IMPROVING THE QUALITY AND FLAVOUR OF RAS CHEESE USING DIFFERENT STARTER CULTURES

By

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ABSTRACT

Fatma Hosny Mohammed Ali: Improving the Quality and Flavour of Ras cheese Using Different Starter Culture, **Unpublished M. Sc. Thesis, Department of Food Science, Faculty of Agriculture, Ain Shams University, 2013.** The ability of some lactic acid bacterial strains to grow in sterilized reconstituted skim milk as single strains and in combination with other different lactic acid bacteria was screened to choice their best suitable for Ras cheese milk culturing. Skim milk powder were reconstituted (12% TS) and autoclaved (1 bar/10 min). Reconstituted skim milk was inoculated with (1% v/v) of activated LAB strains and incubated at optimum temperature for 6 h. LAB counts and pH values were measured after different incubation periods (0, 2, 4 and 6 h) at 37˚C. All data were statistically analyzed.

Lactobacillus casei, Lactobacillus helveticus, followed by *Lactobacillus acidophilus* and *Bifidobacterium bifidum* had the highest ability to grow as single strains in skim milk.

Activated mixed starter culture of Ras cheese (*Streptococcus thermophilus* and *Lactobacillus delbruckii* ssp. *bulgaricus*) and single strain of LAB were incubated at 37˚C for 6 h. Viable counts and pH value development indicated that, *Str. thermophilus* and *Lb. delbruckii* ssp. *bulgaricus* mixed with *Lb. casei* followed by *Str. thermophilus* and *Lb. delbruckii* ssp. *bulgaricus* with *Lb. helveticus* produced the highest growth while pH values were decreased.

Mixture of Ras cheese starter culture and some other lactic acid bacterial strains were used to study the microbial growth and pH value developed in skim milk. LAB counts showed the highest and the growth rate was the fastest in skim milk with the starter culture was containing *Streptococcus thermophilus, Lactobacillus delbrueckii* ssp *.bulgaricus* mixed with *Lactobacillus casei* and *Lactobacillus helveticus,* followed by the starter culture of *Streptococcus thermophilus, Lactobacillus delbrueckii* ssp *.bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium bifidum.*

Ras cheese treatments were produced from cow's milk (3.5 % fat and 8.56 % SNF)**.** Cow's milk was standardized to casein/fat ratio of 0.7, heat treated (69˚C/1 min) and cooled to 35˚C. Activated starter cultures

(control: *Streptococcus thermophilus* and *Lactobacillus delbruckii* ssp. *bulgaricus* as control starter culture, CCB: *Streptococcus thermophilis, Lactobacillus delbruckii* ssp. *bulgaricus, Lactobacillus helveticus* and *Lactobacillus casei*, LL: *Lactococcus lactis* ssp. *lactis and Lactococcus lactis* ssp. *cremoris*, or CAB: *Streptococcus thermophilis, Lactobacillus delbruckii* ssp. *bulgaricus, Bifidobacterium bifidum* and *Lactobacillus acidophilus*) was added at the rate of 1.0% at 35˚C and then allowed to ripen for 20 min before renneting (% acidity developed from 0.18 to 0.2%). Double-strength rennet was added to the cheese milk at the rate of 3 g/100 kg. Green Ras cheese was salted with 1.0% Sodium Chloride for 24h. Cheese was ripened at 15 ± 2˚C and 85% relative humidity for 60 days. Fresh and 60^{th} days ripened cheese samples were chemically and microbiologically analyzed. Volatile compounds profile, Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE), Transmission Electron Microscopy (TEM) and sensory properties were evaluated in ripened Ras cheese. Soluble protein and non protein nitrogen were significantly high. Also protein degradation on SDS PAGE in Ras cheese produced with the use of CCH followed by CAB starter culture. Volatile compounds profile were starter culture dependant in control and all Ras cheese treatments. A lactic acid bacterial count was significantly higher in Ras cheese with CCH followed by CAB starter cultures as compared with other treatments. On the contrary, the yeast and mould, sporeformers, psychrophilic bacterial reported the lowest counts in Ras cheese with CCH and CAB starter cultures. The data suggested that, the development of Ras cheese flavour could be achieved when the bacterial starter cultures contained the strain of (*Streptococcus thermophilus, Lactobacillus delbruckii* ssp. *bulgaricus, Lactobacillus helveticus* and *Lactobacillus casei*, or *Streptococcus thermophilus, Lactobacillus delbruckii* ssp. *bulgaricus, Bifidobacterium bifidum,* and *lactobacillus acidophilus.*

Key Words: Lactic acid bacteria, starter culture, Ras cheese, ripening period, volatile compound profile, GC-MS, TEM, SDS-PAGE.

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LIST OF ABBREVIATIONS

AOAC	American Official Analysis Chemists.
APHA	American Public Health Association.
ATCC	American Type Culture Collection.
B&T	Body and Texture.
c.f.u	Colony forming unit.
DM	Dry matter content.
DSMZ	Deutsche Sammlung Von Mikroorganismen und Zell Kulturen.
EMCC	Egyptian Microbial Culture Collection, Fac. Of Agric., Ain Shams Univ.
Et al.	And others (*et alii*).
FAO	Food and Agriculture Organization.
FDM	Fat in dry matter.
FVFA	Free Volatile Fatty Acids.
NPN	Non protein nitrogen.
SAS	Statistical Analysis System.
SDS	Sodium Dodecyl Sulfate.
PAGE	Polyacrylamide Gel electrophoresis.
SN	Soluble nitrogen.
SWP	Salt in water phase.
TEM	Transmission Electro Microscopy.
TN	Total nitrogen.
TVFA	Total Volatile Fatty Acids.
WHO	World Health Organization.

I. INTRODUCTION

Ras cheese is one of the most important traditional hard cheese in Egypt. The great popularity of Ras cheese by consumers is due to its unique, gratifying, flavor and texture. Usually, Ras cheese is suffering from lack of uniformity. The acceptability of cheese depends on its appearance and sensory properties (flavour, texture and colour). Among these, flavour is the most important attribute for the consumer.

Ras cheese is manufactured in a high proportion under artisan production, in rural areas and small factories. It was produced from raw cow's milk or a mixture of cow and buffalo milks without using starter cultures **(Hofi *et al.*, 1970)**. In such production situation, the fermentation occurs by the wild microflora present in raw milk and surrounding environment. The cheese wheels are usually stored under moist and uncontrolled hygienic conditions, which contaminated by moulds and yeasts. Therefore, the final flavour and texture influenced by the action of the flora.

Recently, the Egyptian organization for standardization and quality control published new standards providing that all cheese varieties should be made from pasteurized milk. These standards aim to produce high quality products for consumer health. This means that cheese makers should use pasteurized milk and starter cultures in the manufacture of such cheese to maintain the typical flavour of produced cheese. Although, the several studies on this type of cheese, the typical flavour and the flavour volatile compounds of cheese have not yet defined.

Ras cheese is the only Egyptian hard cheese that is manufactured in Egypt. It is very similar to the Greek "Kefalotyri" but is made under artisan condition from raw cow's milk or from a mixture of cow's and buffalo's milk. It can consume after more than three months of ripening **(Hofi*et al.*, 1970; Scott, 1981; Phelan *et al.*, 1993 and Abou-Donia, 2002)**.

Ras cheese has been produced from raw cow’s milk or a mixture of raw cow and buffalo’s milks for a long time without using starter cultures **(Sabbour, 1966; Abou-Donia, 2002 and Awad *et al.*, 2003)**. The fermentation always occurs by native microflora that was obtained from raw milk and the environment. Moreover, Ras cheese is stored in moisture and

uncontrolled hygienic conditions. For this reason there are different groups of microorganisms present in the cheese, some of them contribute to the flavor and the texture development and others may be pathogenic or cause defects in cheese **(Darwish *et al.*, 1994; Abou-Donia., 2002; Awad *et al.*, 2003 and Abdella *et al.*, 2006)**.

Cheese flavour believed to result from a balance between a number of components released by enzymic reactions rather than by chemical interactions **(Delahunty and Piggott, 1995)**. The characteristics of the flavour profile of ripened cheeses affected by proteolysis of caseins and in some types also by lipolysis and carbohydrate analysis **(Adda. 1986; Crow *et al.*, 1993)**. The typical cheese flavour results from further degradation of amino acids, due to the pathways for conversion of amino acids by starter bacteria **(Broome and Limsowtin, 1998)**. That is true in cheeses, produced from pasteurized milk and using starter cultures under aseptic conditions. However, cheese ripening influenced by different factors, including the microflora of the raw milk, coagulant, starter cultures and by adventitious contamination of the cheese by non-starter bacteria **(Fox, *et al.*, 1996)**. Based on sensory evaluation and chemical analysis of cheeses, various, groups of volatile compounds had identified as being responsible for the final taste and aroma of cheese. These compounds comprise fatty acids, esters, aldehydes, alcohols, ketones, sulphur compounds and various other components **(Bosset and Gauch, 1993; Engels, *et al*, 1997; Urbach, 1995; Ayad *et al.*, 2004; and Mehanna and Pasztor-Huszar., 2012**).

The major biochemical pathways which occur in cheese ripening are the following: the metabolism of residual lactose, lactate and citrate (sometimes, although erroneously, referred to as 'glycolysis'), liberation of free fatty acids, FFA (lipolysis), associated catabolic reactions and the degradation of the casein matrix to a range of peptides and free amino acids, FFA (proteolysis), and subsequent reactions involved in the catabolism of FAA.

The structural components of the proteolytic system of LAB can be divided into three groups on the basis of their function: (İ) proteinases, which split caseins to peptides, (İİ) peptidases hydrolyzing peptides and (İİİ) transport systems that trans-locate the breakdown products across the cytoplasmic