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Aqueous extracts and suspensions of restructured pork formulated with *Undaria pinnatifida*, *Himanthalia elongata* and *Porphyra umbilicalis* distinctly affect the *in vitro* α-glucosidase activity and glucose diffusion

Alba Garcimartín [a, b], Juana Benedí [b], Sara Bastida [a], Francisco J. Sánchez-Muniz [a, *]

[a] Departamento de Nutrición y Bromatología I (Nutrición). Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal s/n, 28040 Madrid, Spain
[b] Departamento de Farmacología, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramón y Cajal s/n., 28040 Madrid, Spain

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ABSTRACT

Seaweed antidiabetic properties have been linked to changes in carbohydrate digestion and absorption. The effect of aqueous extracts and suspensions of restructured pork (RP) with 5 g/100 g of *Porphyra umbilicalis* (Nori), *Undaria pinnatifida* (Wakame) or *Himanthalia elongata* (Sea spaghetti) on α-glucosidase activity and glucose diffusion were *in vitro* tested. α-Glucosidase inhibition type was also assayed. Sea spaghetti-RP suspensions displayed, through a competitive mechanism, the highest α-glucosidase inhibitory effects (19.8% *vs.* control-RP at 45 min). Control-RP extracts vs. blank increased (15.4%) glucose diffusion while Nori-RP followed by Wakame-RP extracts significantly inhibited glucose diffusion (26.7% and 20%, respectively) and decreased AUC (18–20%) respect to control-RP counterpart. Stepwise analyses showed that maltose and polyphenols in the α-glucosidase inhibition- and Na in glucose diffusion-test, were significantly involved. In conclusion, Sea spaghetti-RP should be selected as α-glucosidase inhibitor while Nori-RP and Wakame-RP should be used as glucose diffusion reducers. As both hypoglycaemic effects were non-concatenated, generalizations about seaweed-RP should be avoided.

1. Introduction

Type 2 Diabetes Mellitus (T2DM) is a lifestyle-related disease that has been strongly associated with dietary habits. Specifically, mild T2DM cases are currently increasing due to decreased physical activity and wrong dietary habits based on diets rich in energy and saturated fat (e.g. fatty meat based-diets) (Zhou, Tian, & Jia, 2013). Meat consumption is very high in Westernized countries. However, some of the potential negative components of meat could be partially removed in order to obtain potentially healthier functional meat (Burger, Beulens, Boer, Spijkerman, & Van der, 2011). In fact, due to their high acceptability, meat products are an excellent alternative to include bioactive compounds in the diet without changing dietary habits. Hence, the design of restructured meat (e.g. restructured pork, RP) may lead to improve composition and potential health benefits ascribed to commercially meat products (Olmedilla-Alonso, Jiménez-Colmenero, & Sánchez-Muniz, 2013).

Seaweeds are a good source of soluble/viscous dietary fibre (Brownlee et al., 2005), minerals and vitamins, and also have associated bioactive compounds such as polyphenols (Bocanegra, Bastida, Ródenas, Benedí, & Sánchez-Muniz, 2009; Jiménez-Escrig & SanchezMuniz, 2000). They exert variable effects on rat cholesterolaemia and antioxidant status, which persist when included as functional ingredient in RP (Moreira et al., 2010) or other food matrices (Dawczynski, Schubert, & Jahreis, 2007). In recent years many studies provide evidence that marine seaweeds and their derivatives show potential antidiabetic properties and could help to partially replace hypoglycemic drugs (Lin & Liu, 2012; Pantidos, Boath, Lund, Conner, & McDougall, 2014).

In vitro models could represent a useful approach to study the

Abbreviations: CVD, cardiovascular disease; CRP, control-RP; GOD, glucose oxidase; Ki, inhibition constant; Km, Michaelis–Menten constant; NRP, restructured pork containing 5 g/100 g of Nori; RP, restructured pork; SRP, restructured pork containing 5 g/100 g of Sea spaghetti; T2DM, Type 2 Diabetes Mellitus; Vmax, maximum velocity; WRP, restructured pork containing 5 g/100 g of Wakame.

* Corresponding author.
E-mail address: frasan@ucm.es (F.J. Sánchez-Muniz).

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inhibition of both α-glucosidase activity (Jo, Lee, Apostolidis, Jang, & Kwon, 2009) and glucose movement across the dialysis membrane into external solution (Gallagher, Flatt, Duffy, & Abdel-Wahab, 2003). In a previous study (Schultz Moreira et al., 2014) we found that some inorganic and organic seaweed extracts (e.g. aqueous extracts from *Himanthalia elongate* and ethanolic from *Undaria pinnatifida*) inhibit α-glucosidase and glucose diffusion, respectively, but both effects were not concatenated.

To the best of our knowledge no studies on the effect of seaweed-RP extracts/suspensions on carbohydrate digestion and absorption have been performed. In the present paper we hypothesised that seaweed-RP aqueous extracts or suspensions maintain or mimic the observed inhibition effects of pure seaweeds on carbohydrate digestion or/and absorption. The aim of this study was to evaluate the *in vitro* effect of aqueous suspensions/extracts of RP enriched with *Undaria pinnatifida* (Wakame), *Himanthalia elongata* (Sea spaghetti) or *Porphyra umbilicalis* (Nori) on α-glucosidase activity and glucose diffusion.

2. Material and methods

Dialysis tubing cellulose membrane (D9277-100FT), NaCl, D-(+)-maltose monohydrate, intestinal acetone powders from rat (I1630), and sodium maleate dibasic were purchased from Sigma–Aldrich (Alcobendas, Madrid, Spain). Anhydrous D (+)-glucose was obtained from Merck (Barcelona, Spain). Glucose-TR kit (1001190) based on glucose oxidase (GOD) reaction was supplied by Spinreact (Barcelona, Spain).

2.1. Restructured pork preparation

Pigs of 6 months of age with a weight range of 80–90 kg from the animal husbandry area of Lleida (Spain) were used. Raw post-rigor (mixture of different muscles: *biceps femoris*, *semimembranosus*, *semitendinosus*, *gracilis and aductor*) and back fat, both pig products (*Sus scrofa domestica*), were purchased in a local store in Madrid. RP were obtained as described by Cofrades et al. (Cofrades, López-López, Solas, Bravo, & Jiménez-Colmenero, 2008). Briefly, raw pig material was homogenized and ground for 1 min in a chilled cutter at 2 °C in a Stephan Universal Machine UM5; (Stephan u. Sóhne GmbH and Co., Hameln, Germany). All the fat and half of the seaweeds, NaCl (2.0 g/100 g for control samples and 0.5 g/100 g for samples with added seaweed), STP and sodium nitrite (the last two previously dissolved in the added water) were added to the ground meat and the whole was mixed for 1 min; then the rest of the ingredients were added and the mixture homogenized for 1 min. Finally, the whole meat batter was homogenized under vacuum for 2 min. Mixing time was standardized at 5 min and the final batter temperature was the below 12 °C in all cases. Concentration of algae in RP was 5 g/100 g. Meat samples were freeze-dried for 48 h in a LyoAlfa 10 freeze dryer (Teltar, Tarrasa, Spain). Samples were frozen to −30 °C and submitted to vacuum at 3 Pa. Four different potential functional meats were obtained: Wakame-RP (WRP), Nori-RP (NRP), Sea-spaghetti-RP (SRP), and control-RP (CRP) elaborated without adding seaweed.

2.1.1. Seaweed and RP analysis

Although seaweeds and seaweeds extracts present a very complex composition, several analyses were performed to obtain information about potential factors affecting enzyme activity and glucose diffusion. Fresh seaweeds were dried in the shade after collection on the Atlantic coast (Algamar C.B., Redondela, Pontevedra, Spain). Then, they were milled (Ultra Centrifugal Mill ZM 200; Retsch GmbH and Company, KG, Haan, Germany) and stored in plastic flasks at 4 ± 2 °C until used.

Proximate analysis of moisture, ash, fat and protein were performed in dried and powered seaweeds following standard methods as indicated by Cofrades et al. (2010). Soluble, insoluble and total fibre contents were determined following indications of Cofrades et al. (2010). Soluble and insoluble fractions were obtained after enzymatic hydrolysis of digestible compounds. Polyphenols were determined using the Folin Ciocalteau reagents as explained by Cofrades et al. (2010). The fatty acid profile was determined by gas liquid chromatography using capillary column and conditions according to López-López et al. (2009). All analyses were performed in triplicate in the different algae (from the same batch).

Three independent samples from each RP type were used for analysis. Moisture, ash, protein and fat RP were analysed as indicated by López-López et al. (2009). The fatty acid profile was determined by column chromatography according to López-Lopez et al. (2009). The mineral content was determined after digestion with HNO_3 and H_2O_2 in the oven by ICP-OES as detailed by López-López et al. (2009).

2.2. Suspensions and extracts preparation

Replicates were made in three independent samples from each RP type. Suspensions of the different RP's were prepared to check the effect of both water soluble and insoluble components on α-glucosidase activity as previous studies reported the effect of insoluble fibre on the inhibition of α-amilase activity (Chau, Chen, & Lin, 2004). Briefly, 250 mg of freeze-dried samples were resuspended in 10 mL of saline solution (0.9 g/100 mL NaCl), previously tempered at 37 °C, and kept stirring for 15 min. Samples were vortexed till homogeneity.

Aqueous extracts instead of suspensions were obtained for glucose diffusion assay to avoid membrane blocking by insoluble compounds. Freeze-dried RP was finely pulverized and stored at −20 °C until analysis. Extraction of components was carried out according to Gray and Flatt method (1998). Briefly, 1.0 g of the powdered RP-samples were placed for extraction in 40 mL of boiling distilled water (100 °C) and stirred thoroughly for 30 min. The mixture was centrifuged (Centrifuge 5804R, Eppendorf, Hamburg, Germany) at 2500 × *g*, 4 °C for 10 min and each supernatant was collected into bijoux tubes. Aliquots (5 mL) of extracts were dried under vacuum (Savant Speed Vac PD 121P, Savant, Labcare, Buckinghamshire, England) and stored at −20 °C. Extracts were reconstituted in 1 mL of PBS, pH 7.4 before analysis.

2.3. α-Glucosidase activity

α-Glucosidase, as a maltase activity, was tested according to the Mai et al. method (2007), to measure the production of glucose from maltose in each of the three independent samples of the different RP. To obtain the enzyme solution, rat intestinal acetone powder (Sigma–Aldrich, St Louis, MO, USA) was homogenized in maleate buffer (pH 6.0) and centrifuged at 1875 × *g* for 10 min at 4 °C. The supernatant was used as a crude enzyme solution for the maltase reaction. Briefly, 100 μL of maltose, 50 μL of enzyme and 50 μL of suspension sample (125 mg RP/mL PBS, pH 7.4) were incubated at 37 °C for 95 min. Every 15 min an aliquot of 20 μL of this incubation sample was introduced 10 min into boiling water to stop reaction. Glucose concentration was quantified by enzymatic colorimetric analysis (Spinreact) reading the absorbance at 490 nm in a spectrophotometer (Digiscan 340, Asys Hitech, Germany). Two different maltose concentrations, 5 mg/mL and 80 mg/mL, were tested. Besides RP suspensions, saline solution was included as a blank. Data are presented as means ± SD. α-Glucosidase activity was expressed as mmol/L glucose produced per min or % activity with respect to blank or CRP.