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Original Article

Amino acid profiles of sufu, a Chinese fermented soybean food

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Abstract

Sufu is a Chinese soybean cheese-like product obtained by solid-state fungal fermentation and ripening of tofu. The resulting "pehtze" is salted, followed by maturation in brine. Total (TAA) and free amino acid (FAA) profiles were determined during consecutive stages of sufu manufacture, i.e., tofu, pehzte (fungal fermented tofu), salted pehtze and in white, red and grey sufu, ripening in dressing mixtures of different salt content (8%, 11%, and 14% w/w). TAA in tofu, pehtze and salted pehtze totalled 547, 551 and 351 mg/g dry matter, respectively. FAA increased from total 1.3 to 15.6 mg/g dm in pehtze after fermentation of tofu by *Actinomucor elegans* and to 11.9 mg/g dm in salted pehtze. During ripening up to 80 days, total FAA in red sufu increased from 28 to 88 mg/g (8% salt), 28–63 mg/g (11% salt) or 26–42 mg/g (14% salt). In white sufu the levels of FAA were generally higher and the effect of salt was less inhibitory. Levels of FAA in white sufu increased in 80 days from 33 to 104 mg/g (8% salt), 27–92 mg/g (11% salt) and 19–73 mg/g (14% salt). The pattern of essential amino acids compared favourably with those of eggs and cow's milk. While FAA increased during ripening of red and white sufu, the ratio of each amino acid remained essentially constant, and glutamic acid, leucine, aspartic acid, alanine, phenylalanine and lysine were found in large quantities. However, in grey sufu the ratio was different, with large proportions of leucine, alanine, isoleucine, valine and phenylalanine found after ripening.

Keywords: Sufu; Ripening; Amino acid; Fermented soybean food

1. Introduction

Sufu, or *Fu-ru* written in hieroglyphics, is a fermented soybean food that originated in China. It is a soft creamy cheese-type product made from cubes of soybean curd (tofu) by the action of a

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mould (Steinkraus, 1996; Su, 1986). This fermented product with its characteristic flavour has been widely consumed by Chinese people as a salty appetizer for many centuries.

There are different types of sufu, produced by various local processors in China (Wang and Du, 1998), with mould-fermented sufu being the most popular type (Han et al., 2001b). Four steps are normally involved in making this type of sufu: (1) Preparing tofu, (2) Preparing pehtze (*pizi*) by fungal solid-state fermentation of tofu using, e.g., *Actinomucor elegans*, (3) Salting of pehtze, and (4) Ripening in dressing mixture (Han et al., 2001b; Wang and Hesseltine, 1970). According to the colour and flavour, sufu can be be classified into four types, i.e., red sufu, white sufu, grey sufu and others, which are mainly based on the different ingredients of dressing mixtures in the ripening.

Hydrolysis of protein occurs mainly during the ripening stage that usually takes 3–6 months. During this stage, protein is hydrolysed and converted to smaller nitrogen compounds such as peptides, amino acids, amines and ammonia (Han et al., 2003), resulting in flavour increase.

Traditionally, the ripening stage took over 6 months since the salt content in some sufu exceeded 14%. Presently, the salt content in most products, especially red and grey sufu, is still > 10% (Han et al., 2001b), with corresponding ripening periods of three months or longer. A reduction of salt content would have the combined advantages of shortening the ripening periods as well as reducing dietary sodium intake.

Like in other fermented foods such as cheese (Messens et al., 1999) and miso (Chiou et al., 1999), salt has a multiple role in sufu. It imparts a salty taste to the sufu, controls enzyme activity and influences biochemical changes in the product.

Since soybean foods serve as a source of protein in the Chinese diet, their amino acid content and pattern are important from nutritional point of view. In addition, amino acids contribute to the taste of foodstuffs (Kirimura et al., 1969; Nelson and Cox, 2000; Nishimura and Kato, 1988) and influence the consumers' acceptance of sufu. Although sufu contains about 40% (dry matter basis) crude protein (Su, 1986), there is little published information on its amino acid profile. Therefore, the objective of this investigation was to determine the levels of total and free amino acids (FAA) of sufu so as to provide data of nutritional and sensory relevance.

From the biochemical point of view, it is of interest to investigate amino acid profiles and chemical changes during red, white and grey sufu production, with special reference to the effect of different salt contents in the dressing mixtures.

2. Materials and methods

2.1. Micro-organism

A. elegans (Academia Sinica AS 3.227) is commonly used as a starter in commercial sufu production in China. Starting from a malt extract agar slant culture, a pure culture inoculum of *A. elegans* AS 3.227 was prepared by liquid substrate culture (approximately 1 cm depth) in Roux bottles as is common practice in Chinese sufu factories. The medium (pH 6.5) consisted of soy whey (by-product from tofu manufacture) to which maltose (2–3% w/v) and peptone (1.5–2.0% w/v) were added prior to sterilization by autoclaving. After incubation at 28°C for 72 h, medium and biomass were harvested and homogenized to obtain an inoculum suspension containing approximately 10^5 colony forming units (CFU)/mL.

2.2. Sufu preparation

Starting from soybeans, soymilk was obtained after soaking, hot grinding, sieving and cooking. Tofu (soybean curd) was obtained by coagulation from soymilk; the tofu was pressed to obtain a firm consistency. The tofu used as raw material for sufu was provided by Beijing WangZhiHe sufu manufacturer, and was cut into pieces ($3.2 \times 3.2 \times 1.6\,\text{cm}^3$). The pieces were inoculated with *A. elegans* by spraying inoculum suspension onto their surface. The inoculated tofu pieces were placed evenly spaced, in plastic trays. The loaded trays were piled up in an incubation room with controlled temperature (around 25°C), relative humidity (around 90%) and air circulation to ensure adequate aeration. Fresh pehtze, i.e., tofu overgrown with *A. elegans* mycelium, was obtained after incubation for 48 h.

The pehtze was transferred into a container (20 L) and salt was spread between layers of pehtze as they were piled up in the container. During a period of 5 d, the pehtze absorbed the salt until its content reached about 14% (w/w).

For the ripening of sufu, 12 pieces of salted pehtze (about 200 g fresh weight) were placed in individual wide-mouthed glass bottles with a capacity of 340 mL, after which dressing mixture (about 140 mL) was added to the pehtze. In order to reach the required final salt level (8%, 11%, or 14% w/w), salt was also added to the dressing mixtures. For red sufu, the dressing mixture consisted of angkak or kojic red rice (Han et al., 2001b), alcoholic beverage (rice wine) (Nout and Aidoo, 2002) to a final alcohol content of 5%, sugar, Chiang (wheat-based miso) (Campbell-Platt, 1987), and spices. For white sufu, the dressing mixture only consisted of alcoholic beverage (final alcohol content 5%). For grey sufu, the dressing mixture has a special recipe without alcohol (not specified due to confidentiality). The filled bottles were incubated at 25–28°C for 80 d.

2.3. Sampling for analysis

Two bottles were drawn randomly from each batch on each sampling day during the ripening. The ripening dressing mixture was decanted and sufu pieces were lyophilized.

2.4. Biochemical analyses

The pH was measured in a homogenized sufu suspension consisting of equal weights of sufu and distilled water, using a digital pH meter. Crude protein was analysed according to the Kjeldahl method (Nielsen, 1998). The lyophilized sample homogenates were hydrolysed in 6 N HCl under vacuum for 24 h at 110°C, and then the hydrolysates were applied to an Automatic Amino Acid Analyser (HITACHI 835-50, Japan) for determination of total amino acids (TAA) (Wu and Ding, 2002). The tryptophan was analysed after hydrolysing in 4.2 N NaOH under vacuum for 26 h at 110°C. The lyophilized sample homogenates were dissolved in sulphosalicylic acid, and supernatants were applied to the analyser using sodium citrate buffer system (pH 2.2, 3.3, 4.3, and 5.4) with post-column ninhydrin detection for FAA (Niven et al., 1998). A known mixture of different FAA was applied to the analyser as an external standard for the calculation.

Of each bottle (2 per sampling time for each treatment), biochemical analysis was carried out in duplicate ($n = 4$).

2.5. Amino acid groupings

Amino acids were grouped (Nelson and Cox, 2000; Sarkar et al., 1997) as acidic (aspartic acid + glutamic acid), basic (lysine + histidine + arginine), total charged (basic + acidic), hydrophilic (total charged + threonine + serine), hydrophobic (valine + leucine + isoleucine + phenylalanine + tyrosine + tryptophan + methionine) and apolar (hydrophobic−tyrosine).

3. Results and discussion

3.1. Changes of pH and crude protein

As the pH of fungal fermented soybean products is related to their taste (Nout et al., 1985), its gradual changes during manufacture would enable a simple monitoring in relation to extent of maturation, as well as impending spoilage (Han et al., 2001a). pH values in tofu, pehtze and salted pehtze, which were intermediate products of sufu prior to the ripening stage, were around 6.9–7.0. During the ripening of sufu, pH values of red and white sufu decreased slightly from 6.6–6.8 after 10 d, to pH 5.9–6.1 after 80 d of ripening time. pH values of grey sufu increased slowly from 6.8 after 10 d, to 7.1 after 80 d of ripening time.

Prior to ripening, crude protein contents in tofu, pehtze and salted pehtze were 63.1%, 62.8% and 37.5% (dry matter basis), respectively. The apparently decreased crude protein level in salted pehtze is caused by the presence of significant levels of salt in the dry matter. During ripening, crude protein in sufu decreased 3–6% gradually, regardless of the type of sufu. This may have been caused by diffusion out of the sufu, into the dressing mixture.

3.2. Amino acid profiles of tofu, pehtze and salted pehtze

Table 1 shows profiles of TAA, FAA and NH_3 prior to ripening in tofu, pehtze and salted pehtze. Whereas TAA and NH_3 in tofu and pehtze are similar (resp. 550 and 11 mg/g dm) the levels in salted pehtze are lower (350, resp. 8 mg/g) because of the presence of salt in the dry matter. However, the ratio or profile of amino acids remained similar. FAA and NH_3 content of tofu was low (1.31, resp. 0.13 mg/g dm), but due to the proteolytic enzyme activity released during fungal growth, a considerable amount of FAA and NH_3 were measured in pehtze (15.6 and 3.6) and salted pehtze (11.9 and 1.6 mg/g dm). Most FAA in salted pehtze was lower than that in pehtze due to the addition of salt. Some FAA such as serine, isoleucine, leucine, tyrosine and phenylalanine increased after the salting stage, which suggests that some degradation of protein could have taken place during the salting stage.

3.3. Amino acid profiles during the ripening of red and white sufu

TAA profile and NH_3 during the ripening of red sufu with 8% salt content, as an example, are presented in Table 2. It can be observed that the total (TAA) remains at a level of approximately 300 mg/g dm. This indicates that no important losses of nitrogen take place during the ripening. Interestingly, some hydrophobic amino acids such as valine, isoleucine and leucine increased, but

Table 1
Profiles of TAA, FAA and NH_3 in tofu, pehtze and salted pehtze

Amino acid	TAA			FAA		
	Tofu	Pehtze	Salted pehtze	Tofu	Pehtze	Salted pehtze
Asp	65.9	66.9	42.6	0.04	0.86	0.74
Thr	21.6	22.1	14.1	0.01	0.46	0.40
Ser	30.6	30.2	19.4	0.06	0.25	0.44
Glu	102.9	101.2	66.1	0.09	4.64	3.00
Gly	24.1	23.8	15.4	0.02	0.50	0.31
Ala	24.0	28.2	17.6	0.04	3.71	2.30
Val	21.5	22.1	14.2	0.01	0.68	0.53
Met	4.2	4.4	2.6	0.01	0.08	0.08
Ile	22.1	25.1	15.5	0.01	0.28	0.39
Leu	42.5	44.9	29.0	0.02	0.34	0.52
Tyr	21.7	21.4	14.1	0.04	0.58	0.63
Phe	31.7	30.8	20.2	0.04	0.36	0.68
Lys	33.3	32.6	21.1	0.04	0.81	0.68
His	13.3	13.2	8.6	0.10	0.78	0.35
Arg	44.2	40.9	25.8	0.76	0.34	0.30
Pro	30.1	29.7	18.8	0.02	0.94	0.59
Trp	6.1	6.5	2.2			
Cys	6.9	7.2	4.0			
Sub-total	546.7	551.2	351.3	1.31	15.61	11.94
NH_3	10.3	11.5	7.9	0.13	3.63	1.64

Data expressed as mg/g sample dry matter; $n = 4$.

all hydrophilic amino acids decreased slightly during the ripening. Probably these amino acids diffuse into the dressing mixture more easily and are therefore lost from the product.

Tables 3 and 4 present the evolution of FAA and NH_3 during the ripening of red and white sufu at 8%, 11% and 14% salt content levels. In red sufu we observed that during the 80 day maturation period, levels of FAA increased 3.2-fold (28–88 mg/g dm) at 8% salt, whereas the increase was less pronounced at higher salt levels (2.2-fold at 11% and 1.6-fold at 14% salt). The relative patterns of distribution of amino acids was not greatly affected by salt levels or ripening time. In contrast, in white sufu we observed that increased salt levels resulted in increased levels of protein degradation. For example, at 8% salt, the level of FAA increased from 33 to 104 mg/g (3.1-fold) whereas 3.4- and 4.0-fold increases were observed at resp. 11% and 14% salt levels. The absolute levels of FAA were also higher in white sufu than in the red type. Further investigations of the proteolytic activities in red and white sufu would be required to understand the different behaviour as influenced by salt concentration.

The very high content of acidic amino acids in red and white sufu was of particular interest. Glutamic acid was the most abundant acid, followed by aspartic acid, together representing around 30% of total FAA. The total of basic amino acids (i.e., lysine, histidine and arginine) constituted only $<10\%$ of the total (arginine was even not detected in white sufu after 80 d of ripening). These results agree with earlier reports (Chou and Hwan, 1994). Glutamic acid in combination with salt (NaCl) contributes to the flavour and hedonic characteristics of foods

Table 2
TAA changes during the ripening of red sufu with 8% (w/w) salt content

TAA	10 d	30 d	60 d	80 d
Asp	39.4	34.2	31.0	32.0
Thr	13.3	11.3	10.6	11.0
Ser	17.4	14.0	13.1	12.9
Glu	58.1	55.0	51.7	54.1
Gly	13.5	12.9	12.1	12.6
Ala	15.1	14.7	15.1	15.8
Val	12.9	13.5	15.4	15.8
Met	1.8	3.0	2.4	1.8
Ile	12.9	15.4	15.1	17.6
Leu	24.7	25.1	26.1	27.7
Tyr	13.3	11.3	11.4	11.8
Phe	19.1	17.0	18.7	19.7
Lys	17.4	16.0	15.7	16.0
His	7.3	6.7	6.5	6.3
Arg	21.1	14.9	12.0	12.3
Pro	17.2	16.0	16.2	16.6
Trp	4.1	4.5	3.4	3.8
Cys	4.2	3.9	6.0	6.9
Sub-total	312.8	289.4	282.5	294.7
NH_3	7.3	7.8	9.7	8.6

Data expressed as mg/g sample dry matter; $n = 4$.

(Halpern, 2000), also referred to as the umami taste. The predominance of glutamic acid has been reported in sufu (Liu and Chou, 1994), in cheese (Omar, 1984) as well as in several fermented soybean foods (Sarkar et al., 1997; Yamaguchi and Ninomiya, 2000). In addition, volatile compounds formed from amino acids by decarboxylation, deamination, transamination and other transformations can make substantial contributions to sufu flavour. This is a major reason for the many different volatile compounds (Chung, 1999, 2000; Hwan and Chou, 1999) encountered in sufu.

The profile of essential amino acids (EAA) of red sufu with 8% salt content at the ripening of 80 days, as an example, is present in Table 5, which shows considerable amounts of all EAAs. Like in all other soybean foods, methionine (6 mg/g total protein) is a limiting amino acid in sufu. The EAA profile of the above mentioned sufu was compared with two high-quality protein foods (eggs and cow's milk) and also with that of the reference pattern (FAO-WHO Expert Consultation, 1990; FAO-WHO-UNU Expert Consultation, 1985). These results indicate that sufu is a good source of almost all EAAs and that the EAA score is as high as that of egg and milk proteins.

3.4. Free amino acid profile during the ripening of grey sufu

Changes of FAA in grey sufu are summarized separately in Table 6, since they evolved in a totally different pattern compared with red and white sufu. All hydrophobic amino acids (except for methionine) increased to a large extent from 6.8 to 33.7 mg/g (5.0-fold), which dominated the

Table 3
FAA and NH_3 changes during the ripening of red sufu with 8%, 11%, and 14% (w/w) salt content

FAA	Time (d)	8% (w/w) salt content				11% salt content				14% salt content			
		10	30	60	80	10	30	60	80	10	30	60	80
Asp		1.6	4.0	6.4	7.0	1.8	2.8	3.6	5.2	1.7	1.9	2.3	3.1
Thr		1.0	2.2	3.3	3.4	1.0	1.4	1.8	2.4	0.9	1.1	1.3	1.6
Ser		1.0	2.0	3.4	3.1	1.0	2.0	2.0	2.6	0.9	1.4	1.7	2.2
Glu		7.2	14.3	19.6	21.3	6.5	9.1	11.8	14.4	6.1	6.8	8.2	9.3
Gly		0.8	1.8	2.7	3.1	0.8	1.2	1.6	2.2	0.7	0.8	1.0	1.1
Ala		2.7	4.0	5.9	6.6	2.6	3.0	3.7	4.9	2.1	2.3	2.8	3.3
Val		1.4	3.1	4.8	4.9	1.5	2.2	2.5	3.5	1.3	1.5	1.8	2.3
Met		0.4	0.8	1.4	1.5	0.4	0.6	0.8	1.0	0.4	0.4	0.5	0.6
Ile		1.4	3.4	4.9	5.5	1.5	2.2	2.6	3.7	1.3	1.5	1.8	2.5
Leu		2.3	5.2	8.0	8.8	2.6	3.7	4.7	6.1	2.4	2.6	3.1	4.1
Tyr		1.8	3.4	4.2	4.5	2.0	2.6	4.0	4.1	1.8	2.0	2.4	2.7
Phe		2.3	4.6	6.3	6.3	2.4	3.3	3.8	4.8	2.4	2.6	3.1	3.4
Lys		1.5	3.4	5.3	5.6	1.7	2.6	3.1	4.0	1.5	1.7	2.0	2.8
His		0.5	0.9	1.4	1.4	0.5	0.8	1.0	1.0	0.5	0.6	0.7	0.8
Arg		0.6	0.9	1.2	1.2	0.6	0.5	0.8	0.2	0.6	0.5	0.6	0.4
Pro		1.3	2.6	3.7	4.1	1.3	1.7	2.2	2.9	1.2	1.3	1.6	1.8
Sub-total		27.8	56.6	82.5	88.3	28.2	39.7	50.0	63.0	25.8	29.0	34.9	42.0
NH_3		2.3	3.5	4.8	5.5	1.9	2.8	3.0	3.2	2.0	2.0	2.4	2.8

Data expressed as mg/g sample dry matter; $n = 4$.

proportion of total FAA. On the other hand, hydrophilic amino acids, which were main amino acid in red and white sufu, remained at the same level (9–11 mg/g dm) or even decreased strongly (glutamic acid, glycine and proline) during ripening. This decrease suggests that they were consumed or transformed somehow at a greater rate than they were formed by proteolytic activity. Grey sufu is not yet described in published literature. Obviously, its different amino acid profile during ripening, as well as its strong, offensive odour, and grey (or sometimes blue) colour that is formed during 5–15 d of ripening, warrant further study. Possibly, a relatively high turnover of amino acids into volatile nitrogen compounds is associated with this smell and the depletion of FAA.

4. Conclusion

Total FAA generally increased during the ripening of sufu and were higher in the lower salt content than in the higher salt content. While FAA increased with the age of red and white sufu, the relative proportion of each amino acid remained essentially constant. Acidic amino acids (i.e., glutamic acid, aspartic acid), leucine, alanine, phenylalanine and lysine were found in large quantities. On the other hand, grey sufu showed a different pattern of changes, with leucine, alanine, isoleucine, valine and phenylalanine as the dominant amino acids at the end of ripening.

Table 4
FAA and NH_3 changes during the ripening of white sufu with 8%, 11%, and 14% (w/w) salt content

FAA	Time (d)	8% (w/w) salt content				11% salt content				14% salt content			
		10	30	60	80	10	30	60	80	10	30	60	80
Asp		2.3	5.6	5.2	6.4	2.0	4.8	6.8	7.9	1.3	2.7	4.2	6.4
Thr		1.4	3.5	5.2	5.3	1.2	2.2	4.0	4.7	0.7	1.4	2.2	3.2
Ser		1.1	0.1	0.1	0.5	0.9	2.4	0.1	0.2	0.8	1.9	2.7	4.5
Glu		5.8	14.7	21.6	22.1	4.8	12.1	16.9	18.5	3.2	4.9	7.9	11.2
Gly		1.1	2.7	4.5	4.8	0.9	2.0	3.1	3.7	0.5	1.0	1.4	2.5
Ala		3.8	6.8	11.3	9.5	3.1	4.5	7.8	7.9	2.3	3.0	4.2	5.7
Val		2.0	4.9	7.3	7.5	1.6	3.2	5.6	6.5	1.0	2.0	2.8	4.8
Met		0.5	1.2	1.4	2.0	0.4	0.9	1.4	1.7	0.2	0.5	0.7	1.3
Ile		1.9	5.2	8.0	8.2	1.6	3.4	6.0	7.3	0.9	2.0	3.2	5.3
Leu		3.2	7.9	11.1	11.5	2.3	5.6	9.1	10.3	1.5	3.6	5.0	7.8
Tyr		2.1	4.0	1.4	1.9	1.7	3.7	4.6	0.9	1.3	2.4	3.4	4.2
Phe		3.1	6.7	8.1	8.2	2.5	4.4	7.7	7.4	1.9	3.6	5.0	6.3
Lys		2.3	5.1	8.4	8.6	1.9	3.6	5.9	7.5	1.2	2.1	2.9	5.4
His		0.6	1.5	2.1	1.5	0.5	0.9	1.7	1.8	0.4	0.7	1.0	1.1
Arg		0.7	1.4	0.2	0.0	0.6	0.2	1.6	0.0	0.3	0.8	1.0	0.0
Pro		1.4	3.7	6.0	5.8	1.2	2.6	4.1	5.2	1.0	1.4	2.1	3.5
Sub-total		33.3	75.0	101.9	103.8	27.2	56.5	86.4	91.5	18.5	34.0	49.7	73.2
NH_3		2.4	5.0	5.7	5.8	2.0	2.9	5.8	4.8	1.7	1.8	2.5	3.3

Data expressed as mg/g sample dry matter; $n = 4$.

Table 5
Comparison of the EAA composition of a sufu with two high-quality animal foods and the suggested EAA pattern of requirements for humans

EAA	EAA (mg AA/g total protein)			
	Requirements for preschool child (2–5 years)[a]	Sufu (8%)[b]	Animal products[a]	
			Eggs	Cow's milk
His	19	22	22	27
Ile	28	51	54	47
Leu	66	89	86	95
Lysine	58	53	70	78
Met + Cys	25	30	57	33
Phe + Tyr	63	107	93	102
Thr	34	37	47	44
Trp	11	13	17	14
Val	35	54	66	64
Total	339	456	512	504

[a](FAO-WHO Expert Consultation, 1990; FAO-WHO-UNU Expert Consultation, 1985);
[b]A red sufu with 8% salt content at the ripening of 80 d.

Table 6
FAA and NH_3 changes during the ripening of grey sufu with 11% (w/w) salt content

FAA	10 d	30 d	60 d	80 d
Asp	0.5	0.8	0.8	0.7
Thr	0.4	0.1	0.0	0.1
Ser	0.1	0.0	0.2	0.2
Glu	5.1	1.2	0.3	0.1
Gly	0.8	0.1	0.0	0.0
Ala	2.7	5.2	7.4	8.9
Val	1.2	3.8	5.3	6.8
Met	0.2	0.1	0.1	0.0
Ile	1.1	3.6	5.8	7.1
Leu	1.7	4.8	7.8	9.6
Tyr	1.0	2.1	3.2	3.7
Phe	1.6	3.6	5.4	6.5
Lys	0.1	0.1	0.2	0.2
His	0.3	0.1	0.1	0.1
Arg	0.4	0.1	0.1	0.0
Pro	1.3	0.2	0.0	0.0
Sub-total	18.5	25.9	36.6	44.0
NH_3	2.9	5.5	6.4	7.8

Data expressed as mg/g sample dry matter; $n = 4$.

The manufacture, flavour and other quality attributes of this relatively unknown product require further study.

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