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# The Influence of Protein Hydrolysates on Changes in Starch Digestibility in Starch-Protein Substrates

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**Abstract** In vitro effects of protein hydrolysates on the change of starch digestibility in the composition of starch-protein substrates were studied in this work. As a result of the study, it was found that when starch was used as a substrate together with both proteins and protein hydrolysates, there was a decrease in starch hydrolysis under the influence of salivary amylase. These changes may be due to the formation of starch complexes with both proteins, protein hydrolysates or peptides, which prevent starch hydrolysis. When the effect on starch of pre-incubation salivary amylase together with both proteins and protein hydrolysates was investigated, a decrease in starch hydrolysis under the influence of salivary amylase was observed. These changes may be due to the inhibition of salivary amylase by both proteins, protein hydrolysates or peptides, which prevent starch hydrolysis.

**Keywords** Salivary amylase, Starch, Casein, Egg white, Albumin serum, Protein hydrolysates, Interaction, Hydrolysis, Digestion

## 1. Introduction

Starch, as one of the major carbohydrates in the daily diet, proved to be a major source of human metabolic energy [3]. It is also a major glycemic carbohydrate, the extent and rate of starch digestion are considered a risk factor for many diet-related chronic diseases [1]. Starch digestibility is determined not only by the structural properties of starch [2], but also by the properties of other components and their interactions with starch [5]. Protein is also a major macronutrient in many types of food such as cereal or legume products. Starch and proteins affect the textural quality and nutritional properties of final food products in different ways [6]. The presence of proteins or their hydrolysates not only changes the functional properties of starch but also affects the digestibility of starch [4]. Hence, understanding the mechanisms of the effect of proteins and their hydrolysates on starch digestibility proved to be an important factor for the design and development of high protein and low glycemic index products. Starch and proteins have different effects on the textural quality and nutritional properties of prepared meals [6].

Although mechanisms underlying the effect of plant proteins or their hydrolysates on starch digestion have been proposed, little information is available on the occasion of direct binding between protein and digestive enzymes and its relationship to starch digestion. In addition, the interaction between proteins or their hydrolysates and α-amylase to determine the effect of this interaction on starch digestibility [7] is of a great interest to study.

**Aim of the study:** to investigate the effect of protein hydrolysates on changes in starch digestibility in starch-protein substrates.

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## 2. Material and Methods

Saliva obtained by spitting from volunteers was studied in this work. In vitro influence of interaction of starch and proteins as well as pepsin hydrolysates of proteins on the change of starch content under the influence of salivary amylase was studied. Starch with proteins as well as starch with 30 min and 60 min pepsin hydrolysates of proteins were used as substrates. In addition, the in-vitro effects of proteins and protein hydrolysates with salivary amylase and further effects on the change in starch content upon exposure to salivary amylase were investigated. Proteins and pepsin hydrolysates of proteins pre-incubated with salivary amylase were used under these conditions. Salivary amylase was used with pre-incubation with 30 min pepsin hydrolysates and 60 min pepsin hydrolysates. The content of residual starch under the influence of salivary amylase was studied by blue staining with iodine reagent, as well as the content of residual starch in the presence of proteins and protein hydrolysates under the influence of salivary amylase, which was expressed as a percentage of the starch content without the influence of salivary amylase.

Statistical processing was carried out by the method of variation statistics with calculation of mean values and their

mean errors, determination of the Student-Fisher (t) difference reliability coefficient. Differences at $p<0.05$ and less were considered statistically significant.

## 3. Results

Based on the data obtained, it was found that when the effect of starch-casein interaction on starch hydrolysis values under the influence of salivary amylase was studied (Tab. 1) using a substrate containing only starch under the influence of salivary amylase, the residual starch value constituted 55±5.1%. This result was significantly ($P<0.001$) lower compared with similar starch values without the influence of salivary amylase. Meanwhile, under the influence of salivary amylase on the substrate containing starch together with casein, the result of residual starch constituted 63±5.9% and was significantly ($P<0.001$) lower with respect to the similar starch result without salivary amylase action. This result was also not significantly higher than the similar value of using starch as substrate under the influence of salivary amylase. In addition, when salivary amylase was applied to a substrate containing starch together with 30 min casein hydrolysate, the residual starch value constituted 71±6.9% and was significantly lower than that of starch without salivary amylase. This index was not significantly higher than the similar result of using starch as substrate under the influence of salivary amylase. At the same time, under the influence of salivary amylase on the substrate containing starch together with 60 min casein hydrolysate, the index of residual starch was at the level of 78±7.4% and was not reliable in relation to the similar result of starch without salivary amylase. Also, this index was significantly ($P<0.05$) higher than the similar result of using starch as a substrate under the influence of salivary amylase (Tab. 1).

In addition, it was found that under the influence of salivary amylase on the substrate containing starch together with egg white, the result of residual starch constituted 59±5.4% and was significantly ($P<0.001$) lower in relation to the similar result of starch without the influence of salivary amylase. This result was also not significantly higher the similar index (55±5.1%) of starch substrate application under the influence of salivary amylase. At the same time, when salivary amylase was applied to the substrate containing starch together with 30 min egg white hydrolysate, the residual starch index constituted 68±6.4% and was significantly ($P<0.001$) relative to the similar result of starch without the influence of salivary amylase. Also, this index was not significantly higher than the similar result of using starch as substrate under the influence of salivary amylase. At the same time, under the influence of salivary amylase on the substrate containing starch together with 60 min of egg white hydrolysate, the index of residual starch remained at the level of 74±6.7% and was significant ($P<0.05$) in relation to the similar result of starch without the influence of salivary amylase. It was also significantly ($P<0.05$) higher than the similar result of using starch as a substrate under the influence of salivary amylase (Tab. 1).

**Table 1.** Investigation of the changing effect of the interaction of starch with proteins and protein hydrolysates on starch hydrolysis

| Proteins used | Composition of interaction components | | | | |
|---|---|---|---|---|---|
| | Starch | Starch + saliva | Starch + protein + saliva | Starch + 30 min protein hydrolysate + saliva | Starch + 60 min protein hydrolysate + saliva |
| Casein | 100 | 55 ± 5,1 | 63±5,9* | 71±6,9* | 78±7,4$^{o}$ |
| Egg white | 100 | 55 ± 5,1 | 59±5,4* | 68±6,4* | 74±6,7*$^{o}$ |
| Serum albumin | 100 | 55 ± 5,1 | 62±5,9* | 73±6,8*$^{o}$ | 81±7,8$^{o}$ |

** - reliably different values of change in starch content in relation to similar index of using only starch as a substrate without the influence of salivary amylase.*
*o - reliably different values of change in starch content in relation to the similar indicator of using starch as a substrate under the influence of salivary amylase.*

**Table 2.** Investigation of the changing effects of salivary amylase interaction with proteins and protein hydrolysates on starch hydrolysis

| Proteins used | Composition of interaction components | | | | |
|---|---|---|---|---|---|
| | Starch | Saliva + starch | Saliva + protein + starch | Saliva + 30 min protein hydrolysate after preincubus + starch | Saliva + 60 min protein hydrolysate after pre-incubus + starch |
| Casein | 100 | 55 ± 5,1 | 63±5,9* | 76±7,1* | 84±7,9$^{o}$ |
| Egg white | 100 | 55 ± 5,1 | 59±5,4* | 72±6,7* | 81±7,7$^{o}$ |
| Serum albumin | 100 | 55 ± 5,1 | 62±5,9* | 74±6,9* | 86±8,2$^{o}$ |

** - reliably different values of change in starch content in relation to the similar indicator of using only starch as a substrate without the influence of salivary amylase.*
*o - reliably different values of change in starch content in relation to the similar indicator of using starch as a substrate under the influence of salivary amylase.*

At the same time it was found that under the influence of salivary amylase on the substrate containing starch together with serum albumin, the result of residual starch constituted 62±5.9% and was significantly (P<0.001) lower in relation to the similar result of starch without the influence of salivary amylase. This result was also not significantly higher than the similar result (55±5.1%) of using starch as a substrate under the influence of salivary amylase. In addition, when salivary amylase was applied to the substrate containing starch together with 30 min serum albumin hydrolysate, the result of residual starch constituted 73±6.8% and was significantly (P<0.05) relative to the similar result of starch without the influence of salivary amylase. Also, this index was significantly higher than the similar result of using starch as substrate under the influence of salivary amylase. In addition, under the influence of salivary amylase on the substrate containing starch together with 60 min serum albumin hydrolysate, the result of residual starch constituted 81±7.8% and was not significantly higher than the similar result of starch without the influence of salivary amylase. At the same time, the result of using starch as a substrate under salivary amylase was significantly (P<0.01) higher than the similar result (Tab. 1).

The influence of interaction of salivary amylase with proteins and protein hydrolysates on starch hydrolysis was also investigated. At the same time at influence of salivary amylase on the substrate containing starch together with casein the result of residual starch constituted 63±5,9% and salivary amylase on starch, accordingly, constituted 55±5,1%. In addition, when salivary amylase was exposed to starch together with 30 min pepsin hydrolysate of casein after their pre-incubation, the result of residual starch constituted 76±7.1%, which was not reliable with respect to the similar result of starch without the effect of salivary amylase. Also, this value was not significantly higher than the similar result of using starch as substrate under the influence of salivary amylase. In addition, when starch was affected by salivary amylase together with 60 min pepsin casein hydrolysate after their pre-incubation, the result of residual starch constituted 84±7.9%, which was not reliable with respect to the similar result of starch without the effect of salivary amylase. Also, this value was significantly (P<0.01) higher than the similar result of using starch as substrate under the influence of salivary amylase. (Tab. 2).

In addition, when salivary amylase was exposed to the substrate containing starch together with egg white, the result of residual starch constituted 59±5.4% and salivary amylase on the starch constituted 55±5.1%.

In addition, when the starch was affected by salivary amylase together with 30 min pepsin hydrolysate of egg white after their pre-incubation, the result of residual starch constituted 72±6.7%, which was significantly (P<0.05) relative to the similar result of starch without the effect of salivary amylase. Also, this index was not significantly higher than the similar result of using starch as substrate under the influence of salivary amylase. At the same time, when the starch was exposed to salivary amylase together with 60 min pepsin hydrolysate of egg white after their pre-incubation, the result of residual starch constituted 81±7.7%, which was not significantly higher than the similar result of starch without the influence of salivary amylase. Also, this index was significantly (P<0.01) higher than the similar result of using starch as substrate under the influence of salivary amylase (Tab. 2).

In addition, when salivary amylase was exposed to starch substrate together with serum albumin, the result of residual starch constituted 62±5.9% and salivary amylase on starch was 55±5.1%. At the same time, when salivary amylase was exposed to starch together with 30 min pepsin hydrolysate of serum albumin after their pre-incubation, the result of residual starch constituted 74±6.9%, which was significant (P<0.05) with respect to the similar result of starch without the effect of salivary amylase. Also, this index was not significantly higher than the similar result of using starch as substrate under the influence of salivary amylase. At the same time, when the starch was exposed to salivary amylase together with 60 min pepsin hydrolysate of albumin serum after their pre-incubation, the result of residual starch constituted 86±8.2%, which was not significantly higher than the similar result of starch without the influence of salivary amylase. Also, this index was significantly (P<0.001) higher than the similar result of using starch as substrate under the influence of salivary amylase. (Tab. 2).

## 4. Discussion of Results

The obtained results showed that there was a significant decrease in starch content in relation to the result of using it without the influence of salivary amylase with the use of a substrate containing only starch, under the influence of salivary amylase. At the same time under the influence of salivary amylase on the substrate containing starch together with proteins the result of residual starch was not significantly higher in relation to a similar indicator of starch under the influence of salivary amylase. Besides, when salivary amylase was applied to the substrate containing starch together with 30 min protein hydrolysate, the residual starch result was not significantly higher with casein and egg white hydrolysates and significantly higher with whey albumin hydrolysates in relation to the similar starch result without salivary amylase. Moreover, when salivary amylase was applied to a substrate containing starch together with 60 min protein hydrolysate, the residual starch index was significantly higher with all proteins used. These changes indicate that there was a decrease in starch hydrolysis under the influence of salivary amylase when starch was used as a substrate together with both proteins and protein hydrolysates. These changes may be related to the formation of starch complexes with both proteins and protein hydrolysates or peptides, which prevent starch hydrolysis.

The effect of salivary amylase interaction with proteins and protein hydrolysates on starch hydrolysis was studied in this research. The results showed that under the influence of salivary amylase on the substrate containing starch together

with proteins without pre-incubation, the result of residual starch was not significantly higher than that of starch under the influence of salivary amylase. It was found that when the starch was exposed to salivary amylase together with 30 min pepsin hydrolysate of proteins after pre-incubation, the result of residual starch was not significantly higher in relation to that of starch under the influence of salivary amylase. At the same time, when the starch was exposed to salivary amylase together with 60 min pepsin hydrolysate of proteins after their pre-incubation, the result of residual starch was significantly higher relatively to that of starch under the influence of salivary amylase. These changes indicate that the effect of salivary amylase pre-incubation on starch in conjunction with both protein and protein hydrolysates was observed to decrease starch hydrolysis under the influence of salivary amylase. These changes may be due to the inhibition of salivary amylase by both proteins, protein hydrolysates or peptides, which prevent starch hydrolysis.

## 5. Conclusions

The results obtained show that a decrease in starch hydrolysis under the influence of salivary amylase was observed with the application of a substrate of starch together with both proteins and protein hydrolysates. These changes may be related to the formation of starch complexes with both proteins, protein hydrolysates or peptides, which prevent starch hydrolysis. When the effect on starch of pre-incubation salivary amylase together with both proteins and protein hydrolysates had been investigated, a decrease in starch hydrolysis under the influence of salivary amylase was observed. These changes may occur due to the inhibition of salivary amylase by both proteins, protein hydrolysates or peptides, which prevent starch hydrolysis.

## REFERENCES

[1] Blaak, E. E., Antoine, J. M., Benton, D., Björck, I., Bozzetto, L., Brouns, F., ... & Vinoy, S. Impact of postprandial glycaemia on health and prevention of disease // Obesity reviews. - 2012. - V. 13. - №. 10. - P. 923-984.

[2] Dhital, S., Warren, F. J., Butterworth, P. J., Ellis, P. R., & Gidley, M. J. Mechanisms of starch digestion by α-amylase-Structural basis for kinetic properties // Critical reviews in food science and nutrition. - 2017. - V. 57. - №. 5. - P. 875-892.

[3] Hardy, K., Brand-Miller, J., Brown, K. D., Thomas, M. G., & Copeland, L. The importance of dietary carbohydrate in human evolution // The Quarterly review of biology. - 2015. - V. 90. - №. 3. - P. 251-268.

[4] López-Barón, N., Gu, Y., Vasanthan, T., & Hoover, R. Plant proteins mitigate in vitro wheat starch digestibility // Food Hydrocolloids. - 2017. - V. 69. - P.19-27.

[5] Singh J., Dartois A., Kaur L. Starch digestibility in food matrix: a review // Trends in Food Science & Technology. - 2010. - V. 21. - №. 4. - P. 168-180.

[6] Torres, M. D., Moreira, R., Chenlo, F., Morel, M. H., & Barron, C. Physicochemical and structural properties of starch isolated from fresh and dried chestnuts and chestnut flour // Food Technology and Biotechnology. - 2014. - V. 52. - №. 1. - P. 135-139.

[7] Xu, H., Zhou, J., Yu, J., Wang, S., & Wang, S. Mechanisms underlying the effect of gluten and its hydrolysates on in vitro enzymatic digestibility of wheat starch // Food Hydrocolloids. - 2021. - V. 113. - P. 10650.