Studies on the Cheese Flavors

V. Changes of Volatile Carbonyl Compounds during Ripening of Cheese Curd by *Aspergillus oryzae* chosen B

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(Received for Publication on March 22, 1965)

In the previous papers of this series⁹⁻¹¹), formation of volatile free fatty acids and volatile sulphur compounds during ripening of cheese curd by *Aspergillus oryzae* chosen B were described. Only a little quantities of these compounds were sufficient to cause characteristic flavors of this cheese. During the past few years, carbonyl compounds also have been detected in the matured other cheeses and volatile carbonyl compound(s) (VCC) have been associated directly with characteristic cheese flavor^{8,14}). The present paper is a report on the change of VCC during ripening of cheese curd by *Asp. oryzae* chosen B. Especially, effects of fat content and starter microbe on formation of VCC were studied.

Materials and Methods

1. Cheese Samples

Preparation of the ripened cheese curd was based on the procedure reported previously⁹⁻¹¹).

2. Analytical Methods

(a) The chemical composition

The contents of fat, moisture, ash, nitrogenous compounds, and acidity in cheese were analyzed by the general methods⁹).

(b) Isolation of volatile carbonyl compound(s) (VCC) from cheese

The method reported by MATSUOKA et al.⁸) was modified to measure the quantity of VCC. One hundred g of cheese sample was shredded into one liter flask containing 200 ml of distilled water and the mixture was steam distilled 200 ml of steam distillate was led directly into 50 ml of 2, 4-dinitrophenylhydrazine solution (1 g/500 ml 2 N HCl) to avoid any loss of the volatiles. The distillate and reagent were held at 45°C for 2 hrs and then stored at 0°C overnight. The produced 2, 4-dinitrophenylhydrazone(s) (DNPH) were continuously extracted with ethylacetate. The ethylacetate solution was concentrated and stored.

(c) Paper chromatographic separation of DNPH

Separation of the mixed hydrazones was carried out by the method of LYNN et al.⁷) and MATSUOKA et al.⁸) using 2-phenoxyethanol impregnated paper (Toyoroshi No. 51) as the stationary phase and heptan saturated with 2-phenoxyethanol as the mobile phase.

(d) Identification and quantitative estimation of VCC

Identification and quantitative estimation of each DNPH were carried out by the method of WALKER et al.¹⁴) and MATSUOKA et al.⁸) Identification was made by R but

values (distance of movement relative to the hydrazone of butanone-2). In order to record the absorption spectra of individual hydrazones, the separated spots on paper chromatograms were carefully cut out and eluted with 5 ml of ethanol. 0.5 ml of alcoholic KOH (10% W/V) was added to this solution and their absorption spectra are plotted over the range 300~600 m μ using a spectrophotometer.

In order to estimate the individual DNPH, 1~10 μ l of concentrated ethylacetate solution of mixed hydrazones was spotted to a chromatogram paper. Individual hydrazones on the paper chromatogram were eluted with 5 ml of ethanol. This absorbance at 360 m μ was measured using spectrophotometer. The amount of DNPH was measured from the prepared calibration curve.

Experimental Results

1. Paper Chromatograms, Absorption Patterns, and Properties of DNPH of VCC Isolated from Cheese Curd Ripened by *Aspergillus oryzae* chosen B.

Paper chromatogram of DNPH of VCC isolated from ripened cheese and authentic DNPH are shown in Fig. 1. Six spots and three spots were detected on paper chromatogram of whole milk curd and skim milk curd after ripening for 192 hrs, respectively. Then, these spots were eluted with ethanol and absorption patterns were obtained as described above (Fig.

Fig. 1. Paper chromatogram of 2,4-dinitrophenylhydrazones of volatile carbonyl compounds from cheese curd ripened by *Asp. oryzae* chosen B.

Solvent system: heptan-2-phenoxyethanol.
A: control (authentic 2,4-DNP hydrazones)
B: 192 hrs aged whole milk cheese curd
C: 192 hrs aged skim milk cheese curd

Fig. 2. The absorption patterns of 2,4-dinitrophenylhydrazones of each volatile carbonyl compounds from cheese curd ripened by *Asp. oryzae* chosen B. (In ethanolic KOH)

2). Properties of DNPH of VCC isolated from cheese curd ripened by *Aspergillus oryzae* chosen B are summarized in Table 1.

As the results, the following six compounds were tentatively identified in the distillates from the ripened cheese curd: acetaldehyde, acetone, butanone-2, pentanone-2, heptanone-2, nonanone-2. In absorption spectra shown in Fig. 2, individual DNPH has two distinctive

Table 1. Properties of 2,4-dinitrophenylhydrazones of volatile carbonyl compounds isolated from cheese curd ripened by *Aspergillus oryzae* chosen B

Chromatogram spot	Carbonyl compound	R but* value	Absorption maxima in ethanolic KOH (m μ)				Colour		First detected (hrs after manufacture)	Concentration of carbonyl compounds in cheese curd 192 hrs after manufacturing (p.p.m.)
			Cheese curd		Authentic		On paper	In alkaline solution		
			Major	Subsidiary	Major	Subsidiary				
I	acetaldehyde	0.30	424	—	425	—	Red-brown	Red-brown	0	0.40
II	acetone	0.69	428	—	430	526	"	"	0	2.43
III	butanone-2	1.00	430	528	432	528	"	"	0	0.35
IV	pentanone-2	1.43	430	528	433	530	"	"	192	0.05
V	heptanone-2	2.28	434	530	435	528	"	"	192	0.14
VI	nonanone-2	2.84	436	533	434	530	"	"	192	0.20

* Rbut = movement relative to butanone-2 DNP hydrazone.

absorption with major and subsidiary maxima. These absorption maxima gave the agreeable results in comparison with authentic derivatives of these compounds. R but values of DNPH from the cheese were identified with the same one of authentic derivatives. The colours of the DNPH of VCC from the cheese both on paper chromatogram and in 10% alkali show very specific red brown.

2. Development of VCC in Cheese Curd and Effect of Fat Content on Formation of VCC

In the cheese curd ripened by *Asp. oryzae* chosen B, following six VCC were detected, that is, acetaldehyde, acetone, butanone-2, pentanone-2, heptanone-2 and nonanone-2. The average levels of the individual VCC of cheese curd during ripening is given in Table 2. The results were obtained by analysing three series of cheese curd which were made from whole milk, skim milk and half skim milk, respectively.

As shown in Table 2, following six compounds: acetaldehyde, acetone, butanone-2, pentanone-2, heptanone-2 and nonanone-2 were contained in whole milk curd after ripening for 192 hrs. But, pentanone-2, heptanone-2 and nonanone-2 were not detected in the whole milk cheese curd immediately after making and after ripening for 72 hrs. At the start of the ripening and after ripening for 72 hrs, acetaldehyde, acetone and butanone-2 were significant in amount. Contents of acetaldehyde, acetone, butanone-2, pentanone-2, heptanone-2 and nonanone-2 were 0.40, 2.43, 0.35, 0.05, 0.14 and 0.20 p.p.m. respectively after ripening for 192 hrs.

Table 2 shows also that pentanone-2, heptanone-2 and nonanone-2 were not detected during ripening of skim milk cheese curd. Acetaldehyde, acetone and butanone-2 were significant in amount at the start of ripening and during ripening of skim milk cheese curd. Contents of acetaldehyde, acetone and butanone-2 were 3.74, 0.33 and 0.18 p.p.m. respectively after ripening for 192 hrs.

Half skim milk cheese curd at the start of ripening and during ripening contained acetaldehyde, acetone and butanone-2. But, this curd did not contain pentanone-2, heptanone-2 and nonanone-2. Contents of acetaldehyde, acetone and butanone-2 were 0.88, 0.80 and 0.33 p.p.m. respectively after ripening for 192 hrs.

3. The Contribution of Starter Microbe to the Development of VCC

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Table 2. Changes of volatile carbonyl compounds in cheese curd ripened by *Asp. oryzae* chosen B (p.p.m.)

Ripening period (hrs)		0	72	192
Analytical items				
Whole milk curd (n=3)☆	acetaldehyde	0.31±0.06	0.20±0.03	0.40±0.12
	acetone	0.55±0.06	0.36±0.12	2.43±0.47
	butanone-2	0.15±0.02	0.08±0.04	0.35±0.10
	pentanone-2	—	—	0.05±0.03
	heptanone-2	—	—	0.14±0.04
	nonanone-2	—	—	0.20±0.08
	Total	1.01	0.64	3.57
	Protein %	23.90	23.73	24.68
	Fat %	23.50	24.80	25.33
	Moisture %	48.20	45.30	44.85
	Ash %	4.03	4.76	4.15
	Acidity %	0.40	2.07	2.09
	*index number %	2.80	34.90	49.90
Skim milk curd (n=3)☆	acetaldehyde	0.27±0.03	2.26±0.24	3.74±0.46
	acetone	0.12±0.03	0.21±0.04	0.33±0.07
	butanone-2	0.01±0.01	0.09±0.04	0.18±0.08
	pentanone-2	—	—	—
	heptanone-2	—	—	—
	nonanone-2	—	—	—
	Total	0.40	2.56	4.25
	Protein %	48.70	48.98	49.37
	Fat %	1.30	1.83	1.99
	Moisture %	47.55	46.20	44.99
	Ash %	2.08	2.36	2.47
	Acidity %	0.72	1.09	1.80
	*index number %	3.20	30.06	41.90
Half skim milk curd (n=3)☆	acetaldehyde	0.25±0.04	0.45±0.08	0.88±0.32
	acetone	0.24±0.13	0.63±0.20	0.80±0.18
	butanone-2	0.07±0.06	0.14±0.07	0.33±0.37
	pentanone-2	—	—	—
	heptanone-2	—	—	—
	nonanone-2	—	—	—
	Total	0.56	1.22	2.01
	Protein %	33.51	33.89	34.32
	Fat %	15.33	15.98	16.37
	Moisture %	47.30	46.03	45.19
	Ash %	3.44	3.40	3.98
	Acidity %	0.85	1.03	1.59
	*index number %	2.74	31.55	40.78

* index number=water soluble N/total N×100.

☆ numbers of sample analysed.

Table 3. The contribution of starter microbe to the development of volatile carbonyl compounds (p.p.m.)

Microbe	Analytical items	Ripening period (hrs)		
		0	72	192
Lactic acid bacteria (control)	acetaldehyde	0.29	0.33	0.38
	acetone	0.46	0.27	0.41
	butanone-2	0.21	0.22	0.15
	pentanone-2	—	—	—
	heptanone-2	—	—	—
	nonanone-2	—	—	—
Lactic acid bacteria + <i>Asp. oryzae</i> <i>chosen B</i>	acetaldehyde	0.29	0.27	0.47
	acetone	0.46	0.39	0.99
	butanone-2	0.21	0.19	0.31
	pentanone-2	—	—	0.09
	heptanone-2	—	—	0.12
	nonanone-2	—	—	0.23

Note: Microbe used { Lactic acid bacteria
 { *Streptococcus lactis*
 { *Lactobacillus bulgaricus*
 Aspergillus oryzae chosen B

Culture medium

Whole milk curd

In order to compare the production of VCC, cheese curd ripened with only lactic acid bacteria and that ripened with both lactic acid bacteria and *Aspergillus oryzae chosen B* were examined.

The change of VCC during ripening of whole milk cheese curd are shown in Table 3. As shown in Table 3, acetaldehyde, acetone and butanone-2 were detected at the start of ripening and during the ripening in the cheese curd ripened with only lactic acid bacteria. Pentanone-2, heptanone-2 and nonanone-2 were not detected during the ripening of this cheese curd.

In the cheese curd ripened with both lactic bacteria and *Asp. oryzae chosen B*, acetaldehyde, acetone, butanone-2, pentanone-2, heptanone-2 and nonanone-2 were detected. Content levels of individual VCC of cheese curd ripened with both lactic acid bacteria and *Asp. oryzae chosen B* were slightly higher than those of cheese curd ripened with only lactic acid bacteria. It is clear that there is close correlation between the formation of VCC and the kind of starter.

Discussion

As volatile carbonyl compound(s) (VCC) which are produced during ripening of cheese curd by *Aspergillus oryzae chosen B*, acetaldehyde, acetone, butanone-2, pentanone-2, heptanone-2 and nonanone-2 were detected. These compounds may have the direct relationship to the development of the flavor. Among the flavor components in this cheese, VCC, volatile free fatty acids¹⁰⁾ and volatile sulphur compounds¹¹⁾ may have some relationship to flavor quality and intensity.

As shown in Table 2, six compounds mentioned above were contained in whole milk cheese curd during ripening. But, pentanone-2, heptanone-2 and nonanone-2 were not detected in

skim milk cheese curd and half skim milk cheese curd during ripening, and in these cheese curd, acetaldehyde, acetone and butanone-2 were significant in amount. The difference is clearly attributed to the fact that milk of different fat content is used in the manufacture of this cheese. This finding will present some interesting and important problems.

It has already been demonstrated that the various methyl ketones found in other cheeses made in aerobic conditions such as Blue cheese are formed by the action of fungal enzymes on the fatty acids in cheese^{4,6,14}. The acidic carbonyl compounds are intermediate compounds in the ripening process and are direct precursors of neutral carbonyl compounds. These products are also associated with the oxidation-reduction system of cheese and are influenced by the ripening conditions. This β -oxidation mechanism of formation of methyl ketones is summarized as follows:

In our study, methyl ketones of odd number carbon atoms such as pentanone-2, heptanone-2 and nonanone-2 would be suggested as the products from free fatty acids in the cheese¹⁰. Butanone-2 of even number carbon atoms maybe produced by the decomposition of acethyl methyl carbinol¹³. Acetaldehyde may be produced by strecker degradation⁶ decarboxylation of pyruvic acid, transamination and decarboxylation of alanine, etc.

PATTON¹² found that methyl ketones such as heptanone-2, pentanone-2 and acetone occurred in Blue cheeses. MATSUOKA et al.⁸ found that acetaldehyde, acetone, butanone-2, pentanone-2, heptanone-2 and nonanone-2 were produced during ripening of the semi-soft white mould cheese. Other studies on VCC have been reported about Cheddar cheese³, Swiss cheese¹ etc. The kind and different distribution of VCC in various cheeses may be attributed to microflora used, manufacturing conditions, storage conditions etc.

In order to compare the formation of VCC, cheese curd ripened with only lactic acid bacteria and that ripened with both lactic acid bacteria and *Asp. oryzae chosen B* are examined. As shown in Table 3, kinds and contents of VCC at the start of ripening and during the ripening are different. It is clear that formation of methyl ketones such as pentanone-2, heptanone-2 and nonanone-2 are influenced by the use of *Asp. oryzae chosen B*. In this cheese curd during ripening, *Asp. oryzae chosen B* plays an important role in the formation of VCC as described above.

Summary

The purpose of this investigation was to determine the quantitative changes in volatile carbonyl compound(s) (VCC) which are produced during ripening of cheese curd by *Aspergillus oryzae chosen B*. The results obtained were as follows:

(1) By use of paper chromatographic procedures, 2,4-dinitrophenylhydrazone derivatives of VCC were isolated and identified. As the results, the following six compounds were detected from the ripened cheese curd: acetaldehyde, acetone, butanone-2, pentanone-2, heptanone-2 and nonanone-2.

(2) Six compounds mentioned above were produced in whole milk cheese curd during ripening. But, pentanone-2, heptanone-2 and nonanone-2 were not detected in skim milk cheese curd and half skim milk cheese curd, and in these cheese curd, acetaldehyde, acetone and butanone-2 were significant in amount.

(3) The kinds and contents of VCC at the start of ripening and during the ripening of cheeses are different. Especially, the formation of methyl ketones such as pentanone-2, heptanone-2 and nonanone-2 is influenced by the use of *Aspergillus oryzae* chosen B.

Acknowledgements

This work was supported by a Grant in Aid for Scientific Research from the Ministry of Education.

References

- 1) BASSETT, E.W. and W.J. HARPER (1958) J. Dairy Sci., 41 : 1206.
- 2) DAY, E.A. et al. (1960) J. Dairy Sci., 43 : 463.
- 3) Department of Agr. Chem., Tokyo Univ. Jikennogeikagaku, (1958) 615.
- 4) FOSTER, J.W. (1949) Chemical Activities of Fungi, 556.
- 5) JACKSON, H.W. and R.V. HUSSONG (1958) J. Dairy Sci., 41 : 920.
- 6) KEENEY, M. and E.A. DAY (1957) J. Dairy Sci., 40 : 874.
- 7) LYNN, W.S. et al. (1956) Anal. Chem., 28 : 132.
- 8) MATSUOKA, H. and T. TSUGO (1963) J. Agr. Chem. Soc. Japan, 37 : 332.
- 9) NAKANISHI, T. and Y. NAKAZAWA (1963) Jap. J. Dairy Sci., 12 : A-148.
- 10) NAKANISHI, T. and Y. NAKAZAWA (1964) Jap. J. Zootech. Sci., 35 : 98.
- 11) NAKANISHI, T. and Y. NAKAZAWA (1965) Jap. J. Zootech. Sci., 36 : 60.
- 12) PATTON, S. (1950) J. Dairy Sci., 33 : 680.
- 13) SCARPELLINO, R. and F.V. KOSIKOWSKI (1962) J. Dairy Sci., 45 : 343.
- 14) WALKER, J.R.L. et al. (1959) J. Dairy Res., 26 : 265.

チーズの風味成分に関する研究

V. *Aspergillus oryzae chosen B* によるチーズカード熟成中における 揮発性カルボニル化合物の消長

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Asp. oryzae chosen B によるチーズカード熟成中の揮発性カルボニル化合物の消長を検討した。とくに、本報では揮発性カルボニル化合物の生成に対するカード中の脂肪含量の影響と使用微生物の効果を検討した。その結果は次のとおりである。

(1) チーズから分離した揮発性カルボニル化合物を2,4-ジニトロフェニルヒドラゾンにして、ペーパークロマトグラフィーによつて定量した。熟成チーズカード中には次の6種の化合物が検出された。すなわち、アセトアルデヒド、アセトン、ブタノン-2、ペンタノン-2、ヘ

ブタノン-2 およびノナノン-2 である。

(2) 全脂乳カードの熟成中には、前記6種の化合物が検出された。これに対して、脱脂乳および半脱脂乳カードの熟成中には、ペンタノン-2、ヘブタノン-2 およびノナノン-2 は検出されず、アセトアルデヒド、アセトンおよびブタノン-2 が検出された。

(3) 熟成開始時および熟成中の揮発性カルボニル化合物の種類と量は異なっており、とくに *Asp. oryzae chosen B* を使用すると、ペンタノン-2、ヘブタノン-2 およびノナノン-2 の生成に効果があつた。