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The Effect of Lipase Enzyme Addition on the Lipolysis of Iranian White Brine Cheese

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ABSTRACT

In this paper, we describe the effect of the addition of lipase on Free Fatty Acids(FFA) of Iranian white brine cheese. FFA was determined at different ripening times in cheeses manufactured with and without commercial lipase. The addition of lipase increased the amount of total FFA, particularly of short – chain FFA. Unexpectedly, lipase utilization significantly ($P<0.05$) affected total FFA concentration. The amounts of Myristic, Oleic and Linoleic acids increased until the day of 45of ripening period and then decreased until the end of ripening period. The slower rate of FFA production in the control cheese (sample without added lipase) was attributed to the inhibitory effect of NaCl on lipolysis.

Keywords: Iranian White Brine Cheese, Lipolysis, Lipase, Free Fatty Acids.

INTRODUCTION

White brined cheeses are almost semi-hard cheeses, which are particularly suitable for hot climates. They are stored in concentrated brine. Many different types of pickled cheeses are produced in Eastern Europe, Balkans and the Middle East [8]. In Iran, pickled cheeses are a major part of the diet of people. The white brined cheeses produced from raw milk are preserved 6-8 months in order to develop a desired flavour and texture and those manufactured from pasteurized milk have a ripening period of about two months [8] World cheese production is almost 14 million metric tons per years, approximately 75% of which is ripened for periods ranging from 3 weeks to more than 2 years. Cheese storage represents a significant proportion of the total cost which is 0.25 to 1.0 US $/mo/kg. The major cost incurred involves the cost of providing refrigerated storage as well as the cost of the investment covering the value of material held in the inventory. Accelerated ripening of cheese has the potential for saving the industry hundreds of millions of dollars annually, and has therefore been of great interest. Methods which are currently being evaluated include starter culture modification, elevated or programmed ripening temperatures, addition of exogenous enzymes, and combinations thereof Lipase, which is often used for production of some cheese varieties (especially Italian cheeses) liberates short and medium chain fatty acids from triacylglycerols[9]. A number of volatile compounds contribute to the aroma which vary according to the kind of milk used (ewe, cow or a mixture) and the method of manufacture. Acetic acid is the most abundant acid; however, upon addition of lipase during preparation of the milk, the cheese will have high levels of short-chain acids (C_4-C_8) are produced, which may subsequently be esterified by ethanol. White pickled cheese made from raw milk ripens more quickly than heat treated milk cheese. However, studies on free fatty acids contents of white pickled cheese made from lipase add milk is scant. The aim of this study was to explore the effect of a commercial lipase on the composition of Free Fatty acids of white pickled cheese made from raw ewe's milk.

Therefore we suppose that enzyme lipase addition can change the FFA composition of Iranian white brined cheese without creating off- flavor.

Cheese manufacture

Ewe's milk from the Zandy breed was supplied from a farm in Varamin. Experimental cheese samples were made in three replications at the Tehran Pegah dairy plant (Tehran, Iran). Lighvan cheese was produced using raw milk. The raw milk was warmed to 36 °C, and then added microbial lipase (%2) (Fluka, Swiss) and coagulated with microbial rennet for 60 minutes. After curdling, the curd was cut into cubes of approximately 1 cm^3 and left to rest for 15 minutes. The slab curd was placed on a mesh table and weighted for draining. After whey separation was completed, the curd was cut into large cubes (approximately 10×10×7 cm) and immersed in brine with 22% concentration for about seven hours at room temperature. The cheese blocks were placed into a tin-plate container with brine salted to about a 12% concentration. The container was sealed and stored for 90 days [1].

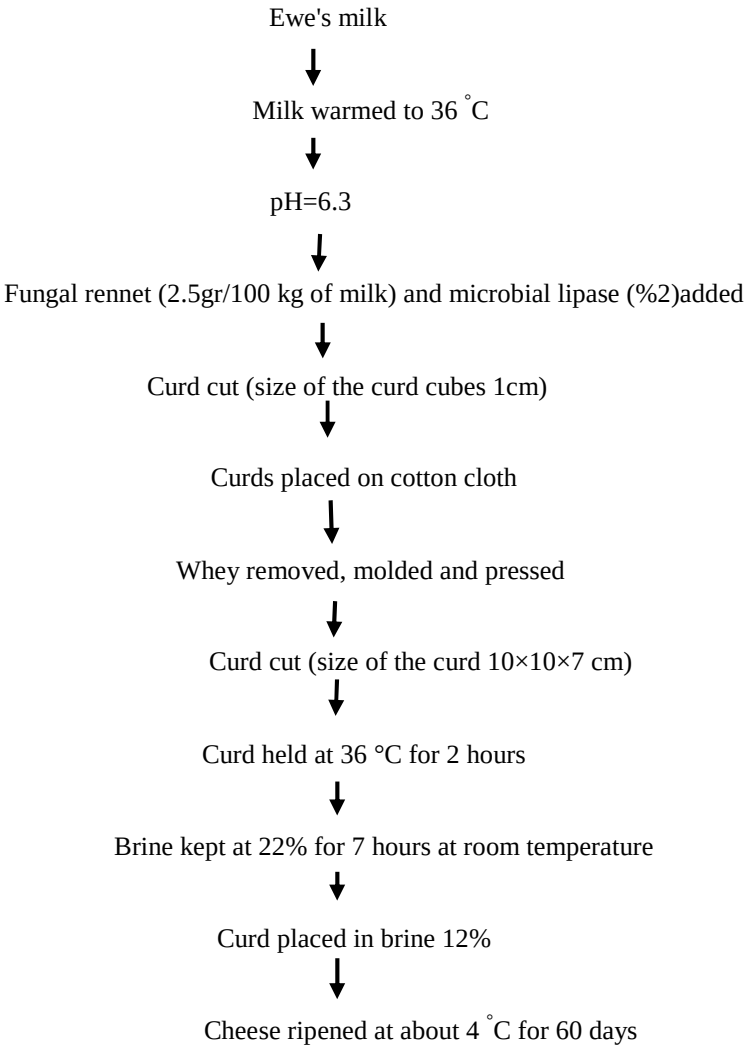

Figure 1.Protocol for the production of White brine cheese using microbial lipase

Lipolysis

The level of lipolysis was assessed in cheese samples 5, 25, 45 and 60 days old by measuring free fatty acids (FFA) content.

Sample preparation

Extraction of cheese lipids and isolation of the FFA were executed by GC as described by other researchers [4]. The sample was prepared as follows: cheese (2.5 g) was ground with anhydrous Na_2SO_4 (4 g), and then 0.4 ml H_2SO_4 (2.5 M) and 1.0 ml internal standard solution containing $C_{13:0,}$ $C_{17:0}$ (0.5 mg ml^{-1}each) were added. This mixture was extracted three times with 3 ml diethyl ether / heptanes (1:1, v/v). After each extraction, the solution was clarified by centrifugation (Beckman centrifuge, Model TJ-6, USA)2000 rpm for 5 min at room temperature and the upper solvent layer was transferred to a screw-capped tube containing anhydrous $NaSO_4$ (1.0 g). The pooled diethyl ether/heptanes extract was applied to a MEGA BOND ELUT NH_2 precolumn (2.8 ml, containing 500 mg of silica modified with aminopropyl group; Varian, Harbor city, CA, USA), which was conditioned with 10 ml heptanes. The neutral lipids were eluted from the column with 10 ml chloroform/2-propanol (2:1, v/v). The FFA was eluted with

10 ml diethyl ether containing 2% formic acid and then FFA was collected in a screw capped tube. A sample (0.1µL) from this solution was taken for GC determination of the FFA. Two chromatographic injections were made from each cheese extract. A gas chromatography model Star 3400 (Varian, Harbor city, CA, USA), equipped with an on-column injector and a flame ionization detector (FID) was used with a capillary column Bp-21(Length 30 m, inner diameter 0.53 mm). Direct cold on-column injection took place at 60 °C for 2 min; the injector temperature was raised 60-220 °C at a rate of 10°C min^{-1}, and then held at 220°C for 25 min. Injector and detector temperature were 200°C and 250°C respectively. Carrier gas was nitrogen and its purity was 99.9%. The pressure for headspace was 15 psig. The identification and quantification of cheese were based on known Concentration of different fatty acids standard (≥99%GC; Sigma, Steinheim, Germany).

Statistical Analysis

The data were statistically analysed using a completely randomized design (CRD) with three replications. Data were subjected to analysis of variance using the SAS statistical software package SAS Institute (1988). Mean comparison was performed with LSDs test at the $P<0.05$ level of significance.

Result and Discussion

Chromatographic separation of underivatized Free Fatty Acid (FFA) allowed the quantitation of all major fatty acids in one run (Figure 2.). The release of FFA in pickled cheese was studied at various stages of ripening (Table 1.).Examination of this table revealed that the addition of lipase had significant ($P<0.05$) effect of FFAcomposition. FFAs were found to be $C_{10:0}$, $C_{18:1,}$ $C_{18:2}$. Capric acid was the most abounded free fatty acid among all released FFAs. This quantity was attributed to tendency of lipase toward capric acid. Short chain free fatty acids (SCFFAs) increased during ripening. The relatively higher increase was viewed in the concentration of SCFFA ($C_{4:0}$ to $C_{8:0}$), which has a significant impact on the development of characteristic aroma of cheese, during ripening than medium chain free fatty acids (MCFFA) ($C_{10:0}$ to $C_{14:0}$) and long chain free fatty acids (LCFFA) ($C_{16:0}$ to $C_{18:2}$) (Table 1.). This could mainly be due to specificity of milk lipoprotein lipase and microbial lipase added to milk of cheese making towards FFA located at the positions sn-1 and sn-3 of the triglyceride. [Shahab Lavasani *et al*., 2012]. Generally SCFFA are predominantly esterified at the outer esters bond of tri- or diacylglycerides [11].

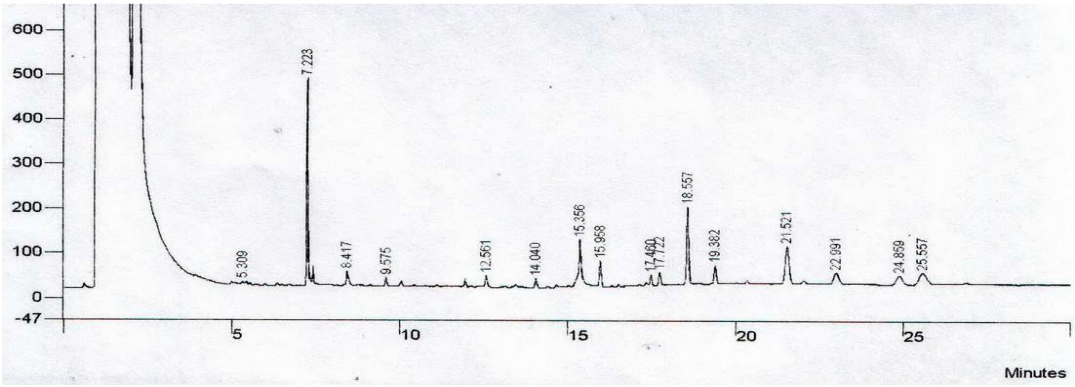

Figure 2. Gas chromatogram of FFA extracted from a white brine cheese spiked with internal FFA standards C_{13} and C_{17}.

Despite the quantitative importance of medium and long chain FFA, they are not the main contributors to cheese flavour [3]. Butyric acid was the main FFA in SCFFA control samples (without added lipase) and caprylic acid was the main FFA among SCFFA samples contains 2% added lipase. Among medium chain FFAs, Caprilic and Lauric acids increased during ripening of Iranian white brine cheeses however myristic acid increased until 45 days of ripening and then decreased until the end of ripening period.

Lipoprotein lipase (LPL) and microbial lipases were most likely responsible for the low lipolysis level found in cheeses without added lipase. As observed in table1. The increase in the lipolytic activity during ripening was primarily related to an increase in the content of short-chain FFA. Cheeses with commercial lipase added had significantly higher amount of total FFA than cheeses made without lipase at all sampling times. The amounts of myristis, oleic and linoleic acids increased until 45 day of ripening and then decreased until the end of ripening period because FFA and alcohols can be converted to short –chain fatty acids, esters, like ethyl acetate or ethyl butyrate [7].

The role of FFA in determining the quality and intensity of flavour in non-rancid Iranian White brine cheese is rather limited and additional factors such as total acidity and degree of protein degradation [5] as well as headspace

volatile compounds, mainly ethanol, propan-1-ol, butan-2-ol and butan-2-one [6] are also important. The slower rate of FFA production in the control cheese was attributed to the inhibitory effect of NaCl on lipolysis.

Table1: Changes of free fatty acids of white pickled cheese made from microbial lipase during ripening time[‡] (mg/100g)

		Free Fatty Acids(mg/100g)														
Ripening period (days)	Lipase level[†]	$C_{2:0}$	$C_{4:0}$	$C_{6:0}$	$C_{8:0}$	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	SCFFA*	MCFFA*	LCFFA*	Total
5	L_0	3.93^{aA}	9.45^{aA}	12.45^{aA}	10.03^{aA}	34.85^{aA}	13.35^{aA}	36.15^{aA}	9.10^{aA}	36.63^{aA}	34.90^{aA}	52.20^{aA}	31.93^{aA}	84.35^{aA}	132.83^{aA}	293.15^{aA}
	L_1	0.98^{bB}	16.88^{bB}	24.88^{bB}	34.28^{bB}	68.65^{bB}	20.53^{bB}	0.93^{bB}	1.65^{bB}	10.70^{bB}	42.08^{bB}	61.50^{bB}	76.03^{bB}	90.10^{bB}	115.93^{bB}	317.39^{bB}
45	L_0	4.38^{cC}	13.47^{cC}	14.02^{cC}	11.45^{cC}	35.20^{cC}	19.90^{cC}	27.98^{cC}	4.35^{cC}	38.48^{cC}	69.08^{cC}	77.20^{cC}	38.95^{cC}	83.08^{cC}	189.10^{cC}	347.42^{cC}
	L_1	2.38^{dD}	27.45^{dD}	28.08^{dD}	43.55^{dD}	90.98^{dD}	25.18^{dD}	1.85^{dD}	5.50^{dD}	4.45^{dD}	51.50^{dD}	57.18^{dD}	99.08^{dD}	118.00^{dD}	118.63^{dD}	367.61^{dD}
90	L_0	15.92^{eE}	40.65^{eE}	30.28^{eE}	20.53^{eE}	50.98^{eE}	57.88^{eE}	1.00^{eE}	2.45^{eE}	42.55^{eE}	29.08^{eE}	69.25^{eE}	91.45^{eE}	109.85^{eE}	143.33^{eE}	385.58^{eE}
	L_1	3.02^{fF}	35.83^{fF}	39.08^{fF}	47.45^{fF}	95.43^{fF}	25.08^{fF}	2.35^{fF}	6.08^{fF}	4.80^{fF}	29.70^{fF}	57.63^{fF}	122.35^{fF}	122.85^{fF}	98.20^{fF}	376.28^{fF}

[†]L_0: Cheese without using lipase as a control sample; L_1: Cheese with using 2% lipase

[‡]Different letters (a-d) in the same columns were significantly different from each other for lipase levels (P <0.05). Different capital letters (A-D) in the same columns were significantly different from each other for ripening periods (P <0.05).

*SCFFA:short chain free fatty acids; MCFFA: medium chain free fatty acids; LCFFA: long chain free fatty acids

CONCLUSION

The present results indicate that the use of lipase in the manufacturing of white pickled cheese enhances the development of free fatty acids but has only a slight effect on the pattern of the free fatty acids formed in white pickled cheese. The high level of lipase addition to cheese milk could be recommended for accelerating the development of flavour in this cheese over a short ripening period and applying 2% of microbial lipase produced better flavour and accelerated ripening period of Iranian white brine cheese.

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