Starter Cultures Effects on Proteolytic Changes and Amino Acid Content in Fermented Sausage

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Abstract **The main objective of this work was to examine the effects of using three types of commercial starter cultures such as *Lactobacillus sakei; Staphylococcus carnosus and Lactobacillus sakei - Staphylococcus carnosus* in fermented sausage. The condition of processing include 27ºC, for 2 days at relative humidity (RH) 80%, for 3 days at 20ºC, RH 80%, 4 days 15ºC, RH 75%, and finals 15ºC, RH 65% for 12 days. Samples were taken at 0, 1st, 3rd, 6th, 9th, and 21st days of ripening for chemical and microbiological analysis. During the fermentation stage, changes of proteolytic characteristics were observed in fermented sausage. Proteolytic activity was high in *Lactobacillus sakei - Staphylococcus carnosus* starter inoculated sausages during processing. Moreover, a slight increase in proteolytic activity was detected during storage in both those sausages. Sarcoplasmic and myofibrillar proteins were also affected by this starter culture addition, during the fermentation, ripening and intense proteolysis were show in both fermented sausages. The content of free amino acids was similar at the beginning of the fermentation stage for all the studied batches. However, the high differences in the content of free amino acids at the end of the process could be attributed to the starter culture activity. Thus, use of starter culture in fermented sausage result a safer product.**

Keywords— **Fermented sausage, starter culture, proteolytic.**

I. INTRODUCTION

The products are found in most parts of the world, although Europe is the major producer and consumer of these products. All these transformations are influenced by ripening conditions, raw meat and ingredients and have a considerable effect on the organoleptic quality of fermented meat products. Breakdown products of lipolysis and proteolysis, i.e. peptides, amino acids, carbonyls and volatile flavor compounds contribute to the characteristic flavor and texture of fermented meats (Diaz, et al, 1997). The pattern of the proteolysis in fermented sausages is influenced by several variables such as product formulation, processing condition and starter culture (Hughes, et al, 2002).

Several biochemical and physical changes occur during the ripening of fermented sausages that determine the flavour and odour of the end product. These changes are mainly acidification as a result of fermentation, pH decrease, changes in initial microflora, reduction of nitrates to nitrites and formation of nitrosomyoglobin, solubilisation and gelification of myofibrillar and sarcoplasmic proteins, proteolytic, lipolytic and oxidative phenomena, and dehydration (Casaburi, et al, 2007).

However, different studies have shown that the increase in the amount of free amino acids is not enough to significantly increase aroma compounds, since the mechanisms of amino acid degradation must also be favored in order to yield higher amounts of flavour compounds (Diaz, et al, 1997). Transamination and deamination reactions are among the primary phenomena through with typical ripened aroma compounds are formed, and the addition to sausages of a commercial amino acid oxidase has been shown to increase significantly both, amino acid breakdown and aroma development of sausages. Nowadays, the consumer pays a lot of attention to the relationship between food and health. Therefore, the marker for food with health promoting properties, so-called functional foods, has shown a remarkable growth over the last few years. The aim of this study was to determine the effect of the proteolytic and amino acid changes produced with and without commercial starter cultures in the manufacture of fermented sausage.

II. MATERIALS AND METHODS

2.1. Preparation of Fermented Sausages. Four separate batches of fermented sausage were prepared included control (without starter culture), of fermented sausages were processed: The ingredients uses; learn pork, salt (2.0% w/w), glucose (1.0% w/w) and Sodium nitrate (0.01% w/w), (Kanto Chemical CO, Inc Japan). Sausage mixes were inoculated 0.1% (v/w) of *Lactobacillus sakei* (MMF-161; San-ei Sucrochemical Co., Ltd Japan); 0.025% (w/w) of *Staphylococcus carnosus* (S-B-61 Bactoferm^{TM}, Chr. Hansen, Inc., Denmark); 0.025% (w/w) of *Lactobacillus sakei - Staphylococcus carnosus* (F-SC-111 Bactoferm^{TM}, Chr. Hansen, Inc., Denmark); Each starter culture was used according to the respective manufacturer's instructions.

The mixtures were stuffed into fibrous casing (40 mm in diameter), at approximately 190 g each. The condition of processing include 27ºC, for 2 days at relative humidity (RH) 80%, for 3 days at 20ºC, RH 80%, 4 days 15ºC, RH 75%, and finals 15ºC, RH 65% for 12 days. Samples were taken at 0, 1st, 3rd, 6th, 9th, and 21st days of ripening for chemical and microbiological analysis.

*2.2. Preparation of sarcoplasmic and myofibrillar proteins.*Sarcoplasmic protein extracts were prepared according to the method of Toldrá, Rico & Flores (1993). 4 g of sausage was homogenized (Ultra Turrax) with 40 ml of 0.03 M potassium phosphate buffer (pH 7.4) for 2 min at 13,500 rpm. The homogenate was centrifuged for 20 min at 10,000*g* at 4 °C. The supernatant included the sarcoplasmic proteins. Myofibrillar proteins were extracted from the resultant pellet by homogenizing (Ultra Turrax) with a solution containing 8 M Urea and 1% (w/v) β-mercaptoethanol for 2 min. The homogenate was recentrifuged under the same conditions and the supernatant contained the myofibrillar proteins.

2.3. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE). The protein concentrations of the sarcoplasmic and myofibrillar fraction were determined by the Bradford (1976) procedure. SDS–PAGE was done using a vertical gel electrophoresis unit (Mini-Protein 3 Cell. Bio-Rad, Lab. Inc) according to the method of Laemmli (1970). Commercial molecular weight standards were obtained from Sigma Genosys Japan (MP-0120), contain lysozyme 16.8 kDa; soybean trypsin inhibitor A, 25.7 kDa; carbonic anhydrase II 32.7 kDa; ovalbumin 47.8 kDa; bovine serum albumin 84.2 kDa; phosphorylase B 110 kDa.

2.4. Amino acid analysis. Samples for free amino acids analysis were prepared according to the procedures described by (Mikami, et al, 1994).

2.5. Statistical analysis. Duncan's multiple range tests was employed to determine any significant difference between treatments (samples without and with starter cultures). All statistical analyses were performed using SPSS statistical program (Version 16 for windows, SPSS Inc., Chicago, USA, 2007).

III. RESULTS AND DISCUSSION

3.1. Proteolysis of sarcoplasmic proteins. The electrophoretic patterns of sarcoplasmic proteins in fermented sausage without and with starter cultures at various stages of fermentation and ripening are shown in Fig 1 The greatest change in sarcoplasmic protein pattern occurred between processing days 0 (before stuffing) and 3 (during fermentation), while only small differences were observed during fermentation and drying in control and single starter-inoculated sausages. Protein bands at 36, 30, and 25.7 kDa proteins disappeared during the first 3 days in sausage with mixed starter cultures.

The band intensity of samples at 0, 3 and 21 days in the control and single starter culture show changes during fermentation and storage. After third day of processing 36, 30, 25.7 and 20 kDa peptides bands decreased. Thereafter, these bands were disappeared in samples used mixed starter cultures *L. Sakei- S. carnosus* during fermentation and ripening of sausage. On the other hand, sausages without starter culture showed a weaker activity of endogenous proteases compared with exogenous proteases reported by Casaburi, et al, 2007.

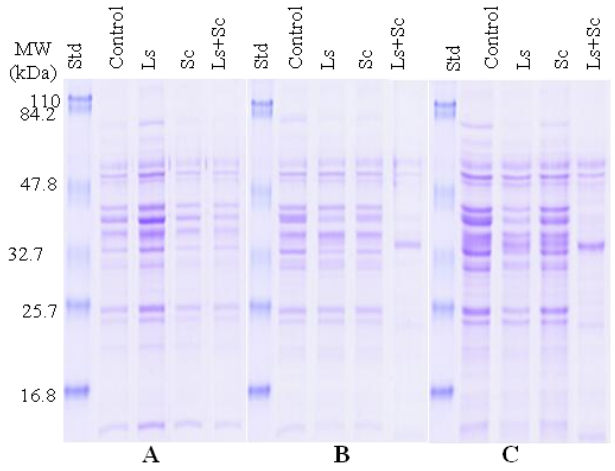

Fig. 1 SDS–PAGE profile of sarcoplasmic proteins. (A) 0 day; (B) 3 day and (C) 21 day; Std: (protein standard); control: (without starter culture); Ls: (*L. sakei*); Sc: (*S. carnosus*); Ls + Sc: (*L. sakei–S. carnosus*).

3.2. Proteolysis of myofibrillar proteins. SDS–PAGE electrophoretograms of myofibrillar proteins are given in Fig 2 for fermented sausage. The intensity of the band decreased gradually after fermentation and drying, control and with single starter culture sausages, but only a few differences were detected during fermentation and ripening in the samples used mixed starter culture *L. Sakei-S. carnosus;* This was probably due to the pH. The main differences were observed in the 80, 40, 34, 30, 18 and 16.8 kDa bands between days 0 and 3. Their intensity decreased during fermentation. The 80 kDa band degraded completely in mixed starter culture *L. Sakei-S. carnosus* samples after the third day, but was not completely degraded in control and single starter culture samples of fermented sausages. The 45 kDa (actin) band intensity decreased between days 0 and 3 in control and starter-inoculated sausages. Degradation of actin (45 kDa) during ripening of fermented sausages was in conflict with findings (Casaburi, et al, 2007 and Hughes, et al, 2002) Tropomyosins (T1 and T2, 38 and 30 kDa) (arrows) decreased during ripening, while tropomyosin T1 and other bands such as 66 and 97 kDa accumulated during storage in control fermented sausages.

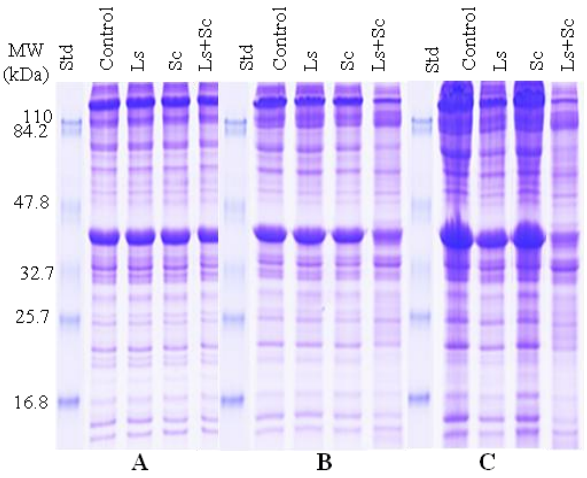

Fig. 2 SDS–PAGE profile of myofibrillar proteins. (A) 0 day; (B) 3 day and (C) 21 day; Std: (protein standard); control: (without starter culture); Ls: (*L. sakei*); Sc: (*S. carnosus*); Ls + Sc: (*L. sakei–S. carnosus*).

This accumulation was also detected in single starter culture sausages, but was not as clear as in sausages used mixed starter cultures. Myosin light chains (MLC1 and MLC2, 24 and 20 kDa) also degraded and disappeared during ripening of sausages. Similar to values reported by (Diaz, et al, 1997), During the fermentation and ripening of dry fermented sausages a large number of biochemical reactions associated to the degradation of myofibrillar and sarcoplasmic proteins take place. These biochemical reactions are promoted by muscle endopeptidases (calpains I and II and cathepsins B, D, H and L) reported by (Toldra, 2006) The role of muscle enzymes in dry cured meat products with different drying conditions and microbial proteinases, are to bound either to the cell wall or to the cell membrane.

3.4. Free amino acids contents of fermented sausages.

The changes in the contents of free amino acids were observed in fermented sausage during ripening are shown in the Table 1. The total free amino acid contents of the sausage constituted 160.6 mg/100g of samples (before stuffing) at 0 day. An increase in the content of amino acids was observed and further increased up to the range of 796.5 to 868.4

mg/100g of total free amino acids at the ripening stage of fermented sausages (21 days). The hydrolysis of meat proteins generates polypeptides that can be further degraded to smaller peptides and free amino acids. This degradation can be produced by endogenous and microbial enzymes as reported by different authors (Hughes, et al, 2002).

Table 1. Amino acid contents (mg/100g)

	0 day	21 day			
	Control	Control	Ls	Sc	Ls+Sc
Asp	5.1	5.1	16	19.4	24.6
Thr	7.6	49	46.1	49.6	33.5
Ser	7.8	52.3	51.5	50	43.1
Glu	23.8	117.5	146.3	127.1	170.1
Gly	14.7	59.1	65.2	61.4	53.7
Ala	32.4	135.1	126.5	124.2	110.2
Cys	1.0	1.5	1.2	0.9	1.9
Val	7.3	47.5	50.6	47.8	47.8
Met	3.1	21.1	22.1	21.2	24.3
Ile	4.1	29.2	34.3	32.5	30.9
Leu	8.8	62.4	65.1	57.1	64.5
Tyr	6.7	22.8	33.4	30.4	24.5
Phe	5.9	33.1	34.6	32.5	42.7
Lys	10.1	83.4	78.2	85.7	69.9
His	6.6	26.8	29.4	26.6	27.8
Arg	10.3	45.7	27.3	8.1	7.8
Pro	5.2	47.5	40.5	45.5	19.3
Total	160.6	839.1	868.4	820.1	796.5

The increase in the total free amino acid concentration was detected in all batches as reported by Hughes, et al, (2002). The differences observed in amino acids which is primarily responsible for the increase in total free amino acids during ripening were Glu (glutamic acid), Ala (alanine), and Lys (lysine) observed in the samples with starter cultures *L. sakei; S. carnosus* and *L. sakei–S. carnosus*, The change occurred during fermentation and ripening process indicating that the highest enzymatic activity took place in these stages. Several authors have reported major release of free amino acids at the beginning of the process in coincidence with the fermentation stage (Diaz, et al, 1997). This increase has been attributed to the higher temperatures applied during fermentation compared to the low temperature applied during drying. The decrease in the content of amino acids may indicate their metabolism by bacteria (Ordoñez, et al, 1999).

IV. CONCLUSIONS

The effect of starter culture on proteolytic changes was followed by a degradation of sarcoplasmic and myofibrillar proteins during the fermentation and drying was detected on the samples inoculated with combined starter culture as of *L.sakei-S. carnosus* were brought about by both endogenous and bacterial enzymes are responsible for the degradation of sarcoplasmic and myofibrillar proteins, although bacterial proteinases contribute to the degradation of these proteins. High value of content of free amino acids at the end of the process was detected on the sample inoculated with *L. Sakei* could be attributed to the starter culture activity.

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