JAP&T
TPA-Timişoara

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Journal of Agroalimentary Processes and Technologies 2011, *17*(4), 410-413

Journal of Agroalimentary Processes and Technologies

Quantification of mucoproteins (glycoproteins) from snails mucus, *Helix aspersa* and *Helix Pomatia*

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Received: 15 November 2011; Accepted: 15 December 2011

Abstract

In this paper the authors have isolated by precipitation with ethanol and lyophilization (after precipitation with acetone) mucoproteins from snail mucus (*Helix aspersa* and *Pomatia*). They were quantified and compared according to the method of isolation (at room temperature and at 4 ^{0}C).

Keywords: glycoproteins, mucoproteins, mucus, mucins, snails

1. Introduction

An important feature of ***modern food processing*** is ***modelling***, respective ***fractioning natural raw materials*** and using the products thus obtained as ingredients to obtain ***"formulated foods"***. Literature suggests insistently that ***invertebrate*** protein fractioning should follow the model supplied by milk processing in protein fractioning by using combined, undenatured methods based particularly on separations with semi-permeable membranes and enzymatic processes, that allow the development of an impressive range of products destined to specific applications in the food industry[1].

The bodies of land pulmonata such as snails and slugs are characterized by rich mucus which covers their surface. Apparently, the mucus may serve in preventing the moisture evaporation, in helping smooth

movements and in protecting the body from mechanical injuries. In addition, some unknown biochemical function may be involved in the mucus, though nothing has been reported so far with this respect [2].

Glycoproteins (GP), physiologically active biomacromolecular structures, widespread in the animal world, are ***heteroproteins (proteins conjugated)*** structured from a carbohydrate (*poly-saccharide* with fragments of ***N-acetyl hexosamine***, different ***monosaccharides***, and ***uronic acids***), is called ***mucopoly-saccharide*** (***immunopoly-saccharide***) as ***prostetic group*** and ***proteins proper***, predominantly quantitatively.

Depending on the nature of the linking, in most glycoproteins, there are three glycosidic bridges: (**O**) – glycosides, (**C**) – glycosides and (**N**) glycosides, respectively.

Mucin glycoproteins are the major macromolecular constituents of epithelial mucus and have long been implicated in health and disease. Mucins historically are large, highly glycosylated, viscoelastic macromolecules that are difficult to isolate and purify All papers will be written in English. The journal publishes original paper and review articles in the mentioned fields [3].

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Proteins are glycosylated mucin composition, over 90% are glycoproteins [4].

Literaturereports the possibility of isolating, purifying, and characterising two categories of glycoproteins (***mucoproteins***) from the snail species (***Helix pomatia)*** (***figure 13***). The first structure was isolated from the mucous secreted by the snail foot and it can be characterised by a low share of the sugar fragment (mainly protein chain). A second protein isolated from the same superficial biological area is characterised by an increased share of the structures with non-protein nitrogen (***6-8%***), called "***sinistrin***" [5].

2. Materials and Method

Mucins isolation from snail mucus. *By precipitation with ethanol.*Collection of mucus is made with a rod glass, by scraping foot, 200 g of snails of each species, Helix aspersa (HA) and Helix Pomatia (HP), snails secrete a large amount of mucus. From H.A. snails is obtain 33 g mucus, and from H.P. 27 g. It is collected in Erlenmeyer and over is added 2 to 3 volumes of distilled water. Mucus mixture with water is stirred overnight at room temperature (RT^0), for the first experiment, and at 4 ^{0}C for the second (***figure 1***).

After stirring, in both cases, the mixture is centrifuged at 11,000 g (gravitational force), 30 minutes at 4 ^{0}C. After centrifugation remove the precipitate and the supernatant WSF (water soluble fraction) is collected, 110 ml for H.A. and 98 ml for H.P. at RT0, and 83.5 ml for H.A. and 100 ml for H.P. at 4 ^{0}C.

Supernatant is precipitated with 3 volumes of cooled ethanol, precipitation performed at -20 ^{0}C for 2 h. Collection of precipitate is made by centrifugation at 2900 g (gravitational force), 30 minutes and 4 ^{0}C.

Before precipitation ethanol and WSF was cool down at -20 0C for 20 minutes. Precipitated with ethanol is kept at -20 ^{0}C. Centrifuge used is Avanti J-30i from BeckmanCoulter company, with the possibility of temperature adjustment.

By precipitation with acetone and lyophilization. Isolation of snails mucins can be done by lyophilization. On the mucus obtained by rubbing is add 2 volumes of dH2O to obtain a mixture of mucus. The mixture is directly precipitated with 2 volumes of acetone, previously cooled. The precipitate is collect, and lyophilized using Ilshin freeze dryers. Freeze time is 24 h, and temperature -53 ^{0}C. Block diagram of operations is shown in ***figure 2*** [4].

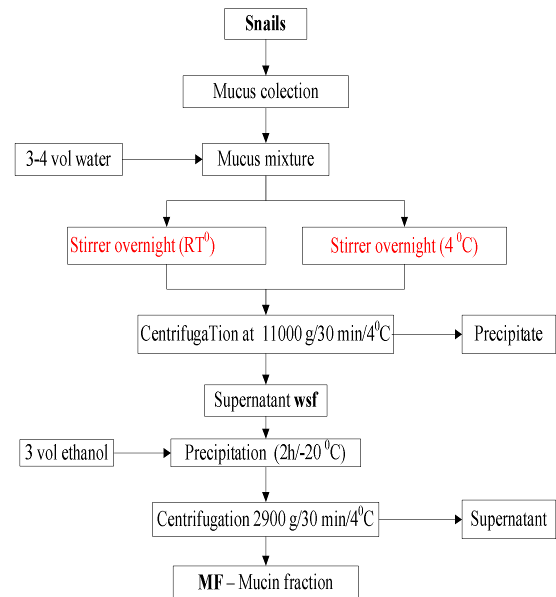

Figure 1. Operations block diagram of Isolation for mucins

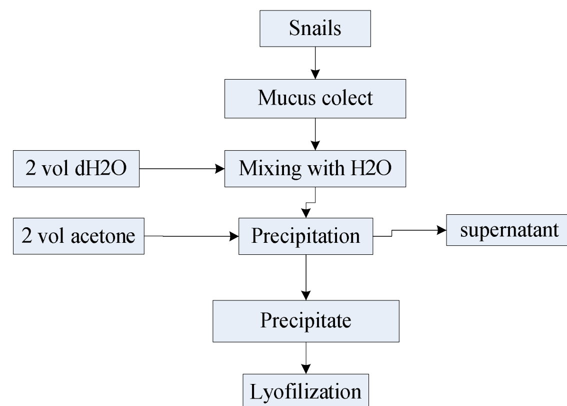

Figure 2. Operations block diagram to obtain lyophilized mucin

Precipitated proteins quantification using the method Bradford

Precipitates, 20 mg of each, is dissolved in 500 ml of urea solution, pH 7.4. For a better dissolve were heated to 90 ^{0}C for 5 minutes, shaken in vortex and / or ultrasonic bath maintained for 5 to 10 minutes.

The dilution, with water, for sample are made: 1:5, 1:10, 1:20, 1:50. Serum albumin, BSA (2 mg BSA / NaCl 0.9% NaN3), is using as standard. Dilution standard are made as follows:

A – 20 µL urea solution
B – 20 µL BSA + 20 µL urea solution
C – 20 µL din B + 20 µL urea solution
D – 20 µL din C + 20 µL urea solution
E – 20 µL din D + 20 µL urea solution
F – 20 µL din E + 20 µL urea solution

On the plate is placed diluted standard and diluted samples, 3 times each in three wells each.

In each well is put 5 µL of sample, over which is added 250 µL of Bradford solution .

The plate is read with Magellan program on Tecan Sunrise. Read plate is made at 595 nm. Is made the average absorbances for standard dilutions, linear regression and the chart standard. With linear regression equation is calculate the amount of protein from each sample. The result is multiplied with the dilution and results expressed in mg / mL protein).

Table 1. The amount of mucoproteic precipitation, respectively lyophilised product

Isolation methods	*Helix Aspersa*	*Helix Pomatia*
Ethanol precipitation, unpurge snails	6.5 g	9 g
Ethanol precipitation, purge snails	10.18 g	13.40 g
Stirred overnight, at RT^0	13.08 g	15.37 g
Stirred overnight, at 4 ^0C	6.7 g	13.90 g
Lyophilization	2.82 g	3.25 g

To determine the amount of protein from precipitated, was used Bradford method. 20 mg of precipitated sample was dissolved in 500 ml urea buffer. For the precipitate obtained by stirring for 12 h at RT^0 and 4 ^0C, protein concentration was calculated using the standard (serum albumin BSA).

Using linear regression ($y = 0.612x + 0.387$) obtained from the graph (***figure 3***) and with values from the below table:

Standard dilution	Absorbans (595 nm)
0	0.39233333
0.5	0.689
0.25	0.54566667
0.125	0.487
0.062	0.397

were calculated values (concentration of protein mg/mL) shown in ***table 2***.

From table 2, figure 4, and figure 5, can see that content mucoproteins is higher in precipitate obtained by stirring overnight at 4 ^0C, compared to those obtained at RT^0, although by stirring at 4 ^0C the amount of precipitation is higher than the amount obtained from RT^0. From the same table can be seen that the amount of mucoproteiens obtained by stirring overnight at RT^0 is higher in HP, instead if stirring at 4 ^0C, the amount of mucoproteins is higher in HA.

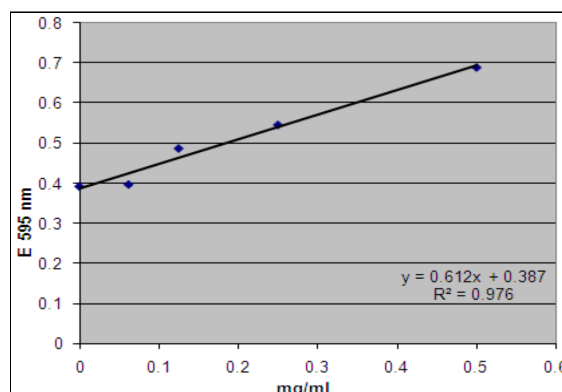

Figure 3. Standard values graph

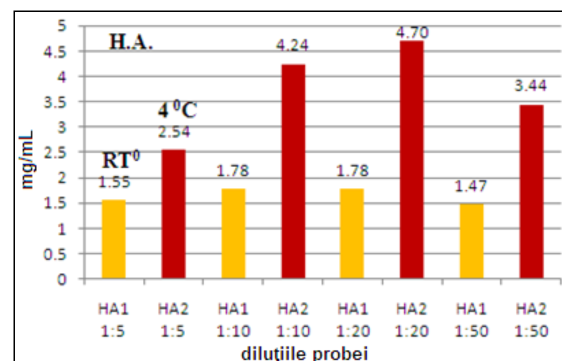

Figure 4. The difference between H.A mucoprotein content of samples at 4 ^0C and RT^0

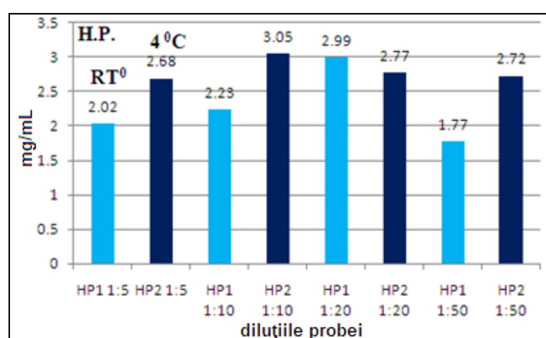

Figure 5. The difference between H.A mucoprotein content of samples at 4 ^{0}C and RT^0

Table 2. The amount of protein, mg/mL, precipitate from HA and H.P. (**20** mg precipitated dissolved in 500 µL urea solution)

Diluţia probelor	**Valoarea absorbanţei la 595 nm**	**Concentraţia de proteine (mg/mL)**
HA1 1:5	0.615	1.55
HA1 1:10	0.51	1.78
HA1 1:20	0.43933333	1.78
HA1 1:50	0.39233333	1.47
HP1 1:5	0.689	2.02
HP1 1:10	0.54566667	2.23
HP1 1:20	0.487	2.99
HP1 1:50	0.397	1.77
HA2 1:5	0.77033333	2.54
HA2 1:10	0.704	4.24
HA2 1:20	0.55466667	4.70
HA2 1:50	0.42333333	3.44
HP2 1:5	0.79333333	2.68
HP2 1:10	0.61	3.05
HP2 1:20	0.47866667	2.77
HP2 1:50	0.412	2.72

Conclusion

The amount of mucin isolated from *Helix Pomatia* species is higher than mucin isolated from *Helix aspersa*;

Also, the amount of mucoproteins isolated from H.P. is higher than the amount isolated from H.A.;

Given the temperature at which the mixture is stirring (mucus and water), the quantity of mucuproteins is higher in samples shaken overnight at 4 ^{0}C (for the same species);

Protein is higher for H.A. snails for cold stirring (4 ^{0}C);

Glycoproteins are also, due to their diversity, biologically active competences, inter- and pluri-disciplinary application fields, of real interest for the science of food processing in the near future.

Acknowledgements

We wish to thank the Banat's University of Agriculture Sciences and Veterinary Medicine Timişoara, Faculty of Food Technology Products, for a generous helpful of different kinds and for financial support by doctoral scholarship programme SOP HRD (Sectoral Opera-tional Programme Human Resources Development) by the Managing Authority of SOP HRD (MA SOP HRD).

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