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REVUE FRANÇAISE
D'**Allergologie**

Revue française d'allergologie xxx (2019) xxx–xxx

Original article

Effect of in vitro hydrolysis on coconut milk digestibility

Effets de l'hydrolyse in vitro sur la digestibilité du lait de coco

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Received 30 November 2018; accepted 13 January 2019

Abstract

Coconut milk has high nutritive value. In this study, we attempted to assess the gastrointestinal digestion of coconut milk proteins in vitro. Cow's milk and coconut milk proteins were subjected to two successive hydrolyses with pepsin then with a mixture of trypsin/chymotrypsin. The kinetics of degradation were determined and SDSPAGE was carried out to evaluate specific protein composition before and after α -NH₂ free-function analysis. Determination of α -NH₂ free functions showed coconut milk proteins to be more susceptible to both pepsin digestion ($^{***}p < 0.001$, $^{**}p < 0.01$) and combined trypsin/chymotrypsin digestion ($^{***}p < 0.05$) in relation to bovine proteins. The electrophoretic profile of native coconut milk proteins revealed the presence of β -Lg and the absence of specific proteins; in addition, no coconut milk proteins were seen in the electrophoretic gels.

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Keywords: Coconut milk; Hydrolysis; β -Lactoglobulin; α -Lactalbumin; Pepsin; Trypsin; Chymotrypsin; Digestion

Résumé

La noix de coco possède une forte valeur nutritive, ainsi que le lait de coco. Dans cette étude, nous avons essayé d'évaluer in vitro la digestion gastro-intestinale des protéines du lait de coco. Le lait de vache et le lait de coco ont subi deux hydrolyses successives avec la pepsine, puis avec un mélange de trypsine et de chymotrypsine. La cinétique de dégradation des protéines a été déterminée et la technique SDSPAGE a été utilisée pour évaluer la composition des protéines spécifiques avant et après l'analyse de la fonction libre d' α -NH₂. La détermination des fonctions libres d' α -NH₂ a montré que les protéines du lait de coco étaient plus sensibles à la digestion par la pepsine ($^{***}p < 0,001$, $^{**}p < 0,01$) et à la digestion par le mélange trypsine/chymotrypsine ($^{***}p < 0,05$) en relation avec les protéines bovines. Le profil électrophorétique des protéines natives du lait de coco a révélé la présence de β -Lg et l'absence de protéines spécifiques. De plus, aucune protéine de lait de coco n'a été observée dans les gels électrophorétiques.

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Mots clés : Lait de coco ; Hydrolyse ; β -Lactoglobuline ; α -Lactalbumine ; Pepsine ; Trypsine ; Chymotrypsine ; Digestion

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<https://doi.org/10.1016/j.reval.2019.01.012>

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1. Introduction

In developing countries, the continuous rise in population coupled with inadequate protein supply have inadvertently increased the occurrence of malnutrition [1].

However, animal protein is also inadequate, and relatively expensive research efforts are geared towards identifying alternative sources of protein from legume seeds [2]. According to Murti et al. [3], soy milk has been recognized as nutritionally beneficial.

The first source of antigens encountered in large quantities in early childhood are cow's milk proteins [4]. Among these antigens, β -lactoglobulin (β -Lg), α -lactalbumin (α -Lac) and caseins constitute the main allergens in cow's milk; other proteins, such as bovine serum albumin (BSA) and even lactoferrin (present in trace amounts) are also considered as potential allergens [5].

Coconut serves as an excellent source of raw materials for the development of dairy-like products. Coconut milk is the liquid from the grated flesh of brown coconuts [6] and has the following nutritional properties; protein (3%), fat (17%–24%), and carbohydrate (2%). It has no cholesterol but contains many vitamins, minerals and electrolytes, including potassium, calcium and chloride. The benefits of coconut milk include strengthening of the body's immune and defence systems [7]. In addition, it contains a large amount of lauric acid, which is also found in significant amounts in breast milk and sebaceous gland secretions [6]. The aim of our study was to determine the specific composition of coconut milk proteins and also to study the characteristics of these proteins as found in cow's milk and coconut milk before and after gastrointestinal digestion.

2. Materials and methods

2.1. Chemicals

All β -lactoglobulin and α -lactalbumin were purchased from Sigma (France). All materials and instruments, polyacrylamide gel, β -mercaptoethanol, glycerol and bromophenol blue, Coomassie blue R-250, acetic acid, bovine serum albumin, caseins, pepsin, trypsin, chymotrypsin and ethanol were purchased from Sigma (France), with phosphate buffer, ninhydrin and acrylamide gel being purchased from Merck (France).

2.2. Milk sample collection and bovine whey protein preparation

For the samples, bovine milk was collected freshly at a bovine breeding farm in the Oran region of Algeria. To remove the fat, the cow's milk and coconut milk were first skimmed by centrifugation at 3500 rpm for 15 min at 4 °C. The skim was then freeze-dried using a 100H SpeedVac concentrator centrifuge [7].

2.3. Gastric-pancreatic digestion

Hydrolysis was performed as described in [8]. Cow's milk and coconut milk whey proteins underwent two successive hydrolyses: first in pepsin then in a trypsin/chymotrypsin

mixture. The whey samples were dissolved in 10 mM HCl pH 2.2 at a concentration of 1 mg/ml. Pepsin “844 E 920792, activity 10 FIP-4/mg” was prepared at 4 mg/ml in distilled water and added to whey samples at an E/SR (Enzyme/Substrate Ratio) of 1/100 then incubated for 120 minutes at 37 °C. Enzymatic kinetics were assessed by sampling 200 μ L from the reacted sample at 0, 30, 60, 90 and 120 minutes. The reaction was stopped by increasing the pH to 7.8 through the addition of 200 μ L of phosphate-buffered saline (100 mM, pH 8.5). To inhibit residual pepsin activity, phosphate-buffered saline (100 mM, pH 8.5) was added. Trypsin/chymotrypsin digestion was conducted by adding a trypsin/chymotrypsin mixture (1:1, w/w [weight/weight]) to the pepsin hydrolysates. Trypsin “818 K 20146079, 404/mg activity” and chymotrypsin “type 2 EC.3.4.21.1” prepared in HCl 10-3 N were added at an E/SR of 1/100, W/W, to pepsin whey samples and incubated at 37 °C, pH 8.2 for 120 minutes. The hydrolysis kinetics were investigated by sampling 200- μ L aliquots from reaction samples at 30, 60, 90 and 120 minutes. The reaction was stopped by heating the samples at 95 °C for 5 minutes.

2.4. In vitro digestibility assessment

For each time point, the concentration equivalents of amino groups (h') in the samples were determined by extrapolating from each standard curve. This gave the rate of equivalents peptide bonds hydrolysed (h'), expressed as mEq/g (milliequivalents/gram) of protein using the following equation: $h' = (A \times b)/m$ [9], where A is absorbance at 540 nm (using a visible spectrophotometer: Jasco V-530 UV/VIS), and b and m are the y-intercept and slope of the calibration curve respectively. For each time point, the quantity of amino groups released (h) was determined separately by subtracting the value of hydrolysed control in the corresponding standard curve ($h = h' - h'$ control).

2.5. SDS-PAGE electrophoresis

Protein hydrolysis was analysed by SDS-PAGE using 15% acrylamide gels. Samples were mixed with sample buffer: (SDS, β -mercaptoethanol, glycerol and bromophenol blue) and heated for 5 min at 90 °C in a water bath. The 15% acrylamide gels containing 10% SDS were prepared and run using the discontinuous buffer system [10]. Protein bands were stained with Coomassie blue R-250, with destaining using a solution of 30% ethanol and 5% acetic acid. Two kits were used: kit (1) containing the reference proteins: BSA (bovine serum albumin, 68 kDa, A-7638 5 g Lot 79H7614); casein (24 kDa, Sigma, 9000-71-9, USA); β -Lg (18 kDa, Sigma, L-O130, USA); α -Lac (14.2 kDa Sigma, L-5385, USA); and kit (2) containing: triosephosphate isomerase from rabbit muscle (26.6 kDa), myoglobin from horse meat (17 kDa), α -Lac from bovine milk (14.2 kDa), aprotinin from bovine lung (6.5 kDa), insulin chain B oxidized bovine pancreas (3.496 kDa) and bradykinin (1.06 kDa).

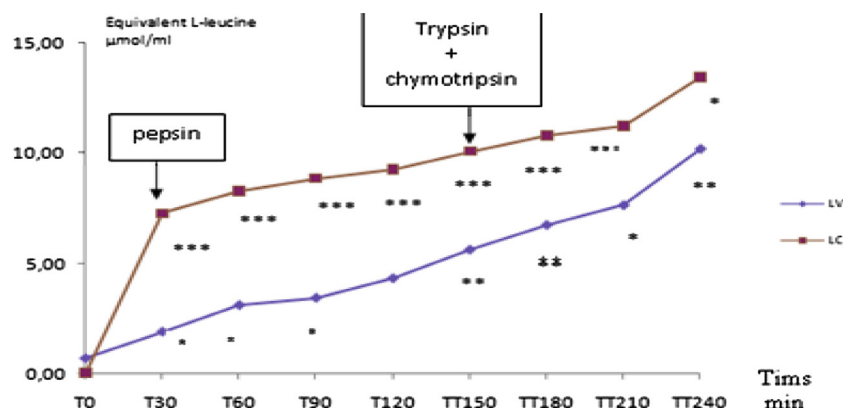


Fig. 1. Kinetics of combined hydrolysis of the wheys by pepsin (E/S R 1:25) and trypsin-chymotrypsin (E/S R 1:100), in each case for 120 min. Values are means $\pm$ SE for five determinations (* $p < 0.05$, ** $p < 0.001$ and *** $p < 0.0001$). LV = Cow's milk; LC = Coconut milk.

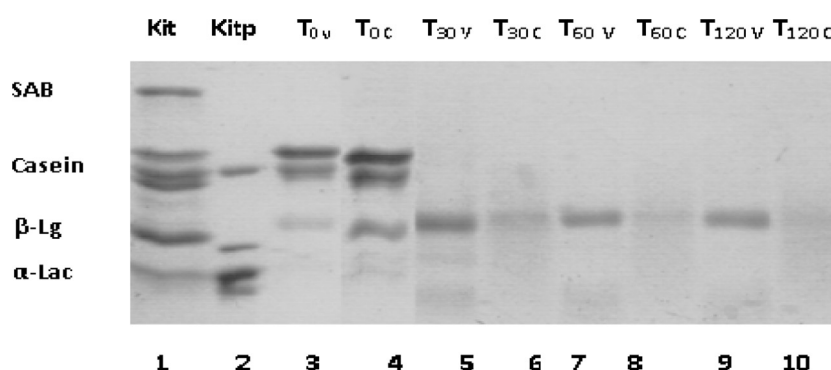


Fig. 2. Electrophoresis on polyacrylamide gel 15% SDS in denaturing conditions of degradation hydrolysates of coconut milk and cow's milk after pepsic digestion. Contains: well 1 T0 of cow's milk, T0 of coconut milk, wells (3,5,7,9) contain hydrolysed cow's milk and in wells (4,6,8,10) milk of coco hydrolysed for 30,60,120 minutes respectively.

2.6. Statistical analysis

Results were expressed as mean $\pm$ standard error (SEM) and $p < 0.05$ was considered statistically significant. The resulting data underwent statistical analysis using Student's test as programmed by Statistica (AXXF307CO20802FA) 200.

3. Results

3.1. Assessment of α -NH₂ functions

Assessment of the α -NH₂ functions released during combined pepsin and trypsin-chymotrypsin hydrolysis (Fig. 1) allowed us to comparatively evaluate the degree of digestion of cow's milk and coconut milk proteins. Our results showed greater gastrointestinal digestion of coconut milk proteins than of cow's milk proteins, with hydrolysis of coconut milk using pepsin combined with trypsin-chymotrypsin being significantly higher than pepsin hydrolysis alone.

Peptic hydrolysis of coconut milk is greater than that of cow's milk and increases in the presence of trypsin-chymotrypsin with less significant release of α -NH₂ functions until the end of T240 digestion: $13.44 \pm 1.007 \mu\text{mol/ml}$, $p < 0.001$, and $10.58 \pm 0.307 \mu\text{mol/ml}$, $p < 0.05$ respectively. These values are

significantly higher than those obtained during gastric digestion, whether for coconut milk ($p < 0.001$) or cow's milk ($p < 0.05$). The α -NH₂ functions released in the hydrolysates resulting from peptic digestion combined with tryptic-chymotryptic digestion were greater than those released from peptic digestion alone. We noted very marked degradation of the two types of whey (coconut milk and cow's milk) immediately after addition of the trypsin-chymotrypsin enzymatic mixture (E/S R 1/100) with a gastric digestion substrate and after 30 minutes. β -Lg is absorbed by the intestine, whether in the native state or slightly hydrolysed, which accounts in part for its strong allergenicity (Chelulei et al., 2010). In the hydrolysed form, the proteins may show improved functional properties depending on the treatment conditions used [11,12]. Allergenic residual protein activity was observed in the formulas resulting from partial and extensive hydrolysis of cow's whey proteins. The principal proteolytic enzymes used on a commercial scale are pepsin, trypsin and chymotrypsin of porcine origin. Pepsin is an endoprotease that is secreted by gastric mucous in the form of pepsinogen and then activated by gastric acidity, but also by autolysis [13]. The peptides resulting from trypsin hydrolysis of whey proteins are of great value in the food industry [14]. Kwon et al., 1996, demonstrated the amino acid composition of the albumin and globulin fraction of coconut protein extracted from defatted coconut flour

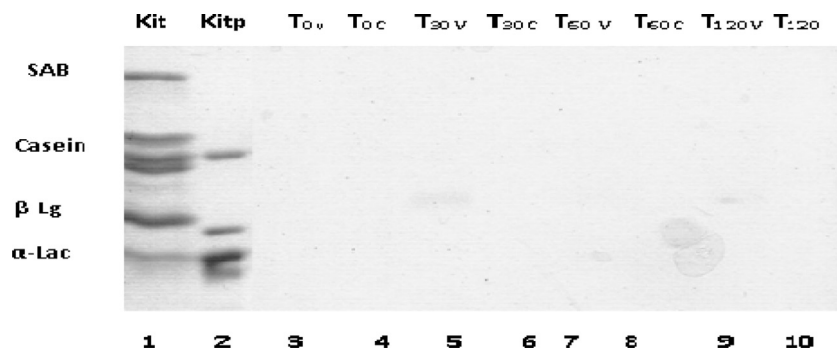


Fig. 3. Electrophoresis on polyacrylamide gel - 15% SDS in denaturing conditions of degradation hydrolysates of coconut milk and cow's milk after pancreatic digestion in vitro. Contains: well 1 T0 of cow's milk, T0 of coconut milk, wells (3,5,7,9) contain hydrolysed cow's milk and in wells (4,6,8,10) milk of coco hydrolysed for 30,60,120 minutes respectively.

[15] while Seow and Goh, 1994, showed that differential scanning calorimetric analyses for undiluted crude coconut milk with a total solid content of 50% revealed endothermic transitions over the 50–130 °C temperature range, reflecting the complex protein composition and the thermal denaturation behaviour of milk.

Comparative analyses of coconut milk and cow's milk after pepsin digestion comparatively with a protein kit and peptide kit, and comparative analyses of coconut milk and cow's milk after trypsin-chymotrypsin digestion comparatively with a protein kit and peptide kit.

The electrophoretic profile of cow's milk hydrolysates compared to the same peptic hydrolysis times as that of coconut milk shows disappearance of the bands corresponding to BSA, caseins and α-Lac and persistence of the β-Lg bands with a notable decrease in the intensity of the strip and the appearance of PM bands of less than 14 kDa corresponding to degradation products (Fig. 2). In this study, polyacrylamide gel electrophoresis was conducted to confirm the presence of the main proteins in cow's milk and to identify the various proteins that compose coconut milk. Protein analysis of cow's milk by electrophoresis allowed the identification of bands similar to those of the kit marker proteins (caseins, SAB, β-Lg, α-Lac). The electrophoretic profile for cow's milk allowed us to identify bands corresponding to β-Lg and caseins (18 kDa and 24 kDa) to hydrolyse the proteins in coconut milk and cow's milk by both gastric enzymes (pepsin) and pancreatic enzymes (trypsin and chymotrypsin) over 3 hours and enabled us to study the biochemical characteristics of these proteins (Fig. 3). The protein assay of coconut milk indicated a higher protein content for cow's milk than for coconut milk. The study by Garcia et al., 2005, showed that the electrophoretic profile of coconut milk proteins presents two main electrophoretic subunits corresponding to globulin 11S and cocosin (24 and 34 kDa), believed to play a significant role in stabilising coconut milk emulsion. In this study, Balb/c mice were used as a model of cow's milk allergy, as described by Chicon et al. [16]. Our results are consistent with those reported in the literature [17], and these studies showed a significant increase in short-circuit current after contact between the sensitizing antigen and intestinal fragments in an Ussing chamber, which suggests that coconut milk constitutes a good alternative to cow's milk allergy.

4. Conclusion

In the present study we examined the effects of pepsin, trypsin and chymotrypsin hydrolysis on coconut milk proteins compared to those of cow's milk using two pancreatic and gastric enzymes. We studied the biochemical characteristics of coconut milk proteins and cow's milk proteins and our results showed that the protein content of cow's milk is higher than that of coconut milk and that enzymatic hydrolysis of coconut milk proteins is more potent than that of cow's milk; the electrophoretic profile of hydrolysates of proteins from both cow's milk and coconut milk revealed the existence of casein and β-Lg in coconut milk, as well as more pronounced degradation for cow's milk hydrolysates.

The preliminary results indicate that coconut milk could provide a useful alternative to cow's milk in the event of allergy to proteins in cow's milk.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgment

This research was supported by the Directorate General for Scientific Research and Technological Development (DGRSDT, MESRS, Algeria). The authors would like to thank Mr. Thierno Aliou Diallo for agreeing to reread their article.

Dr Soualmi Damardji Youcef, Doctor in Cardiorespiratory Physiology, Faculty of Medicine of Oran.

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