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Evaluation of volatile flavouring compounds in Cheddar cheese, manufactured by using *Lactobacillus rhamnosus* as an adjunct culture

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Abstract

Volatile flavouring compounds in Cheese are generated by starter cultures in combination with adjunct cultures that confer distinct flavor and texture properties to cheese. *Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. lactis* are commonly used as preferred starter cultures for Cheddar cheese. Lactobacillus rhamnosus used as adjunct culture improves the flavour, imparts therapeutic properties, decreases bitterness and accelerates proteolysis of Cheddar cheese. For the present project, Cheddar cheese was manufactured from buffalo milk by using *Lactobacillus rhamnosus* with starter cultures. Raw milk used was tested for fat, total proteins, lactose, acidity, total solids, SNF and pH. After manufacturing, cheese was ripened at 6°C for a period of 90 days.. Volatile flavouring compounds were determined at 45 and 90 days of ripening. Results obtained were statistically analyzed to assess the influence of the *Lactobacillus rhamnosus* on Cheddar cheese quality. On the basis of cheese evaluation, it was found that treatment T2 was evaluated to be the best during storage with *Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. lactis* (95:5) @ 1.5% + *Lb. Rhamnosu*s @ 0.5% combination.

Keywords: Cheddar cheese, Physico-chemical analysis, Storage study, Sensory Evaluation

1. Introduction

Cheddar is a hard ripened cheese, and is popular throughout the world due to its distinct flavour, taste and aroma. It was firstly manufactured in a town, Cheddar George (England) but now a days it is being manufactured in many parts of the world. Cheddar cheese is produced by acidification and concentration of milk following gel formation with rennet [5].

It is a complex mixture, consisting of protein, fat, carbohydrates, vitamins and minerals. The changes during ripening depend on the biochemical conditions of curd i.e., water activity, pH, oxidation reduction potential, mineral contents, ripening temperature, level and method of salt addition and nature of secondary microflora [19].

Cheese is also a suitable nutrient for patients who have diabetes or lactose malabsorption, because of the low lactose ratio, it contains [8].The flavour profiles of cheeses are complex and are variety- or type- specific. These profiles are influenced by many substances e.g., organic acids, sulphur compounds, lactones, methyl ketones, alcohols and phenolic substances [31,37]. For flavour development in most cheese types, a period of ripening up to 1-2 years is required for some hard cheese varieties. Attempts to shorten the ripening time have varying degrees of success [20]. Approaches which have been used include the addition of exogenous enzymes or cheese slurries to the crud, use of modified or novel starters or starter adjunct cultures and elevated ripening temperature [39].

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With advancing technology and changing markets, new goals for ripening systems are emerging. These include: flavour improvement of low fat cheeses, enhancement of flavour intensity, production of fast ripening cheese for use in processed cheese products, the development of new uniquely flavour chesses and improvement in flavour consistency. *Lactococcus lactis subsp. lactis and Lactococcus lactis subsp. cremoris* are the main cultures used for Cheddar cheese in combination of 95-98% respectively [35].

Various strains of lactobacilli are used as adjuncts to improve flavour development and accelerate cheese ripening. These adjuncts influence proteolysis, resulting in the formation of high concentration of free amino acid (FAA) and improved flavour as compared to the control cheese [6].

In this project *Lactobacillus rhamnosus* was used as an adjunct culture in Cheddar cheese and its impact on volatile flavouring compounds of Cheddar cheese was studied.

2. Materials and methods

The whole research work was conducted in the Dairy and Food Analysis Laboratories, National Institute of Food Science and Technology, University of Agriculture, Faisalabad.

Raw materials. Buffalo milk of a specific breed was obtained from farm house, Department of Live Stock Management, University of Agriculture, Faisalabad. Commercially available Cheddar cheese cultures and rennet was purchased from scientific stores in Faisalabad.

Milk analysis. The raw milk after collecting from farm house was analyzed for its fat content[23], total protein, pH value, lactose content, total solids, solid not fat and titratable acidity, according to the standard methods of AOAC (2000) [1].

Cheese manufacturing. Cheese was manufactured by following the protocol as described by Scott (1981) [30].

Standardization. For Cheddar cheese milk was passed through the cream separator then standardized at 3.5% fat by mixing the cream and skim milk then mixing it again according to the standard.

Homogenization and pasteurization. After standardization of milk, it was homogenized with a homogenizer and pasteurized at controlled temperature of 65^0C for 30 minutes in water bath. Milk was shifted into processing vat and cooled to 31^0C.

Addition of additives. The milk was divided into five portions. To each portion, additives were added as given in the Table.1

Treatments	**Cultures**	**Rennet**
T_o	*Lactococcus lactis ssp. cremoris* and *Lactococcus lactis ssp. lactis* (95:5) @ 2.0%	0.006%
T_1	*Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. *lactis* (95:5) @ 1.75% + *Lactobacillus rhamnosus* @ 0.25%	0.006%
T_2	*Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1.5% + *Lactobacillus rhamnosus* @ 0.5%	0.006%
T_3	*Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1.25% + *Lactobacillus rhamnosus* @ 0.75%	0.006%
T_4	*Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1% + *Lactobacillus rhamnosus* @ 1%	0.006%

Curd formation. The milk was placed undisturbed for curd formation for 30-45 minutes at 31^0C. The coagulum formation was checked after every 15 min. for this purpose a stem of thermometer was plunged below the surface layer and lifted the coagulum causing it to break in a cleavage line. A clear cleavage with green whey separation at the base of cleft indicated that the curd was ready to be cut.

Cutting. The coagulum was cut into 1/4 inch cubes by stainless steel knife. A smaller cube size will yield the cheese with lower moisture whereas a large cube size will result in a high moisture cheese. The cubes were allowed to set again for 10-15 minutes.

Scalding and stirring. After cutting the coagulum was scalded (cooked) at 39-40^0C for first 15 minutes, then the curd was stirred for 45-50 minutes. The scalding promotes synersis and whey expulsion from the curd, while stirring aids the uniform heat distribution throughout the curd particles.

Whey drainage. After scalding, whey was drained off from the curd.

Firm curd body was washed with hot water at 80^0C and second cooking was carried out at 32^0C for 10-15 minutes to settle down the firm curd and the whey separated during heating was removed continuously from the curd.

Texturing/Cheddaring. Having removed the whey, the curd was divided into blocks and pilled up every 15-20 min. Cheddaring a procedure which involves two basic steps, stretching and in attaining curd particles coalesce, drainage of whey continues and finally the curd attain the characteristic texture. Traditional Cheddaring process involves manually pilling and turning the curd.

Milling. The curd was milled into finger sized pieces, cooled to 25^0C to enclose fat particles before salting.

Salting. Salting was done at 1% by sprinkling the salt on the curd pieces and mixed well. After salting curd pieces were placed for 5 minutes.

Moulding and pressing. After salting firm curd was scooped out and placed in cheese mould and then during pressing, a pressure of 25 Psi was applied for 2-3 hours (the pressure was increased gradually) to give curd the final shape, firm surface and correct final moisture content.

Storage/ripening. Following moulding and pressing, cheese obtained was coated with food grade wax, wrapped in clean alluminium foil and stored for ripening at temperatures 6±2 ^{0}C for a period of 3-months.

3. Results and discussion

Volatile flavoring compounds. The ripening process of cheese consists of 3 primary biochemical processes glycolysis, lipolysis, and proteolysis the relative importance of which depends on the cheese variety [12]. These primary changes are followed and overlapped by secondary catabolic changes, including deamination, decarboxylation, and desulfurylation of amino acids, oxidation of fatty acids, and even some synthetic changes; that is, esterification [13]. Production of volatile compounds in cheese results from catabolism of free amino acid, free fatty acid, lactose and citrate, and is mediated by the complex and intricate enzyme complement of starter, NSLAB and the prevailing conditions of the cheese matrix [16].

Flavour formation in cheeses such as Cheddar or is often associated with the presence of nonstarter lactic acid bacteria (NSLAB) such as *Lactobacillus plantarum, Lb. casei and Lb. paracasei* [28]. Freshly-made curds of various cheese varieties have bland and very similar, flavors but during the ripening the flavor compounds are produced which are characteristic of each variety. It is now generally accepted that the flavor of most cheeses results from the combination of a large number of sapid compounds in the presence of their correct ratios and concentrations [25]. Originally it was thought that cheese flavor resulted from a single compound or class of compounds. But very limited information is available on the characterization of the flavor of most cheese types [32].

It is not clear if the flavouring properties of the adjunct are due to Lactobacillus itself or if they result from an interaction with the starter lactococci [24].

The volatile flavoring compounds identified by GC-MS, in steam distillates from Cheddar cheese ripened for 45 and 90 days are grouped into aldehydes (Table 2), ketones (Table 3), alcohols (Table 4), esters and sulphur compound (Table 5).

Aldehydes. Many flavours in fermented milk products are produced from amino acids, and most of the flavour producing reactions in cheese are enzymatic in nature. Aldehydes mainly originate from the catabolism of amino acids [33,37] and are generated by chemical oxidation of a-keto acids catalyzed by bivalent cations.

The aldehydes found in Cheddar cheese during ripening are given in Table 2. Considerably higher numbers of compounds were extracted after 90 days as compared to 45 days of ripening. After 45 days, only “heptanal” was found in control treatment while most of “pentanal” was extracted from cheese samples prepared by adding *Lactobacillus rhamnosus*. After 90 days of ripening, only “hexanal”, “heptanal” and “acetaldehyde” were noted in T_0. All the aldehydes except “octanal” were found in T_1 while T_2 contained all the compounds listed in Table 4. While “Benzaldehyde” was found in T_2, T_3 and T_4 after 90 days of ripening.. The aldehydes in excess were pentanal and acetaldehyde.

Low molecular weight aldehydes as extracted in the present study are aroma-active compounds in Cheddar cheese [9, 22].

With the ripening process, the number of aldehydes extracted increased noticeably because all the flavoring compounds develop in cheese as a result of complex series of microbiological and biochemical reactions that occur during ripening [15]

Starter cultures and adjuncts added provide the enzymes involved in the biochemical conversions of aminoacids to aldehydes, that give rise to volatile and nonvolatile compounds which contribute to cheese flavor [16].

In present study, the addition of Lactobacillus rhamnosus in Cheddar cheese boosted up the production of aldehydes because the selection of starter systems with high levels of peptides activity and high autolytic abilities results in increasing the level of flavor forming substrates [3]. *Lactobacillus rhamnosus* has previously been shown to have a potent proteolytic system [18] and has been shown to enhance early flavor development in Cheddar cheese, Hannon *et al*., (2007) [16] reported the higher number of aldehydes in Cheddar cheese manufactured by the addition of Lactobacillus besides the Lactococcus species as compared to that having the Lactococcus species only.

Ketones. By enzyme catalysed reactions a-keto acids are converted to ketones, that confer a typical flavour to cheese,cheese is a high-fat food; fresh cheeses contain 30% or more fat [29]. The role of fat fractions of cheese is very important for the development of typical flavour and texture in cheese.

Lipolysis of milk fat triglycerides leads to the formation of free fatty acids, which are further catabolized to volatile compounds, such as ketones [34,40]. Cheddar cheese made from nonfat cheese does not develop full aroma even after 12 months (Ohern and Tuckey, 1969) [27].

Catabolism of amino acids is a major contributor for flavour formation in ripened cheeses. By transamination, methionine (Met), aromatic-(ArAA) and branched chain (BrAA) amino acids are converted to their corresponding a-keto acids, which are further degraded to various aroma compounds i.e ketones [42], according to his studies a-ketoglutarate seems to be the most important amino acceptor. However, the production of a-keto acids by LAB in cheese has been considered to be a limiting step, as only a few natural LAB strains possess glutamate dehydrogenase (GDH) activity. Several Lactobacillus ssp. including *Lb.casei, Lb.rhamnosus* have been shown to possess GDH activity [36].

The ketones identified in steam distillates of Cheddar cheese are listed in Table 3. The ketones produced were considerably affected by the ripening temperatures and the starter cultures used, "2-Butanone" was found in T_1, T_2, T_3, and T_4 after 90 days of ripening. After 45 days "Acetone" found in all cheese sample while 2-Heptanone present in only control treatment (T_0), and 3-Methyl-2-Hexanone present in all treatments after 90 days of ripening, only "Acetone", and "Heptanone" were noted in T_0. All the ketones, except "2-Heptanone", were found in T_1 and T_2. "2-Heptanone" was missing from other treatments after 45 and 90 days.

Table 2. Aldehydes found in steam distillates from Cheddar cheese during ripening

Treatments	**T_0**		**T_1**		**T_2**		**T_3**		**T_4**	
Ripening	**45 days**	**90 days**	**45 days**	**90 days**	**45 days**	**90 days**	**45 days**	**90 days**	**45 days**	**90 days**
Acetaldehyde	-	+	-	+	+	+	+	+	+	+
Pentanal	-	-	+	+	+	+	-	+	+	+
Hexnal	-	+	-	+	-	+	+	+	+	+
2-hexenal	-	-	+	-	+	+	+	+	+	+
Heptanal	+	+	-	+	-	+	-	+	+	+
Octanal	-	-	-	-	-	+	-	+	-	+
Benzaldehyde	-	-	-	+	-	+	-	+	+	+

T_0: *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 2%
T_1: *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1.75% + *Lb. Rhamnosus* @ 0.25
T_2: *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1.5% + *Lb. Rhamnosus* @ 0.5%
T_3: *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1.25% + *Lb. Rhamnosus* @ 0.75%
T_4: *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1% + *Lb. Rhamnosus* @ 1%

Table 3. Ketones found in steam distillates from Cheddar cheese during ripening

Treatments	**T_0**		**T_1**		**T_2**		**T_3**		**T_4**	
Ripening	**45 days**	**90 days**	**45 days**	**90 days**	**45 days**	**90 days**	**45 days**	**90 days**	**45 days**	**90 days**
Acetone	+	+	+	+	+	+	+	+	+	+
2-Butanone	-	-	-	+	+	+	-	+	+	+
2-Hexanone	-	-	-	+	-	+	+	+	+	+
2-Heptanone	+	+	-	-	-	-	+	-	-	-
3-Methyl-2-Hexanone	-	+	+	+	+	+	-	+	+	+

Table 4. Alcohols found in steam distillates from Cheddar cheese during ripening

Treatments	**T_0**		**T_1**		**T_2**		**T_3**		**T_4**	
Ripening	**45 days**	**90 days**	**45 days**	**90 days**	**45 days**	**90 days**	**45 days**	**90 days**	**45 days**	**90 days**
Ethanol	+	-	+	-	+	-	-	-	-	-
2-Butanol	+	+	+	+	-	-	+	-	-	-
Hexanol	+	+	-	-	-	-	-	-	-	-
2-Heptanol	-	-	-	+	-	+	-	+	-	+
Isobutanol	-	+	-	+	+	+	-	+	+	+

Table 5. Sulphur and ester compounds found in steam distillates from Cheddar cheese during ripening

Treatments	**T_0**		**T_1**		**T_2**		**T_3**		**T_4**	
Ripening	**45 days**	**90 days**	**45 days**	**90 days**	**45 days**	**90 days**	**45 days**	**90 days**	**45 days**	**90 days**
Dimethyl Sulphide	-	-	-	+	-	+	-	+	-	+
Dimethyl disulphide	-	-	-	+	+	+	+	+	+	+
Methanethiol	-	-	-	+	-	+	-	+	-	+
Ethyl Acetate	-	-	-	+	-	+	-	+	+	+
Ethyl Benzoate	-	-	-	-	-	+	-	+	-	+
Ethyl Hexanoate	-	-	-	-	-	+	-	+	-	+

T_0 : *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 2%
T_1 : *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1.75% + *Lb. Rhamnosus* @ 0.25
T_2 :*Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1.5% + *Lb. Rhamnosus* @ 0.5%
T_3: *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1.25% + *Lb. Rhamnosus* @ 0.75%
T_4 : *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1% + *Lb. Rhamnosus* @ 1%

Alcohols. Proteolysis is the main biochemical flavor generating process in flavor formation of matured hard cheeses such as Cheddar [14,20]. Citrate in Cheddar cheese are exhausted by *Lb. rhamnosus* , and they continue further growth on lactate. In a study of citrate degradation by NSLAB isolated from Cheddar cheese, Drake et al., 1997; [11] showed that Lb. casei, Lb. zeae and Lb. rhamnosus produced acetate, lactate and ethanol (alcohol) from citrate.As a result of amino acids degradation, Amines and alcohols are produced, that make up a significant portion of cheese flavor and aroma compounds [4]. The free fatty acids act as precursors to produce flavor and aroma compounds leading to the formation of secondary alcohols, as a result of catabolic reactions [2,7].

The alcohols extracted in Cheddar cheese during the present study are presented in Table 4. After 45 days, "2-Butanol" found in both T_0 and T_1 while not present in T_2, T_3 and T_4. After 90 days of ripening "2-heptanal" present in all treatments T_1, T_2, T_3 and T_4 except the control T_0 while "ethanol" was found in T_0, T_1 and T_2 after 45 days of ripening. "isobutanol" was found in all treatments after 90 days of ripening.

Esters and sulphur compound. Esters are formed via the esterification of alcohols and free fatty acids. These precursors are present in cheese at various concentrations [38]. At low concentrations, the esters contribute to cheese flavor, but high concentrations may cause fruity flavor defects in Cheddar cheese [21].

De Figueroa et al (2000) [10] demonstrated that in fact several strains of *Lb. rhamnosus* and *Lb. plantarum* species were capable of using citrate as a sole energy source when grown in complex media. This was confirmed in the study by Jyoti, *et al*. (2003) [17], which showed that *Lb. rhamnosus* could grow on citrate as the only carbon source producing lactate, acetoin, acetate and diacetyl, which contribute to cheese flavour.

The volatile fraction of cheese has several sulphur-containing compounds such as methanethiol, methional, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, dimethyl tetrasulfide, carbonyl sulfide, and hydrogen sulfide [37, 41], and they contribute to the aroma of cheese [26].

The ethyl esters produced in Cheddar cheese are given in Table 5. Only three esters; Ethyl acetate and Ethyl butanate were found in cheese sample. Both Ethyl acetateand and Ethyl butanate were not found in all cheese samples after 45 days ripening. However, Ethyl acetateand was found in both T_1 and T_2 while Ethyl butanate present only in T_2 after 90 days of ripening.

The sulphur compounds identified in Cheddar cheese are listed in Table 5. After 45 days of ripening dimethyl disulphide only present in T_2 while dimethyl sulphide and methanethial were not found in all treatments. Dimethyl sulphide and methanethial were found in both T_1 and T_2 while not found in T_0 after 90 days of ripening.

4. Conclusion

Sensory evaluation of Cheddar cheese revealed that ripening had highly significant effect on all sensory attributes. Treatments had highly significant effect on aroma and flavour. Among and flavour treatments, highest scores were awarded to T_2 after 90 days of ripening and minimum changes were noted in T_0 during the whole ripening period.

On the basis of cheese evaluation, it was found that treatment T_2 was evaluated to be the best during storage. In summary, *Lactococcus lactis* ssp. *cremoris* and *Lactococcus lactis* ssp. l*actis* (95:5) @ 1.5% + *Lb. Rhamnosus* @ 0.5% combination seems to be the best and can be suggested as an ideal combination for Cheddar cheese production.

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