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Original Paper

α-amylase inhibitory effect of several varieties of cheese: Gouda, Gruyere, Edam, Emmental

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Abstract

Introduction : Cheese is a highly nutritious fermented milk product with diverse flavor and texture. As a functional food it has gained a lot of attention in recent times because of their health benefitting properties.
Methods: In the present study, four varieties of cheese (Gouda, Gruyere, Edam and Emmental) are tested for their antidiabetic effect. In fact, an aqueous extract is prepared by maceration of cheese pieces in phosphate buffer. Inhibitory effect against α-amylase of extract, pH and proteins content are investigated.
Results : Results show pH value between 5.2 and 5.9 with protein content between 4.5 and 9.14%. Inhibitory effect against α-amylase is moderate with IC_{50} values of 49.29±4.02, 38.5±6.05, 70.66±12.52 and 25.9±6.82 $mg.mL^{-1}$ for Goud, Gruyere, Edam and Emmental respectively.
Conclusion : In fact, we can conclude that Emmental cheese has the best inhibitory effect on porcine pancreatic α-amylase compared to Gouda, Gruyere and Edam cheeses.. Investigation of active ingredient in cheese especially in Emmental may be interesting.

Keywords: Cheese, Emmental, α-amylase inhibition, diabetes mellitus

Introduction

Cheese is the most common dairy product, which is manufactured from raw pasteurized cow and buffalo's milk, but also from other species such as sheep and goats (Genis et al. 2019). Different types of heat treatments can be used in the dairy industry such as thermization, heating, pasteurization, cooking, and sterilization. Heat treatments of milk are mostly applied to prevent pathogenic microorganisms, to improve stability of products during storage, to increase shelf life, affect the texture of the final product and enhancing the performance of following technological operations (Bezie 2019). Utilization of raw milk for making cheese leads to cheeses with greater variability in comparison to pasteurized cheeses which is characterized by powerful and unique organoleptic profile which sometimes highly accepted by the consumers (De Sainte Marie et al. 2019).
In addition, functional foods have gained a lot of attention in recent times because of their health benefitting properties. In this context, the use of fermented dairy products with particular nutraceutical and probiotic properties has increased profoundly (Mushtaq et al. 2021).
The aim of this study is to investigate the antidiabetic effect of water extracts of four varieties of cheese, Gouda, Gruyere, Edam, Emmental by testing the inhibitory effect on pancreatic porcin α-amylase.

Material and methods

Extraction

In this study, we are interested by four varieties of cheese such as Gouda, Gruyere, Edam and Emmental. All these preparations are made using cow milk.

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To prepare aqueous extracts, 2 g of each cheese are homogenate with 10 mL of sodium phosphate buffer (0.02 M, pH 6.9).
The mixture was incubated for 12 h and centrifuged at 3500 rpm for 20 min. The supernatant represents the aqueous extract and is used to evaluate its inhibitory effect against α-amylase.

Extract characterization

Proteins in cheese extracts (supernatant) were quantified according to the technique employed in the study by Henry et al. (1974) based on the Biuret reaction. The pH of extracts was determined using a digital pH meter (AD 1030) (Lala 1981). Mass concentration was appreciated by drying 1 mL of cheese extract in the oven at 50°C. The obtained residue was weighed to estimate the mass concentration of each extract.

α-amylase inhibitory test

α-Amylase (E.C. 3.2.1.1) inhibition was determined following the method described by Miller (1959) with slight modifications. The assay mixture consisted of 500 µL of cheese extract or acarbose prepared in sodium phosphate buffer (0.02 M, pH 6.9) and 500 µL sodium phosphate buffer (0.02 M, pH 6.9) containing porcine pancreatic α-amylase (3.9 UI/mL). Thereafter, 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 6 mM NaCl) were added to each tube.
Incubation was done at 37°C for 15 min and stopped with 1 mL dinitrosalicylic acid reagent. Thereafter, the mixtures were incubated in a boiling water bath for 8 min and cooled. The absorbance was measured at 540 nm. α-Amylase inhibitory activity was calculated and expressed as a percentage (%).
A control reaction was prepared as the same by replacing the inhibitor (cheese extract or acarbose) with 500 µL of 0.02 M sodium phosphate buffer (pH 6.9).

$$\% \text{ inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

$A_{control}$ is the absorbance of the control reaction
A_{sample} is the absorbance of the test tube with cheese extract or acarbose
The inhibitory concentration at 50% (IC_{50}) was calculated graphically by tracing the linear curve of inhibitory percentage change as a function of inhibitor concentration.

Results and discussion

Cheese extracts characterization

Cheese extracts are characterized for their protein content, pH and mass concentration. The table 1 summarizes the obtained results. We observe a variation in protein content and mass concentration. This later is more important for Edam extract which presents the high value of proteins. Gouda extract has the low value of proteins.
Mass concentration reflects the content of soluble molecules and this variability can be explained by the variability in composition of cheese qualities (Iwaniak et al. 2022).
pH has a great influence on enzymatic activity because it can affect the enzymatic structure. The shape and structure of the enzyme will change if the pH value of the reaction medium changes. For example, pH can affect the ionization state of acidic or basic amino acids. There are carboxyl functional groups on the side chain of acidic amino acids. There are amine-containing functional groups in the side chain of basic amino acids. If the ionized state of the amino acids in the protein is changed, the ion bonds that maintain the 3D form of the protein will change (Moses et al. 2023). This may result in changes in protein function or inactivation of enzymes. For that, all enzymes have an ideal pH value, which is called optimal pH (Liu et al., 2021).

Table 1: characterization of cheese extracts (Proteins content, pH and mass concentration)

	Proteins content (%)	**pH**	**Mass concentration (mg.mL^{-1})**
Gouda	4.56 ±0.02	5.92 ±0.06	50.66 ±8.02
Gruyere	6.31 ±0,17	5.20 ±0.08	42.33 ±7.39
Edam	9.14 ±0.38	5.49 ±0.07	77.66 ±8.38
Emmental	7.61 ±0,18	5.92 ±0.02	51.30 ±6.95

Milk is recognized as an important source of high quality protein with a wide range of nutritional, functional and physiological activities. The total protein content of bovine milk is approximately 3.5% (Mbye et al. 2020). We observe that cheese extracts are rich in proteins than bovine milk. This can be explained by the manufacturing procedure of cheese and the quality of milk (Mladenović et al. 2021). In dairy product, proteins can play the function as bioactive peptides (Ali et al. 2022).

α-amylase inhibitory

One of the stratagems to counter diabetes (type 2) is the inhibition of enzymes responsible for carbohydrate hydrolysis like α-amylase and α-glucosidase. Inhibiting these enzymes will subsequently lead to decreased postprandial glycemia (Kumari et al. 2023).
Table 2 shows values of obtained IC_{50} against porcine α-amylase for Gouda, Gruyere, Edam and Emmental. This later is endowed with the best inhibition (IC_{50}=25.9±6.82 $mg.mL^{-1}$). However this effect is less interesting than acarbose, the reference molecule. There is no correlation with the proteins content or pH in cheese.
Mushtaq et al. (2021) observed a higher inhibition of water soluble peptides extract against α-amylase.

Table 2: IC_{50} values of cheese extracts and acarbose

	Gouda	Gruyere	Edam	Emmental	Acarbose
IC_{50} ($mg.mL^{-1}$)	49.29±4.02	38.5±6.05	70.66±12.52	25.9±6.82	0.16±0.17

Conclusion

In the light of the present work, we can conclude that Emmental cheese has the best inhibitory effect on porcine pancreatic α-amylase compared to Gouda, Gruyere and Edam cheeses. There is no correlation with the proteins content or pH in cheese. Investigation of active ingredient in cheese especially in Emmental may be interesting.

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Conflict of interest

The authors declare that they have no any financial and/or nonfinancial competing interests.

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