proteins, by reducing the access of gastric proteases to proteins in the starch-protein complex. With an increase in the ratio of starch and protein towards an increase in starch, it contributes to an additional decrease in protein hydrolysis, which may be due to the fact that an increase in the amount of starch may be an additional decrease in the access of gastric proteases to proteins, in addition to an obstacle to proteins in the starch-protein complex. In addition, the results of the study show that salivary amylase helps to improve the digestibility of proteins by gastric juice both by reducing starch-protein complexes,

In this way, protein hydrolysis by gastric juice depends on the interaction of starch with proteins as a result of the formation of starch-protein complexes, as well as on the amount of starch, an

increase in the concentration of which is an obstacle to the access of gastric proteases to proteins. In addition, protein hydrolysis in protein-polysaccharide mixtures depends on the presence of salivary amylase.

The use of starch-protein mixtures helps to reduce the hydrolysis of proteins by gastric juice, by reducing the access of gastric proteases to proteins in the starch-protein complex. An increase in starch in starch-protein mixtures contributes to an additional decrease in protein hydrolysis, by reducing the access of gastric proteases to proteins, in addition to obstructing proteins in the starch-protein complex.

In addition, salivary amylase helps to improve the digestibility of proteins by gastric juice due to an increase in the access of proteases to proteins by reducing the concentration of starch as a result of its digestion by salivary amylase and reducing starch-protein complexes.

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