Protein profiling and modulation of digestive proteolytic enzymes by NaDES extract of citrus by-product

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1- INTRODUCTION AND AIM

Food processing generates high waste causing significant disposal expenses. Many of these biomaterials, including citrus wastes, are a good source of valuable compounds that could be used in food industries. Recently, "green" novel extraction techniques have been used to optimize the extraction of bioactive compounds and nutrients. NaDES are composed of two or more components that interact with each other and form a eutectic mixture. The advantages of NaDES are biodegradability, low costs, and low toxicity. This study aimed to characterize the protein profile of extracts from citrus peel obtained using 4 NaDES formulations (Choline Chloride-Fructose (1.9:1) Choline Chloride-Xylose (2:1), Choline Chloride-Glycerol (1:2), Choline Chloride-Glycerol-Citric Acid (1:1:1)) and 1 hydroalcoholic solution (50% Ethanol)). Moreover, since the orange peel is a considerable source of polyphenols that possess an enzymatic modulating activity, the effect of extracts on the hydrolytic activity of pepsin, trypsin, chymotrypsin, and alpha-amylase was also evaluated.

2- METHODS

NaDES were mixed in a flask bottle at a specific molar ratio, with 25% of distilled water. The mixtures were placed in a water bath (80ºC) until a transparent liquid was formed. The orange peel was crushed with a kitchen blender. 1g of orange peel was mixed with 10 mL of NaDES, the extraction was done in a magnetic heating stirrer for 30 minutes at 40 ± 5ºC. The samples were centrifuged (3000 rpm, 10 min), and the supernatant was collected and kept at 4ºC until used (1).

Proteins were collected by mixing the different extracts of NaDES with acetone (ratio 1:1) and put in agitation for 1h at 4ºC. The solution was centrifuged, and the supernatant was collected and mixed with phosphate buffer (PBS). Sample were prepared for the sds-page by adding the reducing agent and then 0,025 ml of each sample were loaded on an 12% acrylamide gel.

For the enzymatic activity, bovine blood hemoglobin (f.c. 2% at pH 2 for pepsin and pH 7 for trypsin, and chymotrypsin) was added to different NaDES extracts volumes (0.0116 ml Choline Chloride-Fructose, 0.0105 ml Choline Chloride-Glycerol, 0.0124 ml Choline Chloride-Xylose, 0.0154 ml Choline Chloride-Glycerol-Citric Acid and 0.0129 ml 50% Ethanol) to reach a concentration of 0.2 mM of total polyphenols in the assay. 0,1 ml of the required enzyme (pepsin, trypsin and chymotrypsin) was then added to start the reaction, which was stopped after 5 or 10 min by adding 1 ml of 5% (w/v) trichloroacetic acid. After centrifugation at 3000 rpm for 10 min at room temperature, aromatic acids released were detected spectrophotometrically at 280 nm. Alpha-amylase activity was evaluated using the Cereal alpha-amylase Assay Kit (Megazyme) following the manufacturer's instruction. Data of different enzymatic activity was expressed as U/mg concerning control (with no NaDES) (2).

3-RESULTS: PROTEIN PROFILE

Protein recovery and profile is depending on NaDES formulation, with a band associated to 66 kDa in Choline Chloride-Xylose and EtOH 50% extracts. Moreover, multiple bands were associated to 60-66 KDa in Choline Chloride-Glycerol-Citric Acid extract (figure 1).

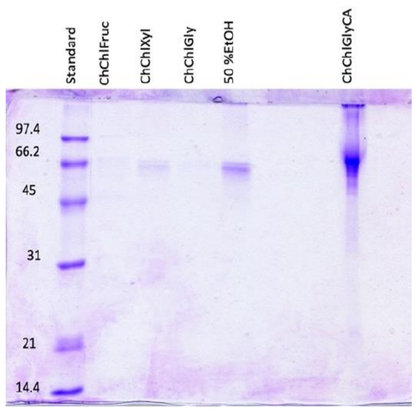

Fig 1: protein profile of different NaDES extracts Choline Chloride-Fructose (ChChlFruc), Choline Chloride-Xylose (ChChlXyl), Choline Chloride-Glycerol (ChChlGly), Choline Chloride-Glycerol-Citric Acid (ChChlGlyCA) and 50% Ethanol (50%EtOH). Pictures are representative of two different SDS-PAGE experiments

3-RESULTS: ALPHA-AMYLASE ACTIVITY

Choline Chloride-Glycerol-Citric Acid extract determined a reduction of alpha-amylase activity with no statistically significant variation for the other extracts (figure 2).

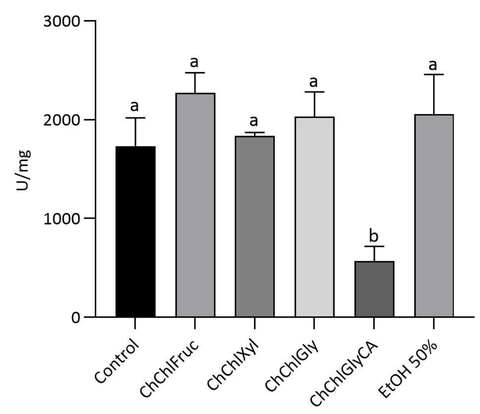

Fig 2: alpha-amylase activity of different NaDES extracts Choline Chloride-Fructose (ChChlFruc), Choline Chloride-Xylose (ChChlXyl), Choline Chloride-Glycerol (ChChlGly), Choline Chloride-Glycerol-Citric Acid (ChChlGlyCA) and 50% Ethanol (50%EtOH). Data are means ± SD of two independent reaction and are expressed as U/mg respect control. Statistical analysis was by one-way ANOVA ($p < 0.05$) with Tukey's post-hoc test (different letters indicate significant differences).

3-RESULTS: PROTEOLYTIC ACTIVITY

All NaDES extracts determined an increase of pepsin activity (figure 3A) without any effect on chymotrypsin activity (figure 3B). On the contrary, Choline Chloride-Glycerol-Citric Acid determined a reduction of trypsin activity (figure 3C).

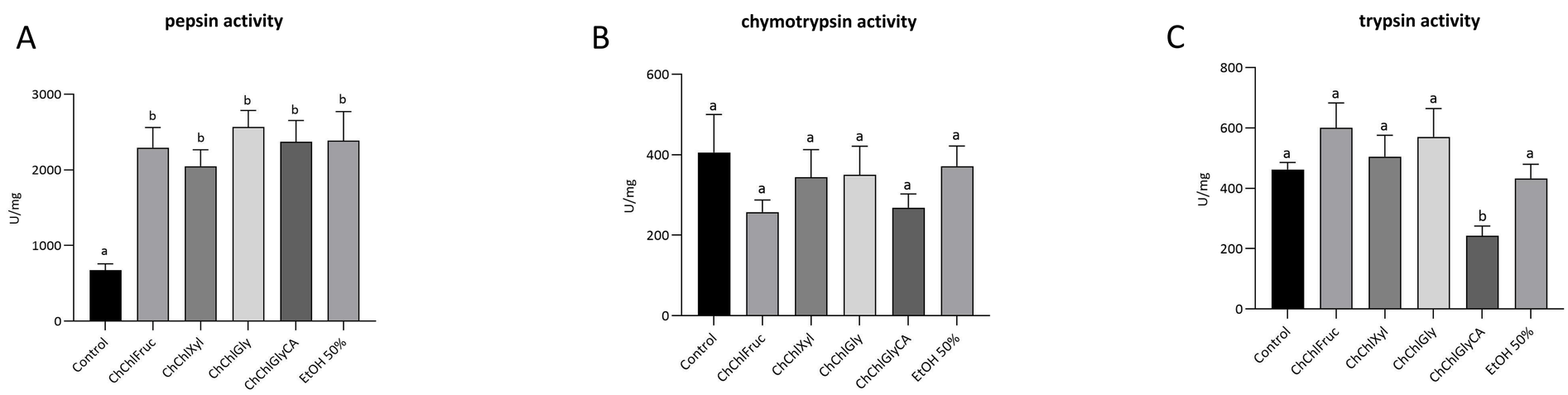

Fig 3: in vitro modulation of pepsin (2A), chymotrypsin (2B), and trypsin (2C) by different NaDES extracts, Choline Chloride-Fructose (ChChlFruc), Choline Chloride-Xylose (ChChlXyl), Choline Chloride-Glycerol (ChChlGly), Choline Chloride-Glycerol-Citric Acid (ChChlGlyCA) and 50% Ethanol (50%EtOH) using hemoglobin as a substrate. Data are means ± SD of two independent reaction and are expressed as U/mg respect control. Statistical analysis was by one-way ANOVA ($p < 0.05$) with Tukey's post-hoc test (different letters indicate significant differences).

4-CONCLUSION

The protein profile of NaDES, in particular the Choline Chloride-Glycerol-Citric Acid (ChChlGlyCA) extract, shows that there are several bands associated with a molecular weight comprised of 66 and 45 kDa. From a nutritional point of view, NaDES extracts can modify the activity of digestive enzymes such as pepsin and trypsin : the reported effect on digestive proteases could improve in vitro protein digestibility at gastric level. Instead, there is no effect on chymotrypsin activity. Furthermore, Choline Chloride-Glycerol-Citric Acid extract showed a reduction in alpha-amylase activity, with no statistical differences for the other extracts.

However further studies are needed to evaluate their biological effects by considering NaDES as active ingredients thus bypassing the difficulties of solute recovery.

5- ACKNOWLEDGEMENT

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