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Research Article

Comparation of the Flavor of Different Cheese Flavouring Agents Produced by Using Surface Ripening Bacterium and/or Enzymes

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Abstract: To accelerate cheese ripening, enhance its flavor types and intensity and make cheese flavoring agent in shorter time, surface ripening bacterium (*Brevibacterium linens* and *Debaryomyces hansenii*) and/or enzymes (*Flavorzyme* 500 MG and *Palatase* 20000 *L*) were used in cheese curd. In this study, aroma compounds generated by using ripening cultures and/or enzymes were analyzed. The control l was made by inoculating ripening cultures, while the control 2 was made through enzymes-modified only. Results showed that cheese flavoring agent made by using ripening strains in combination with enzymes had more volatile flavor compounds (at least 44) than that used just ripening bacterium (26) or just two enzymes (27). Then, through Solid-phase microextraction and Gas Chromatography-Mass Spectrometry analysis, we knew that sample 1, which was made through proteolysis first, next sprayed ripening cultures and last lipolysis, generated 54 flavor compounds. Sample 2, which enzymed cheese curd first, then incubated ripening cultures, had 44 aroma compounds. However, the controls 1, incubated ripening strains only, had 26 volatile compounds, while the control 2, enzymed only, had 27 volatile compounds. This study reveals that ripening bacterium could contribute more to the generation of acids, sulphur compounds, miscellaneous compounds and alcohols, it has a good potential to be used in cheese flavoring agents making. Besides, the combination of surface strains and enzymes, especially using Flavorzyme 500 MG first, then sprayed ripening cultures and at last Palatase 20000 L, could get more volatile compounds.

Keywords: Cheese flavoring agent, enzymes, surface ripening bacterium, volatile compounds

INTRODUCTION

Cheese flavoring agent, because of its rich flavors, convenient application, lower cost and long shelf life, is used in a variety of products including, by way of example only, breads, salad dressings, cheese spreads, pizza toppings, sauces, snack foods and so on (Crossman, 1985). Traditionally, cheese flavoring agent is produced through macerating selected cheeses or a mix of cheeses, rendering in measured amounts of hot water together with various other food grade ingredients and raising the mix to pasteurising/emulsification temperatures before feeding to a conventional spray drier from which the resultant powder is collected and bagged.

However, this conventional process suffers from some disadvantages as follows: First, for large scale manufacture of cheese flavoring powder a considerable inventory of cheese is required, creating cost pressures in financing and storing bulk cheese for a long period under controlled temperatures demanding refrigeration and in costly insulated storage areas. Besides, labour costs are high too, as cheese must be decartoned, unwrapped, cut and macerated before feeding to the mixing tank. Finally, the addition of ingredients such as whey powder and sodium based emulsifying salts, will not only require more water before drying at a solids concentrating, which thereby substantially increases energy costs of batch preparation and eventual spray drying, but also result in increased sodium levels in the end product (Paul and Donald, 1989).

In this study, surface ripening bacterium (*Brevibacterium linens* and *Debaryomyces hansenii*) and/or enzymes were used to make cheese flavoring agent. Brevibacterium linens is a major surface microorganism that is present in orange-red smear of surface-ripened cheeses, such as Limburger, Romadour and Appenzeller and its growth is thought to be an essential prerequisite for the development of the characteristic colour, flavor, aroma and texture of these cheeses (Adamitsch *et al*., 2003; Fergal and Patrick, 1999). Another main ripening strain presents on the surface of smear-ripened cheeses is Debaryomyces hansenii, who can oxidize lactic acid and raise the pH of cheese to a level suitable for the growth of B. Linens (Leclercq-Perlat *et al*., 2000a). Besides, D. Hansenii

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can produce useful compounds that are stimulatory to the growth of B. Linens, such as pantothenic acid, niacin and riboflavin. Therefore, B. Linens and D. Hansenii were chose as the surface ripening bacterium in this research. Although the extracellular proteinases and lipases originating from the two surface strains or raw milk could degradate protein and fat in cheese, their effect to cheese flavor was not obvious. Therefore, two enzymes were also used in this study to intensify cheese flavor and reduce its bitterness. In order to compare the operation sequence of surface strains and enzymes, two groups of samples were made. Sample 1 was made by proteolysis first, next sprayed ripening cultures and last lipolysis while sample 2 was made through hydrolysis first and then surface ripening cultures. Besides, two control groups were also made for showing the advantages of combination of strains and enzymes than strains or enzymes only.

MATERIALS AND METHODS

Biological material: The starters used for cheese-making were frozen TCC-3 cultures (Thermophilic Culture Blend, Chr Hansen, Arpajon, France). TCC-3 contains a mixture of Lactobacillus bulgaricus subsp. Thermophilus and Streptococcus thermophilic subsp. And rennase was NATURETM Stamix 1150 NB (Chr Hansen, Arpajon, France). The ripening cultures used were B. Linens (ATCC 9172) and D. hansenii (ACCC 21352). Two commerical enzymes used in this experiment were Flavorzyme 500 MG (11548 u/g), a fungal proteasel/peptidase cocktail produced from Aspergillus oryzae and Palatase 20,000 L (42500 u/g), a fungal lipase derived from Rhizomucor miehei (Novo Nordisk A/S, Bagsvaerd, Denmark).

Cheese manufacture: Small-scale cheese production (coagulation, cutting, draining and moulding of the curd) following a Mozzarella technology was carried out under aseptic conditions. The raw whole cow's milk (10 L) was filtrated and pasteurized for 30 min at 63°C and cooled to 36°C, then the milk was inoculated with 0.46 g of TCC-3. The milk was held at 35-36°C for starter maturation when the milk was preripened till pH 6.4. It was followed by the addition of the rennet (NATURETM, Stamix 1150 NB, Chr Hansen) at 0.4 g/10 L milk. Coagulation time was 20 min and cutting of the curds started after 30 min of hardening, using a 17 mm knife. The curd was then manually stirred for 5 min at a rate of lo stirs/min while the heating temperature was rised to 38°C. The whey was drained at pH 6.2 and the curds were collected and vacuum packed in sterile bags and stored at -20°C until use. The production process was similar to that described by Xu *et al*. (2011).

Preparation of the surface ripening cultures: B. linens and D. hansenii were precultured in 20 mL Tryptone Soya Broth (TSB) or Malt Yeast Glucose Peptone broth (MYGP), respectively in 150 mL conical flasks. The broths were autoclaved (15 min, 121°C) before incubated at 25°C for at least 48 h at 250 rpm. Afier incubation, propagation cells were harvested by centrifugation (4000×g, 20 min) and then resuspended in 4% NaCl solution (Wafa and Mogens, 2003). Cell concentrations were counted by spread-plate technique and the suspensions were diluted to a final concentration of ~10^8 cfu/mL B. linens and~10^6 cfu/mL D. hansenii. The preparation process of ripening cultures was similar to that described by Leclercq-Perlat *et al*. (2000b).

Curd inoculation: Curds were thawed at 4℃overnight, ground in a mortar and heated at 80°C for 3 min to emulsify the cheese slurry when its temperature was close to 60°C, it was homogenized at 24000 rpm for l min with an Ultra-Turrax T25 grinder (IKA-Labortechnik, Staufen, Germany). The pH of the cheese slurry was adjusted to 7.0±0.05 with sterile saturated $NaHCO_3$. Then, the slurry was pasteurized at 80°C for 10 min and was transferred to sterile glass dishes (95 mm in diameter, 30 mm high) closed with a glass stopper. Aften drying for several hours, the slurry congealed to curd again, then the curd was cut to many small pieces. Then the ripening cultures (~10^8 cfu/mL B. linens and~10^6 cfu/mL D. hansenii) were sprayed onto the surface and transferred into a ripening chamber that had been previously thermostated (20°C) and humidified (relative humidity close to 95%). Cheeses were sprayed with the mixed cultures on 0, 1, 3, 5, 7 d and sampled for analysis before spraying cultures. Then transferred them into sterile bags on d 9 and stored at -20°C until use. Aseptic conditions were employed at all steps.

Preparation of enzyme-modified curd: Curd was thawed, ground and heated to the cheese slurry and after homogenization (T 25 grinder, 24000rpm, 1 min), the pH was adjusted to 7.00 with sterile saturated $NaHCO_3$. Flavorzyme 500 MG was added to the slurry (0.2w/w%), mixed for 3min and the slurry was incubated at 55°C for 6 h. After incubation, samples were heated at 80°C for 15 min, for enzyme inactivation. The pH of resulting cheese slurry was then adjusted to 6.80 and treated with Palatase 20,000 L (0.88v/w%) at 48°C for 4 h. The enzyme was then inactivated again (80°C, 15 min) and the sample (the control) was stored at -20°C for further analysis. The whole process was modified according to the details stated by Martin *et al*. (2002) and Leclercq-Perlat *et al*. (2004).

Spray drying to make cheese powder: Spray drying method to make cheese flavouring was similar as

reported by Jan (2004). First bring cheese into a liquid form (solids content about 35%) and a temperature of 80℃ to get a feed which is not too viscous for atomization. The drying is conducted in a conventional spray dryer with cooling fluid bed with air inlet temperature of 180190°C. The cooling of the powder is done in a Vibro-Fluidizer supplied with ambient air in the first section and cold dehumidified air in the last section. And the integrated belt dryer FMD was used to make cheese powder discharged in an agglomerated form.

Cheese analysis's: Cheese samples were analysed in duplicate before inoculated or enzyme-modified for moisture, total nitrogen, fat and pH. Moisture was determine by the oven drying method at 102°C , total nitrogen by the Kjeldahl method (Bynum and Barbano, 1985) and fat by the Van Gulik method (Ardo and Polychroniadou, 1999). For pH measurement, a digital pH meter (pH 211, Hanna Instruments) was used directly into the thawed cheese slurry. SAS Version 9.1.3 for Windows was used to determine the Analysis of Variance (ANOVA).

Estimation of free fatty acids: The estimation of Free Fatty Acids (FFA) involved three steps, including cheese extraction, solid phase extraction and gas liquid chromatography. Details of the three steps were in accordance with that described by Das *et al*. (2005). Duplicate analyses were performed.

Determination of volatile flavor compounds: Solid-phase microextraction and Gas Chromatography-Mass Spectrometry technology was used to analyse volatile aroma compounds and free fatty acids produced by different cheese flavouring samples. The method of sample preparation, GC-MS operating parameters were similar to that described by Hayaloglu *et al*. (2008). A portion (about 3 g) of cheese powder was stored in a 15 mL vial and allowed to equilibrate at 40°C for 30 min. Then extraction of volatile compounds is achieved by injecting a 20 mm 50/30 um DVB/CAR/PDMS (Sigma-Aldrich, Poole, UK) into the vial and exposing to the headspace for 30 min at 40°C. Desorption of the extracted volatiles was carried out on a Varian 4000 Gas Chromatography-Mass Spectrometry System run in splitless mode. During desorption, the fibre remained in the injector for 15 min at a temperature of 250°C, with helium as the carrier gas at a flow rate of l.0 mL/min. The components were separated on a VF-5 ms column, 30 m×0.25 mm×0.25 ym. The oven was held at 40°C for 3 min (desorption period), then ramped at 5°C /min to 140°C and the temperature was then raised at 10°C/min to 240°C, which was then held for an additional 8 min. The mass spectrometer was set to record 43-500 amu, ionization voltage of 70 eV. Compounds were identified by matching mass spectra with the NBS library of standard compounds. Mass spectrometric identification was further confirmed by comparing GC retention times with authentic compounds.

RESULTS AND DISCUSSION

Properties of cheese curd: The content of total nitrogen, moisture, pH and fat were shown in Table 1.

Changes of pH: At a ripening temperature of 20°C and relative humidity close t0 95%, changes of pH in cheeses on d 0 to d 7 were shown in Fig. 1. The pH of two cheese samples both decreased on d 0 to d 3 and then increased on d 3 to d 7. However, the cheese made with surface bacterium changed obvious than the control (made without surface bacterium). This must be the reason that at the beginning, the residual lactose in cheese was gradually consumed by surface bacterium and turned into lactic acid, which made the pH dropped. And with the time prolonged, yeast deacidified the cheese by the metabolism of lactic acid and lactate to CO_2 and H_2O and by the deamination of amino acids and the production of NH_3. This lead to the increase of pH in cheese. Besides, this made conditions more favorable for the growth of salt-tolerant bacteria (B. linens). Therefore, pH of cheese increased and the orange red appeared (Fig. 2).

Changes of FFA concentration: At a ripening temperature of 20℃ and relative humidity close to 95%, changes of FFA concentration in cheeses on d o to d 7 were shown in Fig. 3. Through the whole ripening

Table 1: Properties of raw cheese curd★

	Total nitrogen/%	Fat/%	Moisture/%	pH
Cheese curd	17.86	24.57	55.50	5.50

★: Average over three replication

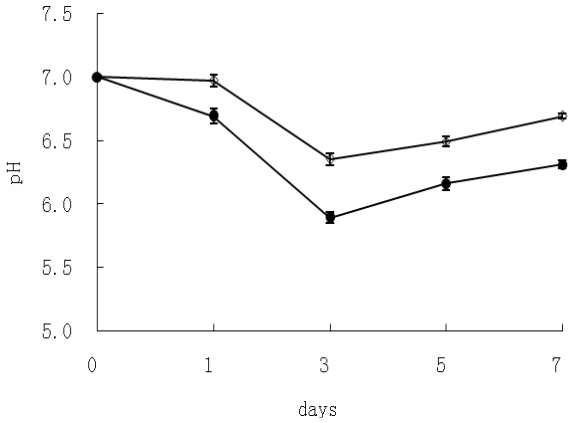


Fig. 1: pH change of cheese made with surface bacterium (●), and the control cheese made without surface bacterium (◇)

Fig. 2: Cheese after 7 days cultured at a ripening temperature of 20℃ and relative humidity close to 95%

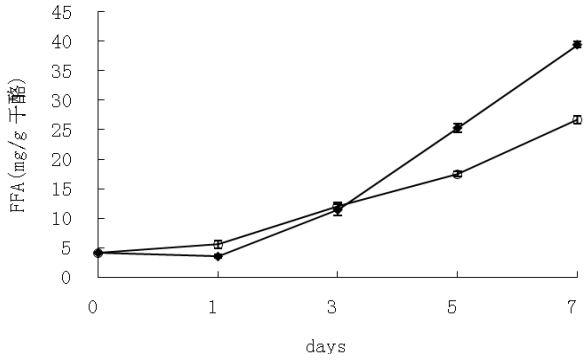


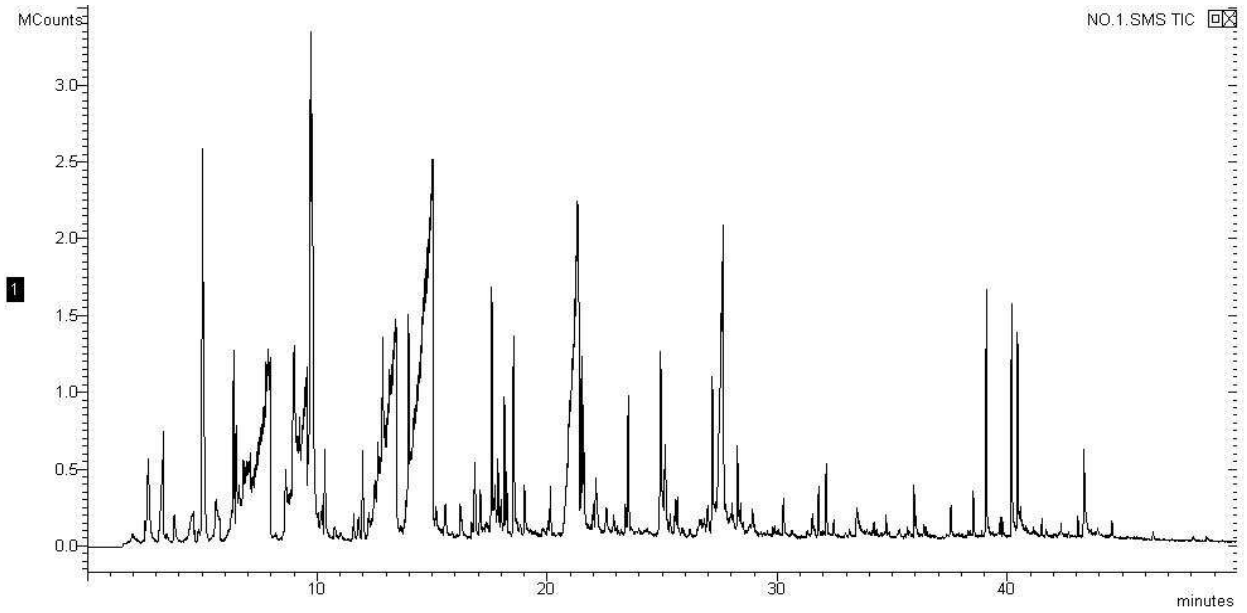
Fig. 3: Total free fatty acids content of cheeses, made with surface bacterium (◆) and the control made without surface bacterium (○)

period, the concentrations of FFA of the two samples were both increased. On d 0 to d 3, the concentrations were similar in two samples. However, after d 3, the concentrations of FFA of the cheese made with surface bacterium increased more rapidly than the control. This must be the reason that surface bacterium grown quickly in these days and secreted many extracellular lipases. This led to the degradation of fat in cheese and generated lots of FFAs. To the control, as some starter cultures (TCC-3), which could also generated some extracellular lipases, lefi in cheese at the process of cheese curd made. For this reason, the concentrations of FFA of the control increased in these days.

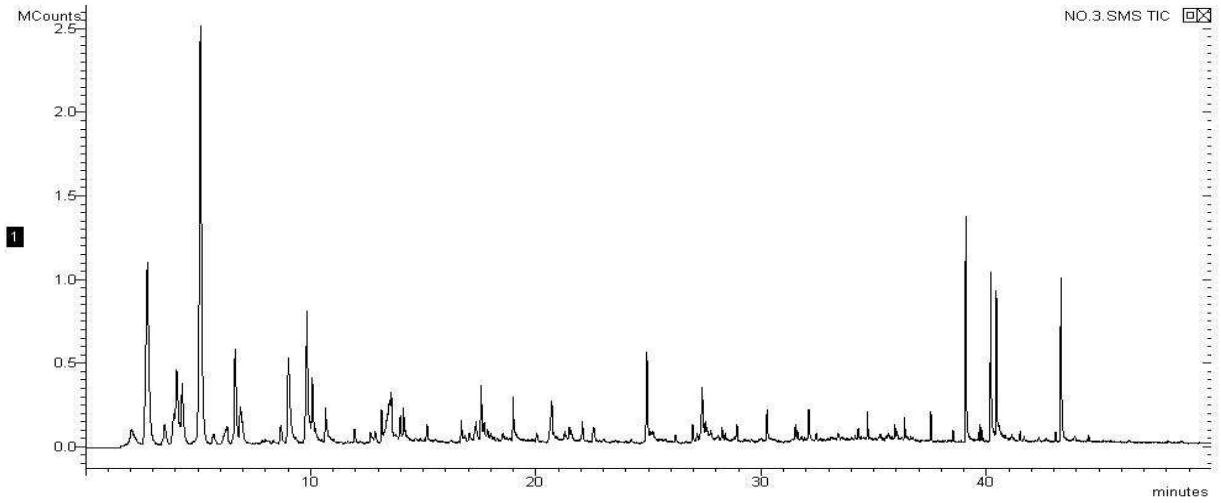
Volatile composition: Four samples were analyzed by Gas Chromatography-Mass Spectrometry for their volatile flavor substances, their total ion chromatograms are showed in Fig. 4.

Total eighty-four compounds were identified in the volatile fraction of the 4 samples, including 14 acids, 30 esters, 6 ketones, 4 aldehydes, 10 alcohols, 5 sulphur compounds, 3 terpenes and 12 miscellaneous compounds. The compounds identified from the cheese flavoring samples are listed by chemical group in Table 2.

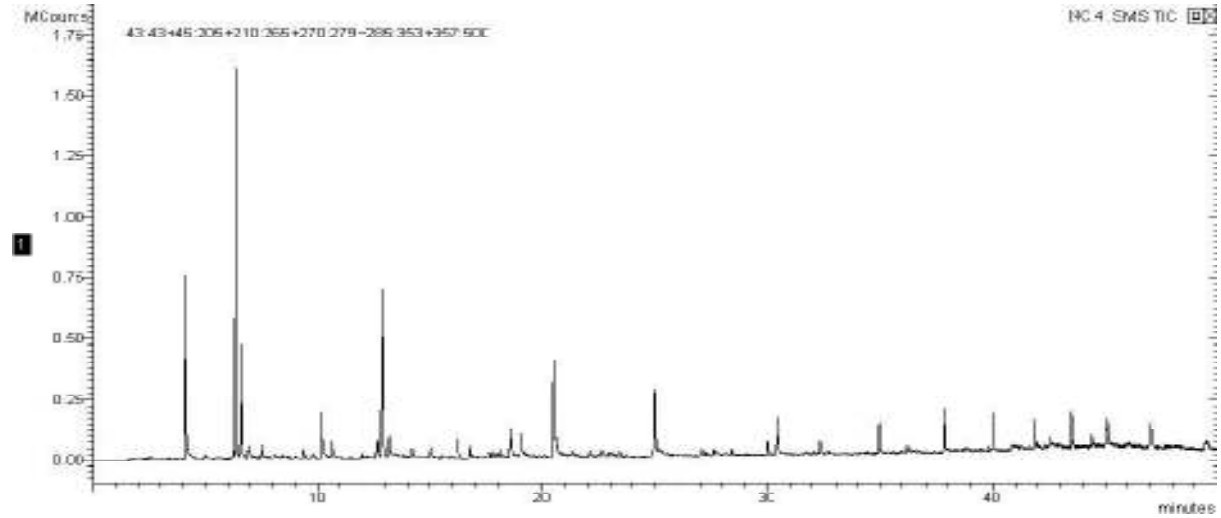
Acids: As can be seen from the table, acids in sample l is similar to that in the control 2, with total relative contents of 47.379 and 32.612, respectively. Their principal acids were both butanoic acid and octanoic acid. The least content of acids (13.078) were identified in sample 2 and most of them were octanoic acid, decanoic acid and butanoic acid. However, the control l had only 3 kinds of acids and most of them were 2-hydroxyl-2-methyl butyric acid. It took up 98.26% of the whole acids in this group. The source of acids is not only originated from lipolysis of the milk fat but also come from metabolism of lactose and amino acids (McSweeney and Sousa, 2000).



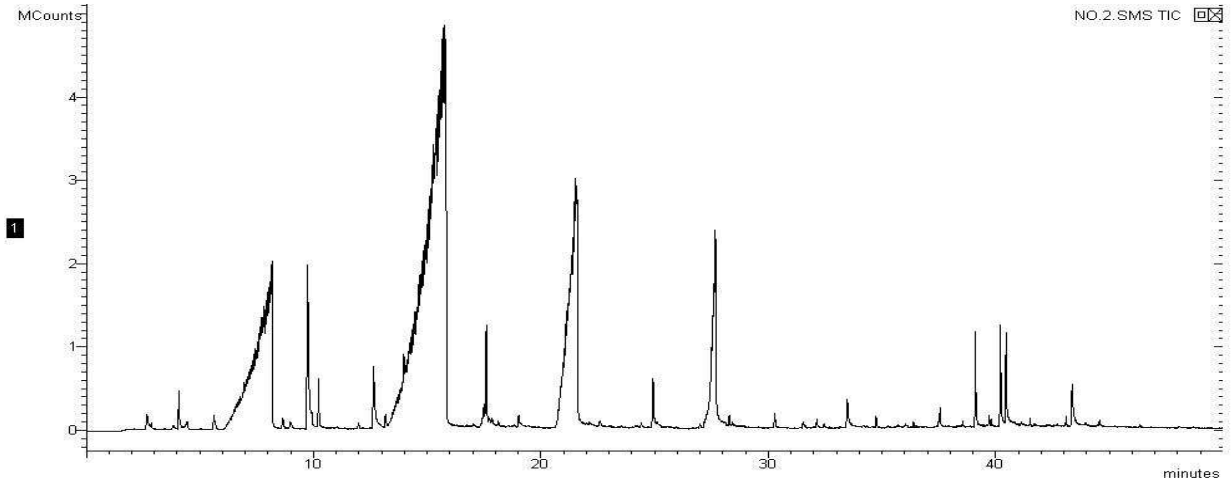
(a)Sample 1



(b)Sample 2



(c)The control 1



(d)The control 2

Fig. 4: Total ion chromatograms of the volatile flavor substances of each sample

Table 2: Aroma compounds identified and quantified in each sample

	Relative content of each sample			
Volatile flavor compounds	Bacterial protein fat	Dual enzyme bacteria	Bacteria bienzyme	The control
Acids (14)	11	6	3	10
Acetic acid	1.601	ND	ND	0.038
Propanoic acid	0.629	2.606	ND	0.093
2-hydroxyl-2-methyl butyric acid	ND	ND	46.01	ND
Butanoic acid	16.13	2.666	ND	20.22
3-Methyl butanoic acid	2.085	ND	0.439	ND
2-Methyl pentanoic acid	6.848	ND	0.375	2.171
Pentanoic acid	0.258	ND	ND	0.061
4-Methyl pentanoic acid	0.917	0.918	ND	ND
Heptanoic acid	ND	ND	ND	0.22
Octanoic acid	12.58	3.48	ND	5.538
Nonanoic acid	ND	ND	ND	0.01
Benzenepropanoic acid	0.022	ND	ND	ND
Decanoic acid	5.97	2.979	ND	4
Dodecoic acid	0.339	0.429	ND	0.261
Total acids	47.379	13.078	46.824	32.612
Alcohols (10)	6	4	3	1
3,3'-oxydipropanol	ND	7.555	ND	ND
4-methyl-3-hexanol	ND	ND	0.81	ND
2,5-dimethyl-3-hexanol	0.135	ND	ND	ND
Benzyl alcohol	0.347	0.141	ND	ND
2-Dodecanol	ND	0.237	ND	ND
3-Nonen-1-ol	ND	ND	ND	0.03
Phenethyl alcohol	1.564	0.457	3.889	ND
Phenylpropanol	0.137	ND	0.284	ND
1-Tridecanol	0.215	ND	ND	ND
1-Undecyl alcohol	0.356	ND	ND	ND
Total alcohols	2.754	8.39	4.983	0.03
Aldehydes (4)	2	3	2	1
3-Methyl-1-pentanal	0.061	0.083	ND	ND
Benzaldehyde	ND	0.446	2.367	0.629
2-Hydroxy-3-methylbenzaldehyde	ND	ND	0.158	ND
4-Hydroxy-3-methylbenzaldehyde	0.368	0.341	ND	ND
Total aldehydes	0.429	0.87	2.525	0.629
Ketones (6)	4	5	1	3
2-Pentanone	0.322	ND	ND	0.059
1-Hepten-3-one	0.122	0.038	ND	ND
2-hydroxy-3-pentanone	ND	3.721	ND	ND
2-Nonanone	1.573	2.396	0.574	0.741
4a-methyl-4,4a,5,6,7,8-hexahydro-3h-napathalne-2-one)	ND	0.075	ND	ND
2-pentadecane ketone	0.273	1.39	ND	0.034
Total ketones	2.29	7.62	0.574	0.834
Esters (31)	19	10	10	7
Propionic acid-2-isopropoxy methyl ester	ND	ND	1.307	ND
2-Methyl ethyl butyrate	0.034	ND	ND	ND
Methyl hexanoate	0.066	0.137	ND	0.012
3-Hydroxy ethyl butyrate	0.177	ND	ND	ND
Methyl (R)-(-)-3-Hydroxyisobutyrate	ND	0.133	ND	ND
2-Oxo methyl butyrate	0.219	0.021	ND	ND
Ethyl hexanoate	1.516	ND	ND	56.63
Propyl caproate	26.08	0.707	ND	ND
2,2-Dimethylpropanoic acid,3-methylbut-2-enyl ester	ND	ND	0.967	ND
Isoamyl butyrate	0.325	0.207	2.108	ND
Pentanoic acid, 2-methylbutyl ester	ND	ND	0.089	ND
Ethyl butyrate	0.207	ND	ND	ND
2-Ethyl-n-butyric acid ethyl ester	ND	ND	0.442	ND
Isoamyl isovalerate	0.935	ND	0.588	ND
Ethyl butyrate	ND	ND	0.042	ND
Isobutyl butyrate	0.393	ND	ND	ND
Methyl caprylate	ND	ND	ND	0.025
(R)-α-(Hydroxymethyl)benzeneacetic acid methyl ester	ND	0.062	ND	ND
Butyl hexanoate	0.095	ND	ND	ND
Ethyl caproate	0.071	ND	ND	ND
Ethyl caprylate	0.764	0.371	ND	8.756
Amyl caproate	0.244	ND	ND	ND
Isoamyl hexanoate	ND	ND	0.783	ND

Table 2: Continue

Volatile flavor compounds	Relative content of each sample			
	Bacterial protein fat	Dual enzyme bacteria	Bacteria bienzyme	The control
Triglyceride butyrate	ND	ND	ND	0.038
Methyl caprate	ND	ND	ND	0.012
Isobutyl benzoate	0.089	0.415	ND	ND
Benzenepropanoic acid, ethly ester	0.086	ND	ND	ND
Ethyl caprate	0.291	0.514	0.639	0.06
Isoamyl octanoate	0.095	ND	ND	ND
Phenyl ethyl butyrate	ND	ND	1.923	ND
Ethyl dodecanoate	0.102	0.155	ND	ND
Total esters	31.789	2.722	8.888	65.533
Sulphur compounds (5)	3	4	2	1
Dimethyl disulfide	4.728	43.17	0.782	0.022
1-Propene-1-thiol	0.803	ND	ND	ND
2-(methylthio)-ethanol	ND	0.22	ND	ND
Allyl methyl sulfide	ND	0.143	ND	ND
Dimethyl trisulfide	6.349	4.807	20.38	ND
Total sulphur compounds	12.023	48.34	21.162	0.022
Terpenes (3)	3	2	0	0
(-)-Limonene	0.189	ND	ND	ND
p-Cymene	0.038	0.152	ND	ND
Longifolene	0.136	0.73	ND	ND
Total terpenes	0.363	0.882	0	0
Miscellaneous(12)	5	10	5	4
3-ethyl-2,2-dimethylpentane	1.206	11.67	ND	0.228
2-methyl Pyrimidine	ND	0.037	ND	ND
4,6-Dimethyl Pyrimidine	0.162	2.686	2.387	ND
2,4 (10)-Thujadiene	0.021	0.056	ND	0.013
2,3,5-trimethyl Pyrazine	ND	1.153	1.434	ND
2,6-diethyl Pyrazine	ND	0.405	ND	ND
2,3,5,6-tetramethyl Pyrazine	ND	1.142	ND	ND
4-ethyl Phenol	ND	ND	10.41	ND
Azulene	ND	0.57	ND	ND
Benzyl butyl ether	1.019	0.187	ND	0.025
Indole	ND	ND	0.554	ND
Butylated Hydroxy Toluene	0.314	0.2	0.317	0.002
Total miscellaneous	2.722	18.106	15.102	0.268

ND: Not Detected

As we can see from the table, the addition of lipase contribute more to the kinds of acids than surface ripening strains? Besides, we can also draw the conclusion that adding lipase in the last step (just as sample l and control 2) could generate more acids through lipolys is in cheese than other groups. However, sample 2, which sprayed surface bacterium at the last step, had less acids than sample l. This could be the reason that the use of acidity regulator (saturated NaHC03) before surface bacterium were inoculated, neutralized some acids generated through lipolysis.

Esters: Esters have been identified as important contributors to cheese flavor due to their high volatility at ambient temperatures and their low perception threshold of volatile compounds (Garde *et al*., 2002). They are formed by the esterification of short- to medium-chain fatty acids and primary or secondary alcohols derived from lactose fermentation or from amino acid catabolism (Curioni and Bosset, 2002). In this study, the kinds of esters in all the aroma compounds in every group were the most and their kinds were 19, 10, 10 and 7 in sample l, sample 2, control l and control 2 respectively. In sample l and 2, propyl caproate was the predominant ester, which has been positively correlated with a sweet fruity flavor. And isoamyl butyrate, a kind of flavor and fragrance agent with an odor like banana, pineapple, cherry and sweet, was detected most in all the esters in control l. Another important aroma ester in control l was phenylethyl butyrate, which tasted like fruity, bacteriumL, sweet and green with a tropical winey nuance. The most important ester in control 2 was ethyl hexanoate, who had an aromatic note resembling pineapple or banana and had also been positively correlated with pungent odor and silage-like, salty, acidic and peppery flavors (Garde *et al*., 2002).

Ethyl hexanoate was also found and played an important role in the aroma profiles of aged Cheddar, natural Gorgonzola, Grana Padano, Pecorino and Ragusano cheeses (Curioni and Bosset, 2002). As can be seen from the table, sample l, 2 and control l have more kinds of esters than control 2. This indicates that the inoculation of surface bacterium could make cheese generate more flavor esters.

Alcohols: The kinds and relative contents of alcohols in different samples were influenced by the addition of

surface bacterium. As we can see, the former 3 samples, which all inoculated surface bacterium, had more kinds of alcohols than the last sample, which enzymed by the two enzymes only. Phenethyl alcohol is among the most odorous aromatic alcohols, whose very pleasant aroma presents rose flower notes and its production from phenylalanine seems to be essentially achieved by yeasts (Lee and Richard, 1984). In this research, except control 2, other groups all identified this alcohol and the three samples were all inoculated yeast (D. hansenii), which agreed with the conclusion reported by Lee and Richard (1984). Besides, some alcohols such as 3,3'-oxydipropanol; 2,5- dimethyl-3-hexanol; 2-Dodecanol; Phenylpropanol; 1-Tridecanol and l-Undecanol, which were detected unfrequently in cheese, were identified in these cheese samples.

Aldehydes: Aldehydes originate from amino acids either by transamination, or by Strecker degradation (Curioni and Bosset, 2002). They were found in the four cheese samples in relatively small quantities and their total relative contents were similar in each sample except control l. Benzaldehyde was the only aldehyde in control 2, which was characterised by a bitter almond odour. Besides benzaldehyde, which was a common component in the volatile fraction of cheese, 3-methyl-l-pentanal, 4-hydroxy-3-methylbenzaldehyde and 2-Hydroxy-3- methylbenzaldehyde were also identified in cheese samples.

Ketones. Ketones are common constituents of most cheese products. Due to their typical odours and their low perception thresholds, ketones are primarily known for their contribution to the aroma of surface ripened cheeses (Curioni and Bosset, 2002). Methyl ketones, such as 2-Pentanone, 2-Nonanone, are the typical flavour compounds in some cheeses and are attributed to p-oxidation of free fatty acids. One important odorant, found in the former two cheese powder samples, was l-Hepten-3-one, which has a Geranium-Iike odour (Michaela and Peter, 2000). However, another research reported that l-Hepten-3-one was identified as responsible for the mushroom note (Cullere *et al*., 2009). 2-hydroxy-3-pentanone was identified in sample 2 0nly, which had a odor of truffle earthy nutty and was found in Kuflu cheese ever (Hayaloglu *et al*., 2008). Besides the above, an uncommon ketone, 4a-methyl-4,4a,5,6,7,8-hexahydro-3h-napathalne- 2-one was also found in sample 2. Sulphur-containing compounds. The most abundant sulphur compounds in these cheese samples were dimethyl disulphide and dimethyl trisulphide, which was converted by methanethiol via oxidative reactions. Many micro-organisms are able to produce methanethiol from methionine, especially B. Linens, P. camemberti and G. Candidum (Jollivet *et al*., 1994). Among these micro-organisms, coryneform bacteria, especially B. linens, are the key agent of sulphur-compound production in smear-ripened cheeses (Molimard and Spinnler, 1996; McSweeney and Sousa, 2000). In this study, dimethyl disulphide was found in every sample, however, dimethyl trisulphide was not detected in the last sample, which didn't inoculated B. Linens on the surface of cheese. Besides, in the last sample, only one sulphur compound was identified, which proofed the critical effect of B. Linens on the production of sulfur compounds. In sample 2, we also identified 2- (methylthio)-ethanol and allyl methyl sulfide. 2-(methylthio)-ethanol, a kind of flavoring agent, had an sulfurous meaty ordor. And another flavoring agent, allyl methyl sulfide, was irritant and smelling like alliaceous garlic and onion.

Terpenes: Terpenes in cheese always originate from plants in the pasture or feed mixture (Curioni and Bosset, 2002). It was reported that the type of feed was an important factor that affected the quality and composition of matured cheeses. Nevertheless, there has no enough evidence in report to state the significant sensory impact of many terpenes on the cheese flavor clearly. Limonene, being associated with citrus-like flavor in some cheeses, is an exception. Three terpenes were identified in sample l, which were Limonene, p-Cymene and Longifolene. And the latter two were also identified in sample 2. However, none were detected in the two control samples. Thus, we can guess that generation of terpenes may also associated with surface bacterium and enzymes.

Miscellaneous: Total 12 miscellaneous compounds including alkanes, alkenes, pyrimidine, pyrazines, ethers, phenols and nitrogen derivatives were identified in the four cheese samples. Sample 2, no matter from the type or quantity, had more miscellaneous compounds than the other three samples, including two pyrimidines, three pyrazines, some alkanes and alkenes and one ether. Phenol is an important compound for surface ripened cheese and its contribution to cheese aroma is perceived as sharp and medicinal notes (Molimard and Spinnler, 1996). In the sample control l, 4-ethyl phenol was detected. It was irritate, but it can give a flavor of smoke, phenolic, creosote and savory. It was often used as a flavor and fragrance agent. Indole, a popular component of fragrances, was identified in control l too. It can be produced by bacteria as a degradation product of the amino acid tryptophan. Furthermore, 4,6-dimethyl pyrimidine, 2,3,5-trimethyl pyrazine, 2,3,5,6-tetramethyl pyrazine and butylated hydroxy toluene were also identified in control l. However, the control 2 had the least kinds and quantities of miscellaneous compounds, including 3-ethyl-2,2-dimethylpentane, 2,4(10)-Thujadiene, benzyl butyl ether and butylated hydroxy toluene. Further graphic analysis was shown in Fig. 5. It is shown that acids, esters and sulphur compounds were the principal volatiles for sample l. For sample 2, the key flavor compounds were sulphur compounds. Besides, acids, alcohols and ketones were also principal volatiles in sample 2. To the control l, acids, sulphur compounds and miscellaneous compounds were its important flavors. However, for the control 2, which didn't spray

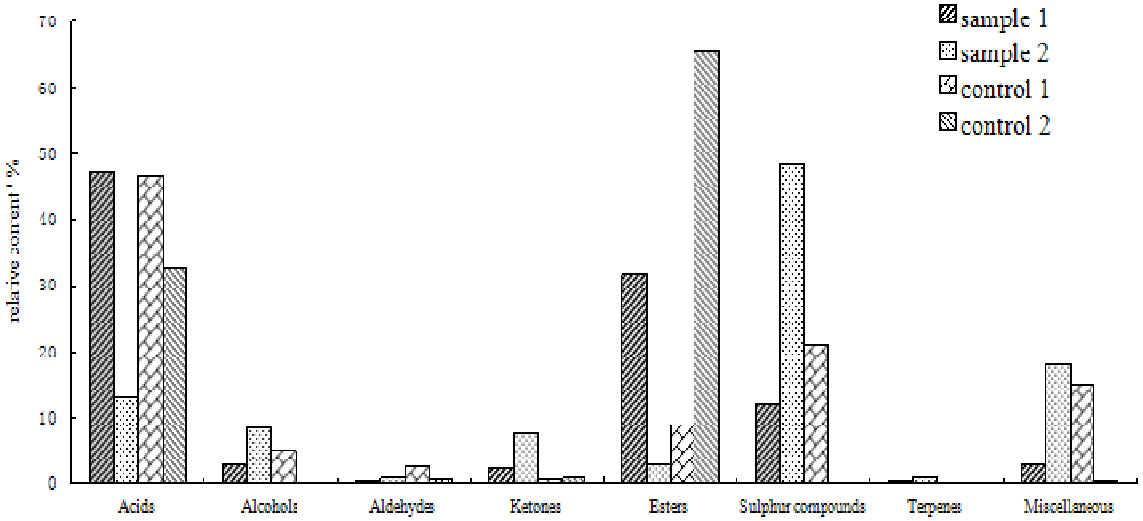


Fig. 5: Comparison of the content of different kinds of flavor compounds in different groups

surface cultures in the processing of making cheese powders, the key flavors were esters, whose relative content were up to 65.533%. Another important volatiles were acids in control 2. Results showed to us, surface strains contributed more to the generation of acids, sulphur compounds, miscellaneous compounds and alcohols. And the combination of surface strains and enzymes could generate more flavor compounds.

CONCLUSION

The paper compares the volatile composition of several cheese flavoring agents made by different methods. As evidenced by this initial study, it is apparent that sample l, made through proteolysis first, next sprayed ripening cultures and last lipolysis, generated 54 flavor compounds. Sample 2, which enzymed cheese curd and then sprayed ripening cultures, had 44 aroma compounds. And the control l, sprayed ripening cultures only, had 26 flavor compounds, while the control 2, enzymed cheese curd only, had 27 flavor compounds. Besides, from the results of those samples associated with surface ripening can not only make pH and FFA of cheese curd change strains, we know the addition of surface ripening strains quickly than control sample, but make cheese appeared orange red. Besides, from sample l and sample 2, we know that the combination of surface bacterium and enz} anes wiU make cheese generate some terpenes, which didn't detected in control l and control 2. This investigation demonstrates that the importance of surface bacterium to cheese flavor and reveals that ripening cultures have a good potential to be used in cheese flavoring agents industry. It contributed more to the generation of acids, sulphur compounds, miscellaneous compounds and alcohols and made cheese appeared orange red, which could driving consumer choice.

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