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Effects of Processing Factors on Biogenic Amines Production in Iranian White Brine Cheese

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Abstract: Simultaneous effects of processing factors such as ripening time (20-60 days), ripening temperature (5-10°C), level of rennet added (1-2 g/100 kg milk), brine concentration (12-16% w v^{-1}), type of brine (NaCl, 25% Kcl + 75% NaCl) and level of starter (1, 3%) on biogenic amines formation in Iranian white brine cheese were investigated. Four biogenic amines i.e., histamine, tyramine, cadaverine and putrescine were determined by HPLC. The main biogenic amine was cadaverine, whose contents were higher than other amines. Among these studied factors, ripening time, ripening temperature and brine concentration were the most important factors. Biogenic amines increased with increasing time and temperature of ripening, while brine concentration had negative effect on biogenic amines content.

Key words: Biogenic amines, white brine cheese, HPLC

INTRODUCTION

Biogenic amines are low molecular weight organic bases that possess biological activity. Biogenic amines are mainly generated by the enzymatic decarboxylation of amino acids by microorganisms. The amine-producing abilities of various bacteria differ widely. The production of biogenic amines in cheese has often been linked to nonstarter lactic acid bacteria like *Enterobacteriaceae* (Novella-Rodriguez *et al*., 2002; Valsamaki *et al*., 2000; Halasz *et al*., 1994).

The presence and accumulation of biogenic amines depend on many factors such as availability of free amino acids (level of proteolysis), pH, water activity, salt-in-moisture level, temperature, bacterial density and synergistic effect between microorganisms (Gardini *et al*., 2001; Stratton *et al*., 1991) and primarily, the presence of microorganisms that have amino acid decarboxylase activity such as lactobacilli, enterococci, micrococci and many strains of *Enterobacteriaceae* (Suzzi and Gardini, 2003; Galgano *et al*., 2001; Joosten and Northolt, 1989; Edwards and Sandine, 1981).

These compounds, such as tyramine, histamine, putrescine, cadaverine, tryptamin and 2-phenyl-ethylamine, have been found in several types of cheese (Santose, 1996). Cheese represent an ideal environment for production of biogenic amines but amine concentration differs widely and depends on several factors as cheese variety, storage temperature, ripening time and microflora (Joosten, 1988; Vale and Gloria, 1997).

The presence of biogenic amines can cause several problems for susceptible consumers, such as nausea, respiratory disorders, hot flushes, sweating, heart palpation, headache, bright red rash, oral burning, hypo or hypertension, whose intensity is depend on quantitative and qualitative differences (Stratton *et al*., 1991). After fish, cheese is the next most commonly implicated food item associated with histamine intoxication (Stratton *et al*., 1991). Besides histamine, tyramine has been implicated in adverse reactions involving headache and hypertensive crisis in patients taking MAOI (monoamine oxidase inhibitors) (Smith and Durack, 1978). Putrescine, cadaverine and agmatine have been identified as potentiators that enhance the toxicity of histamine to humans by depressing histamine oxidation (Taylor, 1986). Furthermore, putrescine and cadaverine can react with nitrites to form carcinogenic nitrosamines (Scanlan, 1983).

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White brined cheese is one of the major items in the diet in Iran and the consumption per capita per annum is about 5.4 kg. At the industrial level, the ripening time is about 45-90 days (Azarnia *et al*., 1997). White brined cheese, like other types of ripened cheese, requires maturation to develop the required sensory properties. In warm climates it is necessary to preserve cheeses in brine. The specific characteristics of brine cheese develop in the salted water and chemical, physical and sensorial properties of this type of cheese are controlled by processing and environmental conditions (Abd El-Salam, 1987; Abou-Donia, 1991).

The objective of the present study, was to determine the contents of biogenic amines in Iranian white brine cheese, as well as to evaluate effects of various processing factors on the formation of these compounds.

MATERIALS AND METHODS

Cheese making: The brine cheese was manufactured for this research according to the method used in Iranian cheese making plants. White brined cheese was prepared from cows' milk. The milk was standardized to a fat content of 2.6%, pasteurized at 72°C for 15 sec and cooled to 32-35°C. $CaCl_2$ was added at a level of 15 g/100 kg of milk followed by the addition of two different starter concentrations (1 and 3%) 30 min before renneting. Cultures of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were used as starter (Hansen's Laboratory, Denmark). Commercial powdered microbial rennet (Meito, Sangyo Co., Japan) with milk clotting activity of 1 g 100 kg^{-1} of milk was added at 2 level of experiment (1 and 2 g 100 kg^{-1} milk) to coagulate milk samples. Following coagulation, the curds were cut and then stirred. The curds were pressed by using weights for 2 h (15 kg weights 30 kg^{-1} final curd). The curds were then cut to a suitable shape and size and soaked in sterile brine (22%, w v^{-1}) for 16 h. The curd pieces were then placed in tins; brines with 2 different concentrations (12 and 16%, w v^{-1}) and two types (NaCl and 25% Kcl + 75% NaCl) were added to cover the curds completely and to fill tins. The filled tins were sealed immediately after brining. The sealed tins were stored at 2 different ripening temperatures (5 and 10°C) for 2 different ripening times (20 and 60 days).

Experimental design and statistical analysis: Six processing factors were studied simultaneously using a factorial split-plot design (Table 1). In split-plot designs there is restriction on randomization and these design types are more effective when there are some hard to change factors (Montgomery, 2001). In our study, 2 of 6 factors (starter and rennet concentrations), were selected

Table1: Factorial split-plot design used to evaluate the effects of process variables

Run	Starter concentration (w v^{-1} %)	Rennet concentration (mg kg^{-1})	Brine Concentration (w v^{-1} %)	Time (day)	Temperature (°C)	Brine type
1	1	10	12	20	10	NaCl+KCl
2	1	10	16	60	10	NaCl+KCl
3	1	10	16	20	5	NaCl
4	1	10	12	60	5	NaCl
5	3	20	16	60	5	NaCl+KCl
6	3	20	12	20	10	NaCl+KCl
7	3	20	16	20	10	NaCl
8	3	20	12	60	5	NaCl
9	3	10	16	60	5	NaCl
10	3	10	16	20	5	NaCl+KCl
11	3	10	12	20	10	NaCl
12	3	10	12	60	5	NaCl+KCl
13	3	20	16	20	5	NaCl
14	3	20	12	20	5	NaCl+KCl
15	3	20	12	60	10	NaCl
16	3	20	16	60	10	NaCl+KCl
17	1	10	16	60	5	NaCl+KCl
18	1	10	12	60	10	NaCl
19	1	10	12	20	5	NaCl+KCl
20	1	10	16	20	10	NaCl
21	3	10	12	60	10	NaCl+KCl
22	3	10	16	60	10	NaCl
23	3	10	16	20	10	NaCl+KCl
24	3	10	12	20	5	NaCl
25	1	20	12	60	5	NaCl+KCl
26	1	20	12	20	10	NaCl
27	1	20	16	60	5	NaCl
28	1	20	16	20	5	NaCl+KCl
29	1	20	12	20	5	NaCl
30	1	20	16	20	10	NaCl+KCl
31	1	20	16	60	10	NaCl
32	1	20	12	60	10	NaCl+KCl

as hard to change factors and we made only 8 vat of cheese and the curd of each vat was randomly divided to 4 parts and other 4 studied factors were applied to these parts. A completely randomized design would require 32 vats of cheese, which was completely unrealistic compared to 8 vats in split-plot design.

Statistical analysis of the data was performed by using the SAS system for windows V9 (SAS institute Inc., Cary, NC, USA).

Biogenic amines determination

Instruments: The chromatographic system consisted of a Wellchrom HPLC pump, K-1001 (KNAVER Germany), dynamic mixing chamber, degasser (KNAVER), Wellchrom solvent organizer K-1500 (KNAVER Germany), UV-detector K-2501 (KNAVER Germany), Autosampler Triathlon type 900 and a personal computer running the software Eurochrom 2000. The column was EC 150/4.6 NUCLEODUR C_{18} Gravity 5 μm (Silica for powerful LC separation).

Chromatographic conditions: The mobile phase consisted of acetonitrile and water and its flow-rate was 0.8 mL min^{-1}. The peaks were detected at 254 nm.

Sample preparation: Samples were prepared by acid extraction and derivatization by slight modifying the method.

A 10 g amount of sample was weighed directly in a centrifuge tube, added with 20 mL of 0.1 M HCl containing the internal standard (1,7-diaminoheptane;10 mg L^{-1}) and then homogenized for 2 min using a disintegrator Heidolph Diax 900 (Heidolph instruments GmbH, Kelheim, Germany). Suspension was centrifuged at 12000 g for 20 min at 4°C. The supernatant was collected and solid residue was extracted for the second time as above. The combined extracts were made up to 50 mL with 0.1 M HCl and filtered.

An extraction with organic solvent (butanol) was then performed. This was carried out in a test tube on 5 mL of acid extract, with 3 portions of 5 mL butanol (vortex agitation). The organic extracts were saturated with NaCl and pH was adjusted to 11.5 with NaOH.

For derivatization of the samples, 1 mL organic extract was mixed with 2 drops of 1 M HCl and dried under vacuum (LABOROTA 4003 Heidolph instruments). Then 1 mL of 0.1 M HCl, 500 μL saturated solution of $NaHCO_3$ and 1 mL dansyl chloride solution (5 mg mL^{-1}) were added. The reaction vessel was transferred to an incubator and kept at 40°C under agitation for 1 h, then the solution was dried under vacuum, acetonitrile (2 mL) was added. The solution was filtered (VARIAN, Bond Elut C_{18}) and injected onto the chromatographic column.

RESULTS

Tyramine: Data analysis showed that ripening time was only significant factor affecting tyramine content ($p<0.01$) and other 5 factors on studied range had no significant effect.

Figure 1 shows the effect of ripening time and starter content on tyramine content in Iranian white brine cheese. This contour plot shows that tyramine content increases by increasing ripening time.

Cadaverine: Figure 2 shows the effect of brine concentration and ripening temperature on cadaverine content. The effect of brine concentration on cadaverine content depend on ripening temperature. In low ripening temperatures, cadaverine content decreased with

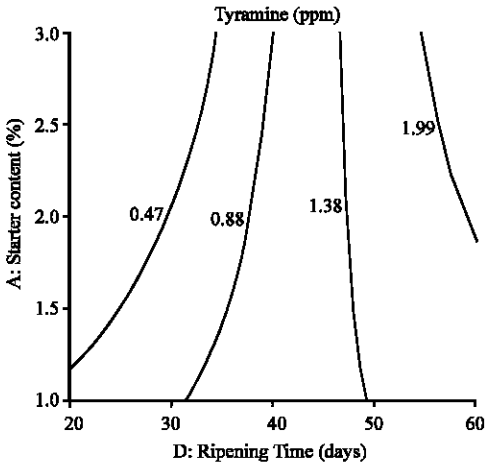

Fig. 1: Contour plot showing the effect of ripening time and starter content on tyramine content

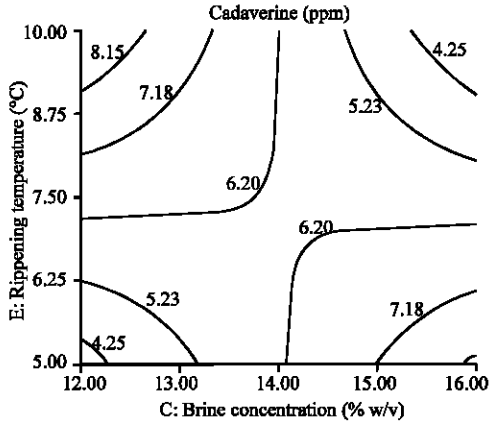

Fig. 2: Contour plot showing the effect of brine concentration and ripening temperature on cadaverine content

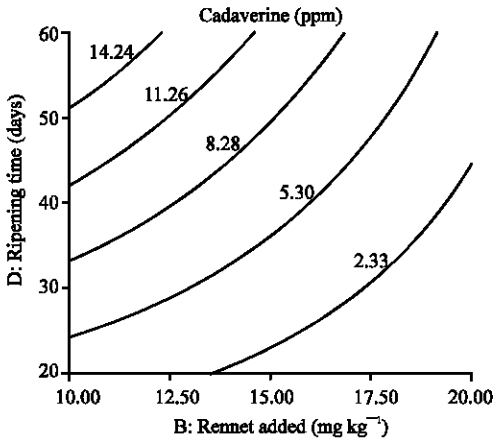

Fig. 3: Contour plot showing the effect of rennet added and ripening time on cadaverine content

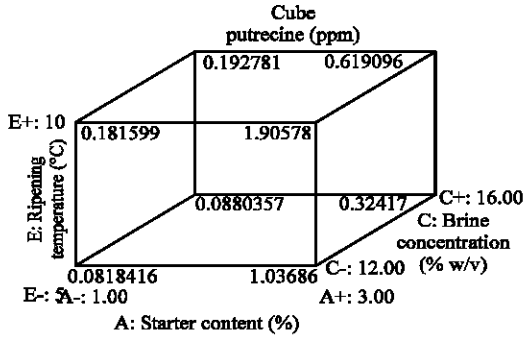

Fig. 4: Interaction effect of ripening temperature, starter content and brine concentration on putrescine content

increasing brine concentration while in high ripening temperatures, cadaverine content increased with increasing brine concentration.

Figure 3 shows the effect of rennet added and ripening time on cadaverine content. Cadaverine content increased progressively throughout ripening.

Putrescine: Figure 4 shows that maximum content of putrescine was in high level of ripening temperature (10°C), high level of starter (3%) and low level of brine concentration (12%).

Histamine: Figure 5 represents the effect of starter content and ripening temperature on histamine content. In low ripening temperature, histamine content increased with increasing starter content while in high ripening temperature, histamine content decreased with increasing starter content.

Figure 6 shows the effect of ripening time and brine concentration on histamine content. Histamine content decreased with increasing brine concentration.

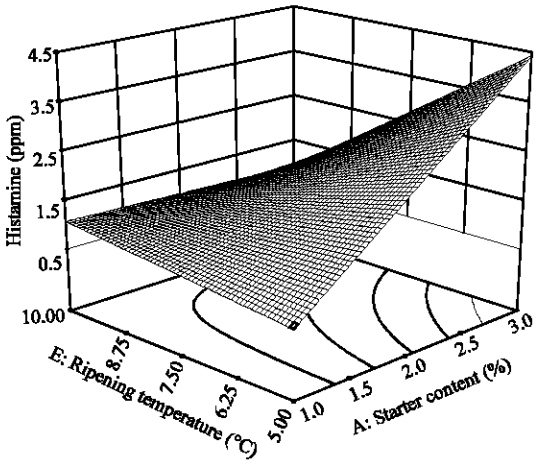

Fig. 5: Three-dimensional plot for histamine of starter content and ripening temperature as a function of response

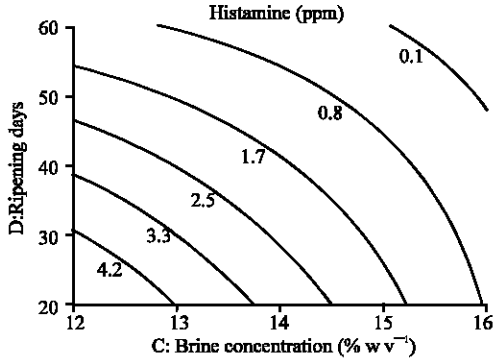

Fig. 6: Contour plot showing the effect of ripening time and brine concentration on histamine content

DISCUSSION

Formation of biogenic amines in food systems is a complex process and there are many factors affecting this process. Furthermore, the effect of one factor may be dependent to another factor. Factorial nature of this study allowed us to explore main and interactive effects of 6 factors on formation of different biogenic amines.

Tyramine: By increasing ripening time non-starter bacteria become dominant and the production of biogenic amines in cheese has been mainly attributed to the activity of non-starter microorganisms (Lanciotti *et al*., 2007; Martuscelli *et al*., 2005; Novella-Rodriguez *et al*., 2002; Valsamaki *et al*., 2000).

As shown on Fig. 1, contour levels change more rapidly on high level of starter relative to its low level. This means high rate of tyramine formation at high levels

of starter content. The starter enzymes were the major contributors to the production of small peptides and free amino acids in cheese during ripening (Lane and Fox, 1996). However, at low levels of ripening time, <35 days, tyramine content in high starter cheese samples was lower than low starter cheese samples. This can be attributed to inhibitory effects of dominant starter bacteria on non-starter types at initial period of ripening.

Cadaverine: Increasing brine concentration had negative effect on cadaverine contents. The biogenic amine production was very low at the higher NaCl concentrations (Gardini *et al*., 2001). *Enterobacteriaceae are* possibly responsible for cadaverine and putrescine build-up (Joosten and Northolt, 1987; Schneller *et al*., 1997). Some mixture of lactobacilli were also found to produce cadaverine (besides tyramine and histamine) and salt-tolerant lactobacilli caused massive formation of cadaverine and, to a less extend, putrescine (Joosten and Northolt, 1987). As discussed earlier, biogenic amines content increased with increasing ripening time (Martuscelli *et al*., 2005; Pinho *et al*., 2001; Valsamaki *et al*., 2000).

Putrescine: It is well known that temperature has a marked effect on the formation of biogenic amines in the fishing industry and in cheese. Several authors report that amine content depends on temperature and increases with time and storage temperature (Klausen and Lund, 1986; Halasz *et al*., 1994). This is not in contradiction with the results obtained in this research. One of the most important factors influencing the formation of biogenic amines in cheese is the presence of precursor free amino acids (Eitenmiller *et al*., 1978). Accumulation of free amino acids can be mainly attributed to the hydrolytic activity of several enzymes such as proteolytic enzymes from starter and non-starter microorganism. The presence of free amino acid can be influenced by several parameters. High temperature, high pH and low salt content have been reported to accelerate the amino acid accumulation and, hence, stimulate amine formation (Joosten, 1988).

Histamine: The presence of starter cultures in cheese delayed the biogenic amine production by wild microflora during the first 2 weeks of ripening. The starter culture used to make cheese did not produce biogenic amine (Martuscelli *et al*., 2005). Use of starter bacteria in cheese manufacture resulted in the accumulation of free amino acids (Hayaloglu *et al.,* 2005; Lane and Fox, 1997). According to Petridis *et al.* (1996), the content of histamine was correlated with the number of lactobacilli. Certain lactobacilli show high histidine decarboxylase activity (Joosten and Northolt, 1989). Roig-sagues and Eerola (1997) found that the use of starter culture did not reduce the formation of histamine and tyramine, even when the amine-producing microorganisms were present in very low concentrations. Sumner *et al.* (1990) found that histamine formation seems to be dependent of the degree of proteolysis. It is well known that high concentration of NaCl in cheese inhibits proteolysis (Guinee and Fox, 1993).

CONCLUSION

The production of biogenic amines in cheese is an extremely complex phenomenon, dependent of several variables, such as the presence of microorganisms, their proteolytic and decarboxylase activities, ripening time and ripening temperature. This research suggests that the most important processing factors influencing the biogenic amines formation are ripening time, ripening temperature and brine concentration. However, the total biogenic amines content was relatively low. It seems that the characteristic features of Iranian white brined cheese (high salt content, ripening and storage in brine, not extended proteolysis) did not create an environment favorable for biogenic amines accumulation. Undoubtedly, further studies are needed in order to evaluate the effect of ripening time, ripening temperature and brine concentration on biogenic amines formation.

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