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Determination of Peptides Caused Bitterness in Turkish White Cheese and Kasar Cheese

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Abstract: The bitterness is a property of flavor created in connection with the accumulation of bitter peptides in cheeses arisen through various effects during ripening. In this study, bitter peptides are isolated from Turkish White Cheese (TWC) and Kasar Cheese by using UF, gel filtration, RP-HPLC techniques. The most bitter fractions in cheese samples were in a molecular weight range between 500 and 4000 Da for TWC, 200 and 700 Da for Kasar cheese. Sensory evaluation on the HPLC eluants revealed that bitterness found in various position but the most bitterness intensity was detected at the hydrophobic region of the chromatograms for TWC and it has been found that the bitter fractions of Kasar cheese contains high level of phenylalanine and tryptophan.

Key words: Turkish white cheese, kasar cheese, bitterness, RP-HPLC, gel filtration

INTRODUCTION

White cheese and Kasar cheese are the most popular cheese varieties manufactured in Turkey. Turkish white cheese is a semi-soft, brined cheese with a slightly acid and salty flavour. It can be consumed while fresh but it is mostly eaten after ripening in a brine solution. It is matured for a period 1-3 months. This type of cheese was manufactured originally from sheeps, goats or cows' milk or combination of these milks (Hayaloglu *et al*., 2002; Topçu and Saldamli, 2006).

Kasar cheese (also called as Kashar cheese) is a Kashkaval like, scalded and kneaded cheese. It has semi-hard or hard texture, manufactured from cows' milk. It can be consumed while fresh or after ripening (2 or 3 months) (Koçak *et al*., 1996).

Proteolysis is the principal and most complex biochemical reaction that occurred during the ripening process of most cheese varieties (Fox, 1989). During proteolysis, proteins are degraded to primary products (polypeptides) and subsequently to secondary products such as small and medium-size peptides and eventually free amino acids (Fox and McSweeney, 1996). However, if the hydrophobic peptides and amino acids are excessive, undesirable tastes such as bitterness may occur which reduce the acceptability and marketability of cheese (Sousa *et al*., 2001).

Bitterness is frequently reported as a flavor defect in different cheese varieties, results from the accumulation of bitter testing peptides formed by action of proteolytic enzymes on casein. These peptides are rich in hydrophobic amino acids. The cheese manufacturing technique, the bacterial profile of cheese, the type and quantity of the starter and rennet used, all affect the development of bitter flavor (Lemieux and Simard, 1991, 1992). However, the bitterness in cheeses is to be accepted as a compulsory consequence of rapid and uncontrolled proteolysis and limits the acceptability and marketability of cheese types and cause economic looses (Habibi-Najafi and Lee, 1996).

To understand these proteolytic products better, bitter peptides needed to be extracted and isolated from White and Kasar cheese matrix. So the objectives of this study were to establish a quantitative method coupled with analytical techniques to isolate bitter peptides from Turkish White and Kasar cheese.

MATERIALS AND METHODS

Cheese samples: For bitter peptide extraction studies, cheese samples were obtained from commercial sources (25 samples for Turkish White Cheese, 17 samples for Kasar Cheese). The cheeses were evaluated organoleptically for the detection of bitterness and then selected bitter cheese samples were treated for extraction process.

Preparation of molecular weight fractions: The method of Kuchroo and Fox (1982) was used to extract the water-soluble nitrogen fraction from Turkish white

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cheese and kasar cheese samples but the ratio of cheese to deionized water was 1:5 (wt/wt) instead of 1:2 (wt/wt).

Water Soluble Extract (WSE) was ultrafiltrated using an Amicon Model 8200 ultrafiltration unit fitted with polysulfone membranes with a nominal molecular weight cutoff of 10 kDa. A pressure of 5 bar was applied with nitrogen during filtration (Sing *et al.*, 1994). The permeate of ultrafiltrated water-soluble extract was then stored at -20°C until use. UF permeate was fractionated by gel filtration chromatography on a column (Bio-Rad 50×2.5 cm) of Bio gel P6 (Bio-Rad, separation range 1000-6000 Da) using deionized water as eluant at a flow rate 0.7 mL min^{-1}.

A standard curve of elution volume vs. log Molecular Weight (MW) was constructed using molecules of known MW ranging from 3496 to 204 Da (Tryptophan (MW:204.2), Angiotensin I (MW:1296), Vitamin B12 (MW:1355), Insulin chain B (MW: 3496)). Sample was applied by using Rheodyme Model 7125 injector with a 2 mL injection loop. Fractions (3.5 mL) were collected and the peptide content was monitored by measuring absorbance at 280 nm. Sensory evaluation of fractions was carried out by using quinine sulphate (10^{-7} M) as a reference. Bitter fractions were lyophilized and kept at -20°C until HPLC analysis.

Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) analysis: The HPLC system (consisting of a SpectraSYSTEM Model SCM1000 vacuum degasser, P4000 quaternary pump) was equipped with a Phenomenex Jupiter RP-C18 wide-pore analytical column (5 μm, 300 A°, 250×4.6 mm) and guard cartridges (Phenomenex, widepore C18, 4×3 mm).

Lyophilized gel permeation fractions dissolved in 1 ml of solvent A and filtrated through a 0.45 μm cellulose acetate filter then injected into the HPLC using a Rheodyme Model 7125 injector with a 100 μL injection loop. Solvent A contained 0.1% (v/v) HPLC grade Trifluoroacetic Acid (TFA) solution in deionized water and solvent B contained 0.1 (v/v) TFA in ultra gradient grade acetonitrile. Solvent A and solvent B were sonicated for 30 min to remove dissolved gases. The samples were eluted initially with 100% A for 5 min, then with a gradient of 0 to 50% B over 50 min and finally with 50% B for 5 min the flow rate was 1 mL min^{-1}. The eluate was monitored at 214 nm using Varian model 9050 UV/VIS wavelength adjustable detector and interfaced with Varian Model 4400 integrator. Eluants of selected separated peaks were collected for 5 repeated HPLC injections and evaporated to dryness, redissolved in deionized water and tasted by the sensory panel for bitterness.

RESULTS AND DISCUSSION

The water-soluble fraction of cheese contributes significantly to the intensity of cheese flavour (Mcgugan *et al.*, 1979; McSweeney *et al.*, 1994) bitterness being most intense in that fraction. Bitter taste of peptides is in relation to their hydrophobicity and molecular weight. Bitter peptides have MW between 100-6000 Da and more hydrophobic peptides have a more intense bitter flavor (Lemieux and Simard, 1992). A plot of absorbance (280 nm) vs elution volume during gel filtration of UF permeate of WSE of Turkish White Cheese (TWC) and Kasar cheese are shown in Fig. 1.

Sensory analysis of the fraction showed that fractions II, III and V of TWC have bitter taste. Their calculated MW were found as 4000, 1000 and 500 Da, respectively. Bitterness intensity of fraction II was low so this fraction has not been identified by RP-HPLC. Before bitterness can be detected, bitter peptide concentration in cheese must exceed a certain level, this increase with the age of the cheese (Lemieux and Simard, 1992; Gomez *et al.*, 1997). For Kasar cheese, fractions IV and VI have bitter taste and their estimated MW were 700 and 200 Da, respectively. Selected fractions were injected to RP-HPLC column and chromatograms are shown in Fig. 2.

Champion and Stanley (1982) concluded that the bitter peptides in Cheddar cheese has a molecular weight approximately 190 Da. In another study, it has been established that the most bitter peptides between 500 and 3000 or >3000 Da (Lee and Warthesen, 1996). Our results show similarities with these findings.

Peaks 1, 2 and 3 were collected from the fractions and tasted by sensory (Fig. 2). Bitter taste was evaluated at these peaks. These peaks were phenylalanine, tryptophan and methionine. These amino acids were identified by comparison of their retention time with that of standard solutions that were injected separately under the same conditions.

In a study (Lau *et al.*, 1991) the retention times of three bitter and hydrophobic amino acids (methionine, phenylalanine and tryptophan) were used to define hydrophilic and hydrophobic zone of RP-HPLC chromatograms, at a detection wavelength of 214 nm.

Fraction III of TWC was very heterogeneous and was not very well resolved by RP-HPLC. This fraction contains high level of phenylalanine, tryptophan (peak 1, 2) and hydrophobic peptides in hydrophobic zone (after 26 min). Gomez *et al.* (1997) concluded that hydrophobic peptide level was an indicator of bitter defect in semi-hard cheeses, as evaluated by a tasting panel. Fraction V showed that this fraction contained no

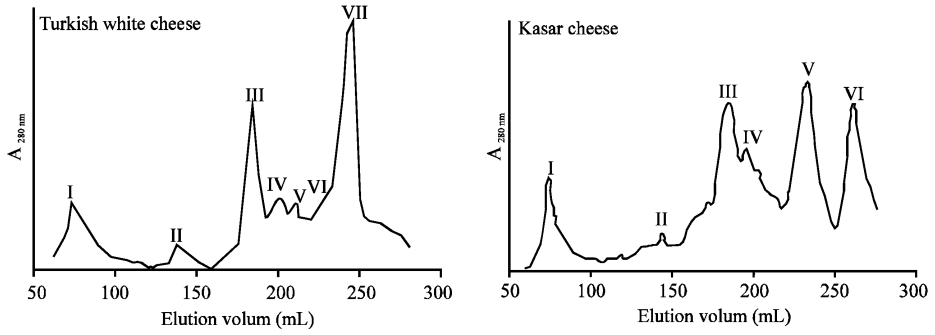

Fig. 1: Gel filtration chromatogram of ultrafiltrate permeate (10 kDa) of water soluble extracts on Bio-Gel P6. Fraction size 3.5 mL, flow 0.7 mL min^{-1}; solvent, deionized water

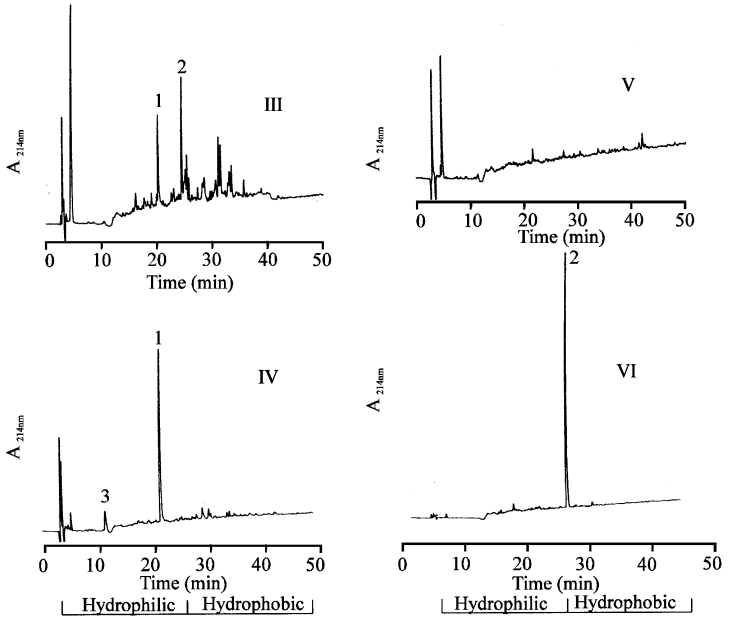

Fig. 2: RP-HPLC chromatograms of fractions III, V of Turkish white cheese and fractions IV, VI of Kasar cheese from Bio-gel P6

main peak in hydrophobic zone but it has two major peaks with retention times at 3 and 5 min in hydrophilic zone. Early eluted peaks show free amino acids and hydrophilic peptides of fractions III, IV, V.

Fractions IV and VI from Kasar cheese contain high level phenylalanine, tryptophan and methionine (peaks 1, 2, 3, respectively) and these amino acids have bitter taste. Cheese flavor was associated with low molecular weight fractions containing free amino acids and peptides (Lemieux and Simard, 1992). Free amino acids and peptides released by proteolytic enzymes such as chymosin and starter lactic acid bacterial proteases and non-starter proteases on casein, during the ripening period (Fox, 1989; Grappin *et al.*, 1985). This affects cheese quality and acceptability.

CONCLUSION

From our results we can conclude that hydrophobic peptides and free amino acids especially phenylalanine and tryptophan are responsible bitterness in TWC. However, in Kasar cheese, the bitter fractions contain high level of phenylalanine and tryptophan. These results greatly extend our knowledge on proteolysis in cheese and mechanisms involved in the development of cheese flavor.

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