

INTRODUCTION

Cardoon thistle (*Cynara cardunculus*) is a Mediterranean plant known as cardo, frequently used as a coagulant in Portuguese cheese production, as a water-based extract called thistle-rennet, particularly in the region of Serpa, Serra da Estrela and Azeitão. Since cardoon is added to the milk, potential bioactivities of this plant could be beneficial as an added value in this type of cheese. The goal of this study was to test the potential bioactivity of cardoon and thistle-rennet, specifically against foodborne pathogens, matrix metalloprotease 9 (MMP-9), an endoprotease which has for long been recognized as playing important roles in inflammation and cancer.

MATERIAL AND METHODS

Cardoon was obtained from a cheese factory in Serpa, as dry plant and flowers. The thistle-rennet was prepared in the same manner as the manufacturer would use to produce cheese. By request of the producer the details of the method will not be disclosed.

Antibacterial activities were assessed using the serial dilution method, with *E. coli* O157 as a model bacteria. Bacterial growth was evaluated in the presence of several dilutions of the cardoon extract, in Müller Hinton media. Optical density (OD) was measured after 24 h growth.

The inhibitory potential against MMP-9 was tested both in the presence of commercial isolated MMP-9 or *in vitro*, in colon cancer cells (HT-29) using the fluorimetric kit DQ-gelatin. For the *in vitro* assays, HT-29 cells were maintained in RPMI growth media, using standard procedures. The filtered cardoon extract was added as a final volume of 1% of total media. After 48 h exposure, the extracellular media containing MMP-9 was collected and its gelatinolytic activities were evaluated.

RESULTS/DISCUSSION

CARDOON INHIBITS *E. coli* GROWTH

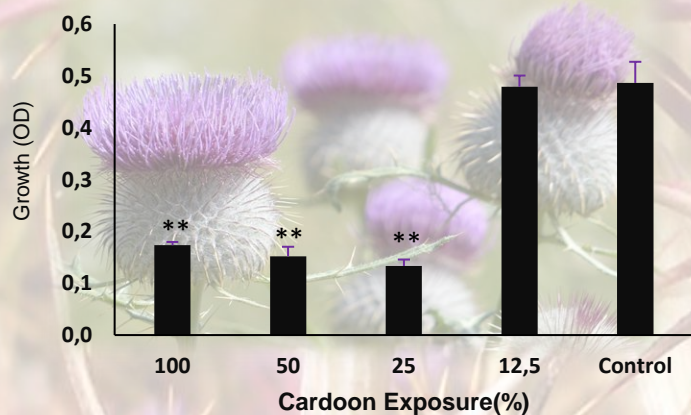


Fig. 1: *E. coli* growth in the presence of sequential dilutions of the cardoon extract. Results are a media of at least three replicate experiments +SD. ** p<0.001

Cardoon significantly reduced bacterial growth, even at the lowest dilution of 25%. These results suggest that the use of cardoon in cheese production can improve shelf life and be of added benefit for health.

CARDOON REDUCES MMP-9 ACTIVITY

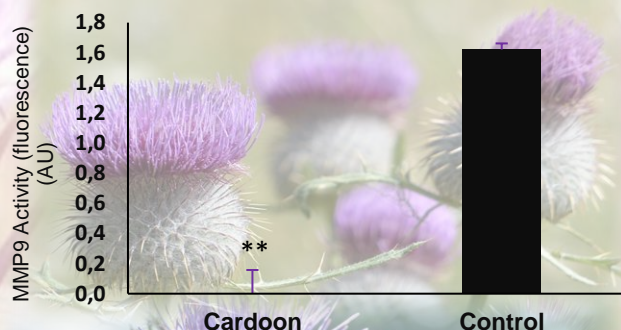


Fig. 2: Gelatinolytic activity of isolated MMP-9 in the presence of the cardoon extract, measured by the DQ-gelatin kit. Results are expressed in arbitrary units of fluorescence (the higher the fluorescence the higher the activity) and as a media of at least three replicate experiments +SD. ** p<0.001

CARDOON INHIBITS MMP-9 ACTIVITY IN COLON CANCER CELLS

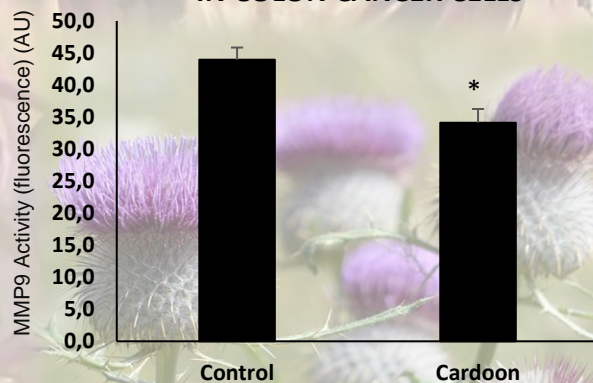


Fig. 3: Effect of the cardoon extract on MMP-9 activity levels in HT-29 cells after a 48-h exposure, measured by the DQ-gelatin kit. Results are expressed in arbitrary units of fluorescence (the higher the fluorescence the higher the activity) and as a media of at least three replicate experiments +SD. * p<0.01

The cardoon extract reduced MMP-9 activity in both isolated MMP-9 and in HT-29 colon cancer cells. Since MMP-9 activity is related to cancer development and inflammation, overall, these results suggest that cardoon may have potential as a novel functional food or nutraceutical.

CONCLUSION:

Cardoon appears to have bioactivities with high potential for food preservation and consumer's health. Although more studies should be performed, our results open promising perspectives for traditional cheese industries in Portugal. Considering cardoon is frequently used in the cheese industry and is completely GRAS (generally accepted as safe), this could be of great importance not only as an added value to the cheese industry but also as a functional food for human health purposes.

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