STUDIES ON ACCELERATION OF RAS CHEESE RIPENING BY AMINOPEPTIDASE ENZYME FROM BUFFALOES' PANCREAS

By

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B.Sc. Agric. Sci. (Food Science), Fac. Agric.,Ain Shams Univ .,2000

THESIS

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APPROVAL SHEET

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Title of Thesis: Studies on Acceleration of Ras Cheese Ripening by Aminopeptidase Enzyme from Buffaloes' Pancreas.
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ABSTRACT

Aminopeptidase (EC. 3.4.11) enzyme of buffaloes' pancreas was isolated, purified and its characteristics were studied. Maximum enzyme specific activity and fold of purification were obtained at 30- 40% saturation of ammonium sulfate. Purification with gel filtration led to specific activity, yield, and fold of purification of 0.96 unit /mg protein, 20%, and 9.6 respectively for buffaloes' pancreas enzyme. The optimum pH for enzyme was 6.0 while the optimum temperature was 40ºC. The enzyme was found to be relatively heat stable. The enzyme was strongly activated by 1 mM of Ca^{+2} and Na^{+}, while 1 mM of Cu^{+2} and Cd^{+2} were inhibitors. The enzyme activity was not significantly affected by 1 mM of EDTA and 1,10 Phenanthroline . The enzyme had a molecular weight (MW) of 20 kDa approximately .

Ras cheese was made from mixture of fresh cows' and buffaloes' milk (1:1), the aminopeptidase was added at levels of 0.03 (T1), 0.06 (T2), 0.09 (T3) and 0.15 (T4) unit / kg milk. Changes in cheese chemical composition, electrophoretic pattern and organoleptic properties were followed throughout the ripening period (120 days / 14 ± 1C). From the results obtained the 0.06 unit of aminopeptidase (T2) was recommended.

Key words: Slaughterhouse wastes, Buffaloes' pancreas, Aminopeptidase, Ras cheese, Cheese ripening.

DEDICATION

I dedicate this work to my MOTHER and my FATHER souls for all the support through their life, as well as to my dear wife EMAN and my dear daughter GANAA for their patience and help to complete my work. Also, I would like to thank my brothers and sisters for all the support and help in all my life.

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INTRODUCTION

Cheese maturation may take 6 months to 2 years depending on the cheese variety. Shorting the maturation period has several an advantage in reducing the cost of cheese production. Several attemps have been made to reduce the ripening period by addition of proteases. The use of proteases to accelerate cheese ripening is promising because of their specific action and potential for low production cost. Proteases are seprated into 2 main groups: endoproteinases and exopeptidases. Endoproteinases cleave the polypeptide chain at specific susceptible peptide bonds within the chain, while exopeptidases hydrolyze 1 or a few amino acid(s) at a time from either the N terminal (aminopeptidases) or C terminal (carboxypeptidases) of the polypeptide. Aminopeptidases are an exopeptidase that catalyzes the hydrolysis of amino acid residues from the N-terminus of peptide or protein substrates, these are believed to act in concert to completely degrade the products of proteolysis into amino acids. Leucine aminopeptidase (EC 3.4.11.1) was preferentially releases leucine amino acid (Kilcawley *et al*., 2002 and Jankiewicz and Bielawski , 2003). Leucine aminopeptidase was purified and characterized from microbial sources such as lactobacilli (Fernandez de Palencia *et al*., 1997; Sanz and Toldra, 1997; Williams*et al* ., 1998 and Magboul and McSweeney , 1999 a, b), *Brevibacterium linens* (Rattray and Fox, 1998 and Fernandez *et al*, 2000), *Pseudomonas fluorescens* ATCC 948 (Gobbetti *et al*., 1995 a), marine psychrophile *Colwellia psychrerythraea* Strain 34H (Huston *et al*., 2004) and *Schizosaccharomyces pombe* (Herrera-Camacho *et al*.,

2007). A wide variety of aminopeptidases have been reported in animal tissues but leucine aminopeptidase reported previously from swine kidney (Spackman *et al*., 1954), calf lenses (Spector, 1963), shrimp (*Penaeus indicus*) muscle (Doke and Ninjoor, 1987), decapods hepatopancreas extracts from two species, crayfish (*Pacijistacus astucus*) and langostilla crabs, *Pleuroncodes pfunipes* (Garcia-Carreno and Haard, 1994), bovine skeletal muscle (Nishimura *et al*., 1994), squid (*Illex illecebrosus*) hepatopancreas (Raksakulthai and Haard, 1999), tilapia intestine, *Oreochromis niloticus* (Taniguchi and Takano, 2002) and scallops, *Patinopecten yessoensis* (Umetsu *et al*., 2003). Aminopeptidase had been purified recently from carp skeletal muscle, *Cyprinus carpio* (Liu *et al*., 2008). Aminopeptidase has been used to accelerate the ripening process of cheddar cheese (Kosikowski and Iwasaki,1974; Hayashi *et al*,1990 and Raksakulthai *et al*,2002).

Isolation, purification and characterization of buffaloes' pancreas aminopeptidase was the first objective of this study. Pancreas is a source of many digestive enzymes such as peptidases, lipases, amylases, and nucleases. Pancreas is a complex organ of both exocrine tissue and endocrine tissue that performs several functions. The exocrine part consists of acini, which produce digestive enzymes (pancreatic juice) and duct system which carries pancreatic juice to the small intestine. The endocrine part consists of pancreatic islets (islets of langerhans), which form less than 2% of the pancreatic tissues (Seeley *et al*., 1998). The anatomy and histology of the pancreas are shown in Figure (1). The second objective of this study was application of

buffaloes' pancreas aminopeptidase to accelerate ras cheese ripening in order to reduce the cost of ras cheese production.

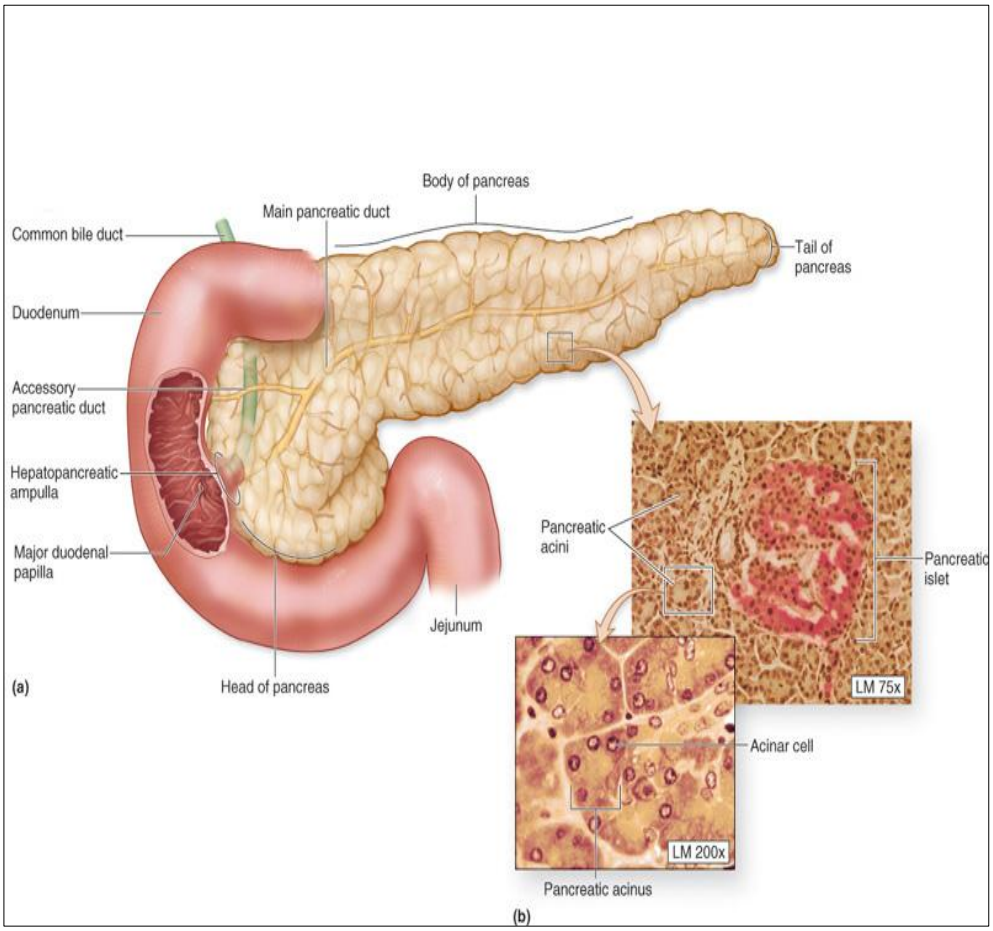

Fig.1.Anatomy and histology of the doudenum and pancreas .

a, The head of the pancreas lies within the duodenal curvature, with the pancreatic duct emptying into the duodenum. b, Histology of the pancreas showing both the acini and the pancreatic duct system. (http://www.6abib.com/anatomy/ant-27.html)