

## Improvements in the Flavour of Soy Cheese

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### Summary

A review of biochemical and technological similarities and dissimilarities between soy cheese and Cheddar cheese is presented to provide guidelines for the improvements in the flavour of soy cheese. Processing technology as well as the final product of soy cheese have many similarities with Cheddar in terms of appearance, texture, mouth feel, chemical nature, biochemical processes, *etc.* Soy protein has many useful amino acids like Asp, Ile, Leu, Met, Phe, Trp, Tyr, Val, *etc.*, which are precursors of flavouring compounds and the right choice of microbial cultures is necessary to benefit from them. Using low levels of sodium chloride, without the use of ethanol, and introducing new milk cheese starter and non-starter cultures like *Lactococcus lactis* ssp. *lactis* (formerly *L. lactis* ssp. *lactis* biovar. *diacetylactis*), *Lactobacillus helveticus*, *Lactobacillus casei*, *Streptococcus lactis* var. *maltigenes* and *Lactococcus lactis* ssp. *cremoris* that enhance flavour will be helpful to improve the flavour of soy cheese.

*Key words:* soy cheese, starter cultures, flavour, flavouring compounds, lactic acid bacteria

### Introduction

Soy cheese, also known as sufu, furu or fermented soybean curd, is a traditionally important and popular fermented food product in China. The historical evidence confirms that the making of sufu started centuries ago in China (1) and the processing conditions differ a little in various localities of China. Originally sufu was a product of fungal fermentation but bacterial fermentation has also been carried out successfully (2–4). Soybean, the basic raw material for the product, has great nutritional (source of proteins, minerals, *etc.*) and therapeutic values (*e.g.* prevention of chronic diseases such as menopausal disorder, cancer, atherosclerosis and osteoporosis), and it is also beneficial in products like soy milk and

soy cheese (5–8). Popular and commonly available types of soy cheese in China are red, white and gray sufu. NaCl and ethanol are the basic components of sufu, which provide the traditional flavour and the product safety against pathogenic microorganisms (1).

The physical appearance, texture, composition and the basic processing technology of soy cheese are quite similar to the milk cheese in many aspects. Unfortunately, unlike milk cheese, comprehensive scientific studies of soy cheese leading to further developments and improvements were not carried out in the past. This study aims to point out the chemically and microbiologically active constituents of soy cheese and propose some changes in the process to improve the flavour. Soy cheese is compared with Cheddar cheese for further studies

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and developments, so that soy cheese can compete with milk cheese in various organoleptic, microbiological, biochemical and technological aspects. Flavour of cheese is a complex of many amino acids, fatty acids, esters and volatile compounds, *etc.* but few of them are more important and responsible for the characteristic flavour of the cheese. Considerable studies (details of which will follow in this paper) have been carried out to determine the major flavour contributors in milk cheese and results showed that certain starter and non-starter cultures have the ability to produce the desirable flavour. We are emphasizing more on Cheddar cheese because it is a famous cheese with huge productions worldwide and sufficient literature about it is available. The aim of this work is to identify the important flavour-producing compounds of Cheddar that are also present in soy cheese, and to find the ways for better utilization of these compounds in order to improve the soy cheese flavour.

### Comparison of the Processing Technologies of Two Kinds of Cheese

Nutritionally, soybean milk, tofu and soy cheese serve the same role for the people of Asia as cow's milk and cheese do for the Western people. Asians like the salt-coagulated soybean curd, not only because of the texture, but also as an important source of calcium and protein (5,6). Comparison of important physicochemical and nutritional aspects of soy cheeses and hard cheeses is given in Table 1. Several types of soy cheese can be distinguished based on the processing method or colour and flavour. The choice of processing method can result in mould fermented, naturally fermented, bacterially fermented, or enzymatically ripened soy cheese, while the choice of dressing mixture classifies it as red, white and gray sufu. Traditional soy cheese is made by solid-state fermentation of tofu (soybean curd) with fungal starters including *Actinomucor*, *Mucor* and *Rhizopus* species (1), while some other moulds, bacteria and yeasts also take part during ripening in brine containing salt and alcohol.

Table 1. Physicochemical characteristics of commercial soy cheese and Cheddar cheese

Component	Soy cheese	Cheddar cheese
Moisture/g	58–70	36.7
Crude protein/g	12–17	24.9
Crude lipid/g	8–12	33.1
Carbohydrate/g	6–12	1.3
Ash/g	4–9	3.9
Calcium/mg	100–230	72
Sodium chloride/g	10–16	1.8
Phosphorus/mg	150–300	51
Iron/mg	7–16	–
pH	5.2–7.3	5.5

Data expressed per 100 g of cheese

### Coagulation

Coagulation is a process to remove water and carbohydrates from milk in order to make a cake-like structure, which can provide a suitable medium for fermentation. This fermentation is responsible for the production of a product with good flavour, texture and mouth feel that meets the food safety standards. Like with milk cheese, coagulation of soy milk (*i.e.* water-extracted autoclaved soybean) is also the key step in soy cheese making. Milk has a complex protein called casein, while rennet contains enzyme chymosin, which is commonly used for coagulation in commercial production. Microbial, acid and heat coagulation methods are also in practice. Soybean protein molecules are relatively simple and can be easily coagulated by acids and salts like calcium sulphate, magnesium sulphate (1) or glucono-delta-lactone (GDL). GDL has good coagulation ability and also contributes towards the formation of silky and good-flavoured curd (9). Some bacterial cultures like lactic acid bacteria (LAB) and bifidobacteria (2–4,10–14) have also shown good results in coagulation of soy milk, and the resultant products have better acceptability.

### Sodium chloride and ethanol

In soy cheese, high concentrations of NaCl (about 14 %) and ethanol (about 10 %) are used in the commercial and traditional recipes in China and some people do not like it due to its very strong salty taste. High levels of NaCl and ethanol ensure product safety from pathogenic organisms (15), but on the other hand, both of them inhibit the enzymatic ripening process and cause a prolonged ripening time. Sodium is directly related to the blood pressure and its high intakes result in high blood pressure. According to the epidemiological surveys hypertension has become a major health problem not only in industrialized nations but also in developing countries like China and Pakistan (16). Thus, the use of low concentrations of NaCl in soy cheese must be promoted. A modified process to avoid the use of ethanol and reduce the NaCl level has been used earlier (4) but it results in the loss of good flavour because the flavour impact of fungal fermentation in traditional recipe was not substituted. In this review, we want to describe the suitable LAB cultures that could substitute this deficiency. A comparison between traditional and modified process of soy cheese and Cheddar cheese making is given in Fig. 1.

During cheese making, commercial lactic cultures are stimulated by low levels of NaCl, but are strongly inhibited at more than 2.5 % NaCl. *L. lactis* ssp. *cremoris* is more salt-sensitive than *L. lactis* ssp. *lactis* in the curd, while non-starter lactic acid bacteria (NSLAB) are less sensitive than these two (17). Another study shows that pH decreases after salting due to the enhanced action of starters at S/M (salt to moisture) levels lower than 5 %, but starter activity decreases abruptly and the pH remains high at higher values of S/M. The quality grade of the cheese also decreases sharply at S/M levels higher than 5 % (18).

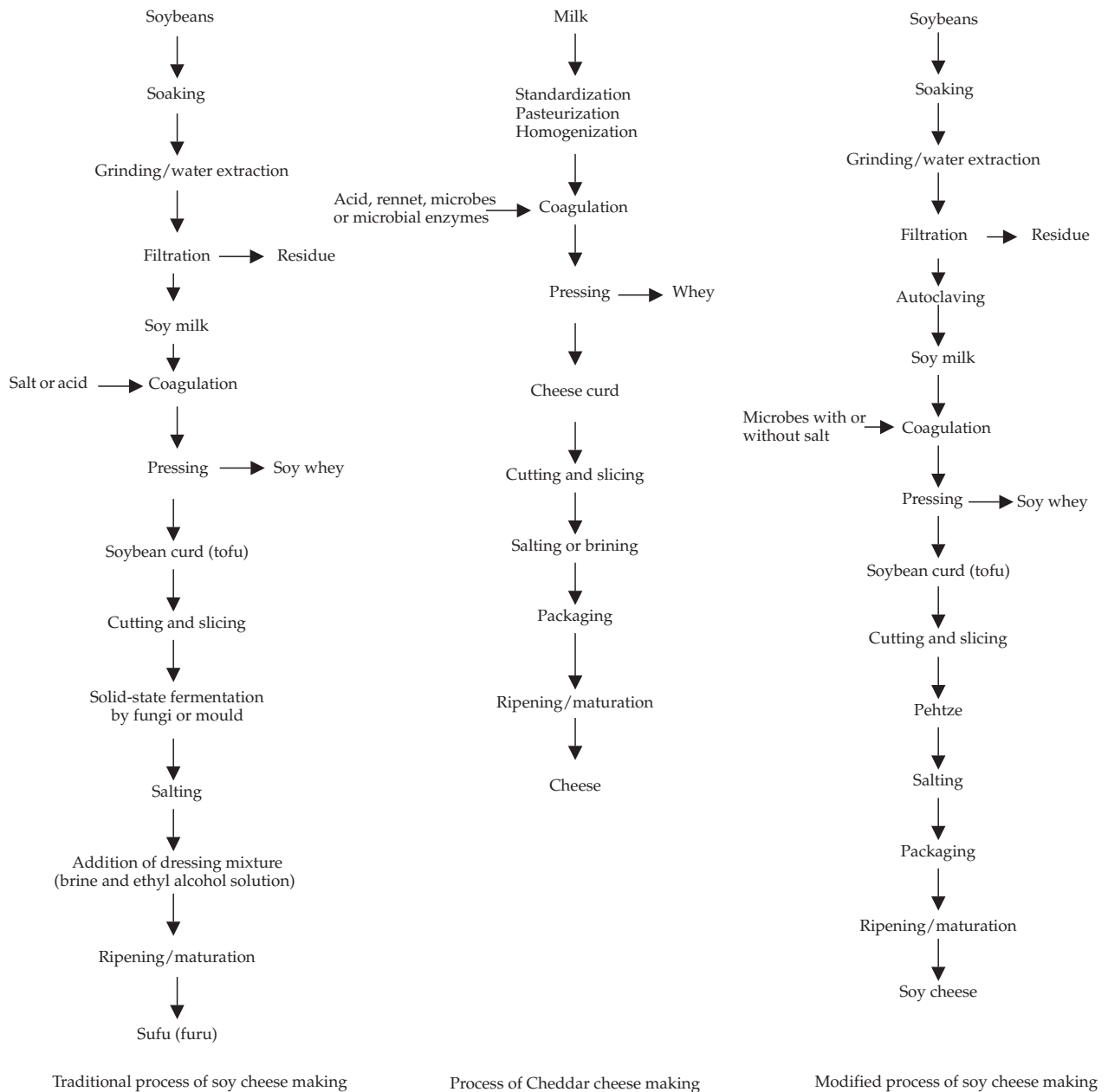


Fig. 1. Comparison of the soy cheese and milk cheese processing technology

### Comparison of the Flavour Ingredients of the Two Kinds of Cheese

Cheese flavour is a combined effect of a large number of chemical compounds that are produced during ripening of the curd. But the presence of the compounds that are precursors of these flavouring compounds and suitable medium conditions are compulsory. Comparing the compounds present in both kinds of cheese, it can be seen that both have a large number of compounds. These compounds are similar in composition or have similar properties. The major difference between them is due to the use of different cultures or different processing technology. A detailed comparison between the constituents of soy cheese (19,20) and Cheddar cheese (21,22) related to flavour is given in Table 2.

### Amino acids and their conversion products

Proteins play an important role in cheese manufacturing. They not only give it shape and texture by coagulating but also contribute to the development of flavour by producing amino acids. For the development of an acceptable Cheddar cheese flavour, a well-balanced breakdown of the curd protein into small peptides and amino acids is necessary (23,24). These products of proteolysis themselves are known to contribute towards flavour or act as precursors of other flavour components during the formation of cheese flavour (25–29). Recent studies show that LAB cultures use amino acids for their metabolic activities and as a result of these activities, flavouring compounds are produced during cheese ripening. A list of flavouring amino acids and their metabolic

Table 2. Important biochemical constituents of Cheddar cheese and soy cheese related to flavour

Chemical compounds	Soy cheese	Cheddar cheese
Free amino acids	Asp, Thr, Ser, Glu, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, Lys, His, Arg, Pro, Trp, Cys	Asp, Thr, Ser, Asn, Glu, Gln, Pro, Gly, Ala, Citr, Val, Cys, Met, Ile, Leu, Tyr, Phe, GABA, His, Trp, Orn, Lys, Arg
Free fatty acids	C16:0, C18:0, C18:1, C18:2, C18:3	C2:0, C3:0, I-C4:0, N-C4:0, N-C5:0, C6:0, C12:0, C14:0, C16:0, C16:1c9, C18:0, C18:1, C18:2
Aldehydes	<i>n</i> -hexanal, benzaldehyde, benzeneacetaldehyde, (E)- and (Z)-2-phenyl-2-butenal, 4-methoxybenzaldehyde, cinnamaldehyde, 5-methyl-2-phenyl-2-hexanal	hexanal, heptanal, nonanal, tetradecanal, hexadecanal, furancarboxaldehyde, benzaldehyde, phenylacetaldehyde
Esters	ethyl-2-methyl propanoate, ethyl butanoate, ethyl-2-methyl butanoate, ethyl-3-methyl butanoate, isoamyl acetate, ethyl pentanoate, ethyl-2-butanoate, ethyl hexanoate, ethyl heptanoate, ethyl lactate, ethyl octanoate, ethyl-3-hydroxybutanoate, ethyl benzoate, diethyl succinate, ethylphenyl acetate, ethyl-3-phenylpropionate, ethyl myristate, ethyl cinnamate, ethyl palmitate, ethyl oleate, ethyl linoleate, ethyl linolenate	ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, ethyl tetradecanoate, ethyl lactate
Ketones	2,3-butanedione, 2,3-pentanedione, 3-pentane-2-one, 2-heptanone, 3-octanone, 3-hydroxy-2-butanone, 1-hydroxy-2-propanone, 2-nonanone, 3-hydroxy-2-methyl-4-pyrone	3-hydroxy-2-butanone, 2-hexanone, 2-heptanone, 2-octanone, 2-nonanone, 8-nonen-2-one, 2-decanone, 2-undecanone, 2-dodecanone, 2-tridecanone, 2-pentadecanone
Alcohols	ethanol, 2-butanol, 2-methyl-1-propanol, 2-pentanol, 1-butanol, 1-penten-3-ol, 3-methyl-1-butanol, 1-pentanol, 2-heptanol, 3-ethoxy-1-propanol, 2-butoxyethanol, 1-octane-3-ol, 2-ethyl-1-hexanol, 2,3-butanediol, 2-methoxyphenol, benzenemethanol, phenol, benzeneethanol, 4-ethyl-2-methoxyphenol, 4-methylphenol, 4-ethylphenol, 2-methoxy-4-vinylphenol, 3-phenyl-2-propenol	2-methylpropan-1-ol, 1-butanol, 3-methylbutan-1-ol, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol, 1-nonanol, 2-pentanol, furan methanol, phenethyl alcohol
Lactones	not reported so far	$\delta$ -octanolactone, $\delta$ -decanolactone, $\delta$ -dodecanolactone, $\delta$ -tetradecanolactone, $\delta$ -hexadecanolactone, $\gamma$ -decanolactone, $\gamma$ -dodecanolactone, $\gamma$ -dodecenolactone
Sulphur compounds	3-(methylthio)propanal, ethyl-3-(methylthio)propanoate, 3-(methylthio)propanol	dimethyldisulphide, dimethyltrisulphide, methional

products by LAB cultures (30) is given in Table 3. A lot of studies have been carried out in milk cheeses but only the studies useful for the soy cheese making will be discussed here.

Gummalla and Broadbent (31,32) studied the catabolism of Phe, Tyr and Trp under artificially created cheese ripening conditions by *Lactobacillus helveticus* and *Lactobacillus casei*, which are widely used as starters or flavour adjuncts. Under near Cheddar cheese ripening conditions (pH=5.2, 4 % NaCl, 15 °C, no sugar), Phe, Tyr and Trp transamination and dehydrogenation pathways were active in both species and these reactions were found to be reversible. Major products of Phe catabolism were phenyllactic acid, phenylacetic acid (flowery, rosy) and benzoic acid, while Tyr degradation resulted in the formation of *p*-hydroxyphenyllactic acid and *p*-hydroxyphenylacetic acid (32). The authors also showed that some of these products were likely to be formed by

non-enzymatic processes, since spontaneous chemical degradation of Tyr intermediate *p*-hydroxyphenylpyruvic acid produced *p*-hydroxyphenylacetic acid, *p*-hydroxypropionic acid and *p*-hydroxybenzaldehyde, while chemical degradation of Phe intermediate phenylpyruvic acid resulted in the production of phenylacetic acid, benzoic acid, phenylethanol, phenylpropionic acid and benzaldehyde (almond). Trp degradation by both *Lactobacilli* was assessed under carbohydrate starvation (pH=6.5, 30/37 °C, no sugar) and under near Cheddar cheese ripening conditions (31). Cell-free extract of both species of *Lactobacilli* catabolized Trp to indole-3-lactic acid. Intact cells of *Lactobacillus casei* metabolized Trp under both conditions and the reaction was also found to be reversible. In contrast, Trp catabolism by strains of *Lactobacillus helveticus* showed different behaviour: (i) it was detected under near cheese-ripening conditions, (ii) cells of *Lactobacillus helveticus* did not catabolize Trp under both conditions, but converted indole-3-pyruvic acid to Trp in

Table 3. Flavouring compounds produced through anaerobic catabolism of amino acids by LAB\*

Amino acids	$\alpha$ -Keto acids	Aldehydes	Alcohols	Carboxylic acids	Others
Leu	$\alpha$ -keto-isocaproate	3-methylbutanal	3-methylbutanol	3-methylbutanoic acid	–
Ile	$\alpha$ -keto- $\beta$ -methylvalerate	2-methylbutanal	2-methylbutanol	2-methylbutanoic acid	–
Val	$\alpha$ -keto-isovalerate	2-methylpropanal	2-methylpropanol	2-methylpropanoic acid	–
Phe	phenylpyruvate	phenylacetaldehyde	phenylethanol	phenylacetic acid	–
Tyr	<i>p</i> -OH-phenylpyruvate	<i>p</i> -OH-phenylacetaldehyde	<i>p</i> -OH-phenylethanol	<i>p</i> -OH-phenylacetic acid	<i>p</i> -cresol
Trp	indolepyruvate	indole-3-acetaldehyde	tryptophol	indol-3-acetic acid	skatole
Met	$\alpha$ -keto-butyrate	3-methylthiopropional	3-methylthiopropional	3-methylthiopropionic acid	methanethiol
Asp	oxaloacetate	–	–	malate	diacetyl, acetoin

\*Courtesy of Y. Ardö (30)

carbohydrate starvation medium and Trp to indole-3-lactic acid under near cheese-ripening conditions. The Cheddar cheese starter *Lactococcus lactis* initiated Trp catabolism *via* transaminase under some of the conditions found in cheese, but did not convert indole-3-pyruvic acid to indole-3-lactic acid (33). Instead, indole-3-pyruvic acid formed by the starter culture underwent enzymatic or spontaneous degradation to indole-3-aldehyde and indole-3-acetic acid. These secondary reactions may be important because some *Lactobacilli* can convert indole-3-acetic acid to skatole, which is responsible for unclean flavour of cheese (34).

The volatile cheese fraction has several sulphur-containing compounds such as methanethiol (cabbage), methional, dimethylsulphide, dimethyldisulphide (onion), dimethyltrisulphide (garlic), dimethyltetrasulphide (cabbage), carbonyl sulphide and hydrogen sulphide (35–37), which contribute to the aroma of cheese (38). Methanethiol has been associated with desirable Cheddar-type sulphur based flavour notes in good quality Cheddar cheese (39–41). However, alone or in excess, methanethiol does not produce typical Cheddar cheese flavour (37). Two enzymatic pathways potentially leading to the formation of methanethiol from Met have been postulated to exist in lactococci. A pathway for Met catabolism *via*  $\alpha$ - and  $\gamma$ -elimination was proposed by Alting *et al.* (42). According to this pathway, a lyase catalyzes deamination and demethylthiolation of Met simultaneously, resulting in the formation of methanethiol and  $\alpha$ -keto-butyric acid. Cystathionine  $\beta$ -lyase and cystathionine  $\gamma$ -lyase have also been purified from *Lactococcus lactis* and characterized (42,43); however, both of these enzymes have relatively low activities on Met. The other potential pathway is initiated by transamination of Met to 4-methylthio-2-oxobutyric acid (KMBA). The characterized aromatic aminotransferases from lactococci exhibit substantial activity with Met (44). Intact cells and autolyzed cells are capable of producing methanethiol, but they utilize different pathways. KMBA is converted to methanethiol in the whole cells. The release of aminotransferases from lactococci by autolysis could result in the accumulation of KMBA from Met. The KMBA may be decomposed to form methanethiol directly or enzymatically converted by the whole cells (45). Lactococcal cell autolysis plays a role in the flavour development in Cheddar cheese, and the balance of autolyzed and intact

cells is believed to be important for the desired cheese-ripening events (46,47).

Transamination is the first step in the degradation of amino acids by *Lactococcus* (32), leading to the formation of  $\alpha$ -keto acids ( $\alpha$ -KA). Aromatic aminotransferase enzymes have previously been characterized from *Lactococcus lactis* ssp. *cremoris* (44,48) and *Lactococcus lactis* ssp. *lactis* (45). These enzymes initiate the degradation of Val, Leu, Ile, Phe, Tyr, Trp, and Met; all of these amino acids are known as precursors of cheese flavouring compounds. Inactivation of aminotransferase enzymes involved in the breakdown of amino acids by *Lactococcus* reduces aroma formation during cheese ripening (49).  $\gamma$ -Keto acids correspond to almost every amino acid in Cheddar cheese; while  $\gamma$ -keto-3-methylbutyric acid and  $\gamma$ -keto-3-methylvaleric acid (50,51) have an intense cheese-like odour. Phenylpyruvic acid formed from Phe by transamination is further degraded to the flavouring compounds *i.e.* phenyllactate and phenylacetate by lactococcal cells *in vitro* (44). This degradation of phenylpyruvic acid to phenyllactate, phenylacetate, and also to benzaldehyde in semihard cheese was also confirmed by Yvon *et al.* (52).

Cheddar cheeses with unclean-type flavour described as subtle floral or rose-like aftertastes were aged for the longest period of time. This flavour note is attributed to compounds like phenylethanol and phenylacetaldehyde, which originate from the hydrophobic amino acid Phe, through enzyme-mediated transamination, decarboxylation, and reduction reactions. The addition of phenylacetaldehyde (50 to 500 ppb) to clean-flavoured mild Cheddar cheese imparted distinct intensities of unclean or rosy taint (53).

A higher dissolution ratio of protein and higher content of peptides and amino acids were observed in sufu aged with brine and ethanol, while high contents of Glu and Leu were present among the free amino acids (54). On the other hand, alcohol retards the degradation of soybean proteins and in order to accelerate the ripening of sufu, stem bromelain was used as a coagulant of soy milk and a hydrolytic enzyme (55). Yang *et al.* (56) studied the debittering properties of microorganisms in a Chinese white sufu and suggested that peptidase of *Mucor* and bacteria is valuable in reducing the bitter peptide of soybean proteins.

### Fatty acids and their catabolic products

Cheese is a high-fat food; fresh Cheddar cheese contains 30 % or more fat (wet mass) (57). The fat fraction of cheese is important for the development of typical flavour and texture. It is well known that a higher fat content leads to a less firm and more elastic body, while low-fat products tend to be harder, more crumbly, and less smooth than the characteristic cheese texture (58). Flavouring compounds like thiols, hydrogen sulphide, esters, aldehydes, alcohols and ketones are present in the lipid phase of ripened cheese (59). In low-fat products, there is an increased cross-linking within the curd, which is also carried into the cheese. Increasing the moisture content as an attempt to overcome these defects leads to weak body and encourages undesirable flora as well as atypical flavour. Cheddar cheese made from non-fat milk does not develop full aroma, even after 12 months (60), but substituting vegetable or even mineral oil for milk fat seems to favour a certain aroma development in Cheddar (61). It is suggested that the fatty acid composition and natural emulsion of milk fat are important for flavour development. However, in recent years there has been an increased interest in low-fat cheeses due to consumer trends and health issues (62). Therefore, soy cheese with a good flavour can be a good substitute.

Like all types of food with a high fat content, lipolytic (enzymatic hydrolysis by lipases and esterases) and oxidative (chemical) changes are likely to occur in cheese. Triglycerides constitute more than 98 % of cheese fat and their hydrolysis is the principal biochemical transformation during ripening. This process leads to the production of free fatty acids (FFA), monoglycerides, diglycerides and possibly glycerol. FFA contribute to the aroma of cheese and individual FFA, particularly between C4:0 and C12:0, have specific flavour (rancid, sharp, goatly, soapy, coconut-like). The flavour intensity of FFA not only depends on the concentration, but also on the presence of certain cations (*i.e.* Na<sup>+</sup> or Ca<sup>2+</sup>) and protein degradation products (63–66) in the medium. The above-mentioned primary reactions are mainly responsible for the basic textural and flavour changes in cheese curd during ripening. However, the secondary transformations are mainly responsible for the finer aspects of cheese flavour and they also modify cheese texture.

The pH of cheese has a major influence on the flavour impact of FFA. A considerable portion of FFA is present as nonvolatile salts at the pH of Cheddar (pH≈5.2), which reduces their flavour impact. In most cheese varieties, relatively little lipolysis occurs during ripening and too much is considered undesirable because most consumers would consider Cheddar, Dutch and Swiss-type cheeses rancid even with moderate levels of free fatty acids. However, in the cheeses such as Italian cheese (Romano, Provolone), Blue, and Feta, extensive lipolysis is desirable as part of overall flavour development (63). Cheddar cheeses of different flavour intensity showed only small differences among the concentrations of individual FFA (67). The lipolytic activity of LAB produces low levels of FFA that can contribute to the background flavour of Cheddar cheese (59).

Soybean lipids in sufu are also hydrolyzed to some extent into fatty acids. The added alcohol reacts with the fatty acids chemically or enzymatically to form esters, providing the pleasant odour of the product (54). It is assumed that soy cheese, being a low-fat cheese, can also use the cultures of low-fat milk cheeses, which were found to be helpful in enhancing the flavour and overall acceptability.

### Other flavouring compounds

Aliphatic and aromatic esters play an important role in the flavour and, sometimes, the off-flavour of Cheddar cheese. This synthesis mainly concerns the above-mentioned short or medium chain fatty acids and the alcohols, which may be aliphatic (ethanol), aromatic (phenylethanol), or thiols (methanethiol). Esters can be produced either enzymatically by LAB or from a purely chemical reaction (68,69). Amides have been identified in cheeses (70) like Cheddar, Emmental and Manchego, but no mechanism has been proposed for their formation.

Chou and Hwan (54) found higher amounts of esters present in sufu aged with ethanol than in that without it. Chung (71) detected 111 volatile compounds present in 3 different samples of fermented soybean curd and 63 of them were common in all the samples. Major classes observed were alcohols (32 in number) and esters (25 in number). It was concluded that the quantity of detected ethanol might produce a large number of ethyl esters that contribute to the floral and fruity notes of the final product.

Lactones are cyclic esters resulting from the intramolecular esterification of hydroxy acids through the loss of water to form a ring structure. Lactones possess a strong aroma, which is not specifically cheese-like but may be important in the overall cheese flavour impact. The accepted mechanism of the formation of lactones in cheese presumes the release of hydroxy fatty acids, which are normal constituents of milk fat, followed by lactonization. Important flavour constituents  $\gamma$ - and  $\delta$ -lactones have been identified in cheeses, particularly in Cheddar (72).

Alkanals, alkenals, and dienals were found in the volatile components of 33 % reduced-fat Cheddar cheese containing deoiled granular soy lecithin and calcium. Among these, (E,E)-2,4-nonadienal (fatty/sweet), (E,Z)-2,4-decadienal (corn chip/stale/hay), (E,E)-2,4-decadienal (fatty/fried), and (E)-2-nonenal (stale/bitter) were detected at high odour intensities. Based on the quantitative data, (E,Z)-2,4-decadienal and (E,E)-2,4-decadienal were the main compounds contributing to the off-flavours in reduced-fat Cheddar cheeses when soy lecithin was added (73).

Production of 2/3-methylbutanal by LAB in milk causes malty flavour, which was considered a defect (74–77). Addition of 0.34 ppm of 3-methylbutanal to good quality homogenized milk was enough to develop a malty defect (73,74). Cheeses made with co-encapsulated cell-free extracts of *Gluconobacter oxydans*, which produces acetic acid from ethanol, and *Streptococcus lactis* ssp. *maltigenes*, which converts Leu to 3-methylbutanal (dark chocolate) and 3-methyl-1-butanol (fruity/al-

cohol) in a milk-fat coat, exhibited a stronger malty flavour than the cheese made with broken capsule or incomplete cell-free extract mixture (78). Recently, high intensities of nutty flavours have been found associated with concentrations of 2/3-methylbutanal and 2-methylpropanal in aged Cheddar cheeses (79). The addition of 2/3-methylbutanal and/or 2-methylpropanal to mild or aged Cheddar cheese resulted in the sensory perception of the nutty flavours in these cheeses.

Chung (19) identified 89 flavouring compounds in red fermented soybean curd (FSBC or sufu) and reported that ethyl-2-methylpropanoate, 2,3-butanedione, ethylbutanoate, ethyl-2-methylbutanoate, 3-(methylthio)propanal (cooked potato), benzeneacetaldehyde and ethyl-3-phenylpropionate were found to be the most important components contributing to the characteristic aroma of red fermented soybean curd.

### Sugars and their conversion products

The major sugars present in soybean are sucrose, raffinose and stachyose. The latter two are not digested by human beings and may cause flatulence (80). Soy milk sugars are fermentable by the most of LAB, bifidobacteria, fungi and yeasts (1,81). *Lactobacillus acidophilus* and *Streptococcus thermophilus* are capable of metabolizing stachyose and raffinose, causing the decrease of sucrose and pH, and increase of fructose, glucose and galactose in soy milk. These activities were enhanced when bifidobacteria were used along with the latter two LAB cultures (13). Some other studies have also reported similar results (82–85).

Lactose is the major sugar present in the milk of animals but its role in cheese making is limited and can be substituted. About 95–98 % of milk lactose is lost during curd making as a part of cheese whey and a very small amount is used by LAB to generate glucose, which is necessary as an energy source for the growth of flavour producing microorganisms. Galactose produced during this process has a negative effect on the cheese colour and can sometimes cause a brownish shade in cheese. Even then, 0.8–1.5 % of lactose was found in the final product of Cheddar cheese (86). During cheese manufacturing, mesophilic starter bacteria ferment lactose to lactic acid. In the case of Cheddar-type cheeses, most of the lactic acid is produced in the vat before salting and moulding. Some bacterial cultures like LAB and bifidobacteria have the ability to produce acid in soy milk (2–4,10–14), thus the role of lactose can be substituted in the soy milk for cheese production.

### Comparison of the Microbiology of the Two Kinds of Cheese

Microorganisms are one of the essential components of all natural cheese varieties and play an important role during the cheese manufacturing as well as the development of flavour. They can be divided into two main groups: starter and non-starter (secondary flora) cultures. The starter flora is responsible for acid development during the cheese production, which results in coagulation. They can work either individually or in various combinations depending on the cheese variety. The non-

-starter flora is composed of complex mixtures of bacteria, yeasts and moulds. They are specifically associated with the particular flavour and specific characteristics of that variety (87). The flavour and texture development of cheese is largely controlled by complicated biochemical reactions, principally by the activities of starter cultures and their enzymes. These reactions include the degradation of proteins, carbohydrates and fats in the curd (88). A lot of studies have been carried out in Cheddar and other milk cheeses but in the case of soy cheese, comprehensive studies have not been reported yet. The following studies have the potential to help the flavour improvements in soy cheese.

Flavour of the reduced-fat Cheddar cheese was enhanced to non-bitter, mildly acidic and buttery by introducing an integrated starter culture system. This system consisted of *Lactococcus lactis* ssp. *cremoris* SK11, *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* JVI and *Lactobacillus casei* 7A. *Lactococcus lactis* ssp. *cremoris* SK11 showed substantially lower intensity of bitterness than the commercial starters, while *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* JVI significantly increased diacetylacetoin and acetate concentrations resulting in buttery flavour (89). In another study, *Lactococcus lactis* ssp. *cremoris* SK11 showed the ability to cause sufficient free amino acid release during the cheese making process (90). *Lactococcus lactis* ssp. *lactis* (formerly *L. lactis* ssp. *lactis* biovar. *diacetylactis*) has the ability to produce  $\gamma$ -aminobutyric acid in skim milk due to its glutamate decarboxylase activity. Optimum production of  $\gamma$ -aminobutyric acid was obtained at pH=4.7, but the production was not available above pH=5.0 (91).

*Lactobacillus helveticus* shows good proteolytic system in cheese and extensive lysis results in a large increase of flavour precursors and some volatile compounds (92). *Lactobacillus helveticus* adjunct exhibited the highest rates of free amino group formation, while the cheese treated with freeze-shocked *Lactobacillus casei* showed the highest rate of free fatty acid formation in Cheddar cheese (93). *Lactobacillus casei* is a well known probiotic culture (94–96) with high peptidase activity (97). It has a broad system of enzymes responsible for its hydrolyzing activity towards a number of peptides, including bitter and methionine-containing peptides. Moreover, *Lactobacillus casei* IFPL731 shows methionine aminotransferase activity that leads to the production of typical cheese aroma (98).

Yang *et al.* (56) also reported that Bacterium JS3 and Yeast JSBB2 were helpful in reducing the bitter amino acids in Chinese white sufu. The successful growth of bifidobacteria and LAB (2–4,10–14) in soy milk and soybean curd indicates that these cultures can be used successfully for soy cheese production.

### Conclusions

Soy cheese making has close resemblance to that of milk cheese production in terms of biochemistry, microbiology and processing technology. Even the final products have great similarities in appearance, texture and chemical constituents. Nutritionally both products have the same quality because they are rich in protein with

pretty good amounts of fat and minerals, while deficient in carbohydrates. Biochemistry of both kinds of cheese shows quite similar compounds including proteins, lipids, carbohydrates, esters, alcohols, aldehydes and minerals. The list of volatile flavour compounds identified in Cheddar cheese and soy cheese is quite extensive and includes a wide range of compounds; mainly acids, alcohols, esters, aldehydes, ketones and sulphur-containing compounds. The physicochemical changes caused by processing of soy cheese are quite similar to those of milk cheese, *e.g.* proteolysis, *i.e.* formation of amino acids from larger protein molecules, lipolysis, *i.e.* conversion of fats into smaller units called fatty acids, and production of aldehydes, alcohols, esters, *etc.* The processing steps like coagulation, pressing, cutting, salting, ripening, *etc.* are almost similar, with some exceptions due to the use of different kind of cultures.

The main difference between animal milk and soy milk is that soy milk does not contain lactose and casein, but this does not affect cheese making because soy sugars can serve the role of lactose, while soy milk proteins have the same role like milk proteins. Soy curd also has useful amino acids like Asp, Ile, Leu, Met, Phe, Trp, Tyr, Val, *etc.*, which are required for LAB cultures to produce flavouring compounds.

Fat percentage is an important point to be focused on because Cheddar cheese has almost 30 % fat, while the soy cheese contains 8–12 %. Fat contributes to flavour in three ways: (i) it serves as a reservoir for flavour compounds by dissolving them and avoiding their loss during processing, (ii) it produces flavouring free fatty acids during lipolysis reactions, (iii) it masks the bad flavour of cheese by dissolving off-flavour and bitter flavour compounds. However, selection of suitable cultures will be helpful to overcome this problem in soy cheese.

In order to decrease the dietary intake of sodium and shorten the sufu maturation time, low-salt sufu products could be a healthy option. At the same time, this will also provide suitable conditions for the LAB and other cheese cultures to grow well and result in improved flavour of the final product. As NaCl and ethanol provide inhibition of the growth of undesirable microflora in traditional Chinese recipe for soy cheese, the cultures with probiotic activities like *Lactobacillus rhamnosus* 6013 or other LAB cultures with the ability of doing the same by producing acid in the medium will be useful. Removal of ethanol from the recipe will not only allow the growth of desirable starter and non-starter cultures for flavour improvements, but it will also decrease the ripening time of the soy cheese.

Due to the key role of starter and non-starter cultures in flavour development of cheese, considerable number of cultures and many of their combinations have been studied in milk cheeses and these studies can also be effective in soy cheese. Soy cheese as a medium has all the necessary conditions to facilitate LAB and other flavouring microorganisms to produce good cheese flavour and reduce bitter flavour. Thus, introducing new cultures and new combinations of cultures will be helpful for flavour improvements in soy cheese. The review of literature indicates that flavour-enhancing cultures like *Lactococcus lactis* ssp. *lactis* (formerly *L. lactis* ssp. *lactis* biovar. *diacetylactis*), *Lactobacillus helveticus*, *Lactobacillus*

*casei*, *Streptococcus lactis* ssp. *multigenes* and *Lactococcus lactis* ssp. *cremoris* have the potential to improve soy cheese flavour.

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