**Physical, Microbial and Chemical Quality of Dangke as Response to Different Temperatures and Papain Concentrations**

**A.N. Mukhlisaha, E. Taufikb\*, & I.I. Ariefb**

aStudy Program of Animal Production and Technology, Faculty of Animal Science, Graduate School, Bogor Agricultural University

bDepartment of Animal Production and Technology, Faculty of Animal Science,

Bogor Agricultural University

Jl. Agatis, IPB Darmaga Campus, Bogor 16680 Indonesia

\*Corresponding author: epitaufik@apps.ipb.ac.id

**ABSTRACT**

Dangke, a soft cheese made from cow or buffalo milk without fermentation process, is an authentic local dairy product of Enrekang, South Sulawesi. The enzymes papain naturally changes milk as main ingredients into solid due to the separation between protein and water. This present study was aimed to evaluate effects of processing temperatures and papain concentration on physical, chemical, microbiological and organoleptic quality of dangke. Research steps included preparation of papain and determination of optimum processing temperature and papain concentration. The study resulted in higher papain concentration (401.672 mg/100g), as compared to commercial papain from Merck (360.63 mg/100g), but these enzymes had similar molecular weight (19.17 kDa). Based on chemical properties and hedonic evaluation, the best dangke was processed at 80 °C and added with 0.3% of papain. In addition, high yield and hardness of dangke were obtained at 70 °C and papain concentration 0.4%. Microbiological quality of dangke processed at 90 °C and papain concentration 0.3% was under threshold of coliform, mold, and yeast contamination. Further study is needed related to the activity of papain based on this study and its economic value to be applied in the manufacturer.

***Keywords: dangke, heat temperature, papain concentration, quality of dangke***

**ABSTRAK**

Dangke merupakan produk olahan susu sapi atau kerbau, sejenis keju lunak yang dihasilkan tanpa proses fermentasi dan menjadi makanan khas di Kabupaten Enrekang Propinsi Sulawesi Selatan. Enzim papain secara alamiah akan mengubah susu sapi atau kerbau itu menjadi padat akibat terjadinya pemisahan protein dan air. Penelitian ini bertujuan untuk melihat optimasi suhu dan konsentrasi papain terhadap kualitas dangke terutama pada uji fisik, kimia, mikrobiologi dan mutu hedonik. Penelitian ini terdiri dari 2 tahap yaitu pembuatan enzim papain dan penentuan suhu pemanasan optimal dan konsentrasi papain. Hasil penelitian menunjukkan bahwa konsentrasi papain penelitian adalah yang tertinggi dibanding dengan komersil dan papain Merck (360.63 mg/100 g) dengan kadar konsentrasi papain 401.672 mg/100 g. Hasil elektroforesis enzim papain hasil penelitian terlihat sama berat molekul antara enzim papain kommersil dan enzim papain penelitian ini yatu 19.17 kDa. Kualitas dangke terbaik berdasarkan uji kimia dan mutu hedonik diperoleh pada suhu 80 °C konsentrasi papain 0.3 %. Uji fisik dilihat dari rendemen dan tingkat kekerasannya tinggi terdapat pada suhu 70 °C konsentrasi papain 0.4 %. Uji mikrobiologi terdapat pada perlakuan suhu 90 °C konsentrasi papain 0.3% dibawah ambang batas cemaran koliform, kapang dan khamir. Diperlukan penelitian lebih lanjut terkait aktivitas enzim papain hasil penelitian ini dan kajian tentang nilai

***Kata kunci : dangke, konsentrasi papain, kualitas dangke, suhu pemanasan,***

**INTRODUCTION**

Dangke is a soft cheese which is local dairy product of Enrekang, South Sulawesi. It is made from cow or buffalo milk. The process employs enzymes that naturally change the milk as main ingredients to solid due to the separation between protein and water. The common-preservation method applied is the salt solution addition ([Marfiyanti *et al.* (2013)](#_ENREF_13).

The dangke has been traditionally produced, which potentially affects to the hygiene during the process and final product safety that is lack of concern by either producers or consumers. In general, dangke production involves many food aspects including the milking, fresh milk transportation, the use of food additives, and packaging. In dangke presence of lactic acid bacteria was reported by ([Syah *et al.*, 2016](#_ENREF_20)). A total of 30 LAB isolates were successfully isolated from dangke, and 5 isolates were successfully identified by 16S rRNA gene sequencing. However, contamination from pathogenic bacteria may occur in each production process. The common heating process at ± 70 °C for ± 20 min enables to induce detrimental effects on pathogens. The contaminants before and after heating also contribute to the bacterial contamination. Although dangke is very popular in South Sulawesi, its shelf life and quality still remain challenges, mainly due to the unstandardized processing and handling such as coagulation processing and heat temperature. Therefore, different temperatures and papain concentrations in dangke production were investigated to obtain desirable and pathogen-free dangke. This present study aimed to evaluate the effects of temperatures and papain concentration on physical, chemical, microbiological and organoleptic quality of dangke.

**MATERIALS AND METHODS**

Dangke was prepared in Laboratory of Livestock Product Technology, while the dangke quality was evaluated in Integrated Laboratory of Department of Animal Production Science and Technology, Faculty of Animal Science, Bogor Agricultural University (IPB).

**Isolation of Papaya Latex (**[**Marfiyanti *et al.*, 2013**](#_ENREF_13)**)**

The papaya (var. California, age 2.5-3 months) latex was isolated in Cihideung Kramat, Bogor The incisions were made in fruit surface (1-2 mm in depth) from base to end of the fruit every 4 days for 28 days in the morning (5.30 – 8.00 am). The latex was immediately collected and stored for next steps.

**Purification of Papaya Latex into Papain (**[**Nitsawang *et al.*, 2006**](#_ENREF_16)**)**

Papaya latex was diluted by adding a solution of 40 mM cysteine​ (3:1), and adjusted to pH 5.6. The suspension was filtered and adjusted to pH of 9.0. Precipitated fractionwas then centrifuged (9000×g, 30 min) and protein was precipitated by adding (NH4)2SO4 until saturation of 45% to obtain salt solution. The solution was then stirred and centrifuged (9000×g, 30 min). Precipitate obtained was dissolved in 20 mM cysteine ​​and added by NaCl (10% w/v). The mixture was centrifuged to precipitate papain. The enzyme obtained was tested using Lowry method and SDS PAGE.

Determination of Optimum Heat Temperature and Papain Concentration

The pasteurized cow milk at 70, 80 and 90° C were added by papain that was previously diluted with distilled water (1:9) with the concentration of diluted papain of 0.2, 0.3, and 0.4%, respectively. The milk were then stirred until a syneresis (separation of curd and whey) was done. The curd was then filtered and shaped using coconut shell, then pressed until solid, and the products were subsequently tested for the quality.

**SDS PAGE Electrophoresis (**[**Monti *et al.*, 2000**](#_ENREF_15)**).** Electrophoresis was performed to determine the molecular weight and confirm purity. The selected fraction was collected and analyzed by SDS PAGE (Bio-Rad, Hercules, CA, USA) using polyacrylamide gel 15%, followed by staining with *Coomassie* Brilliant Blue R250 (Sigma, St. Louis, MO, USA).

**Protein Content Analysis of Lowry (Lowry *et al.* 1951).** The first reagent of 0.1 M NaOH was added by 10 mL H2O then added by Na2CO3 2%. The second reagent of Na+K+tartrate 1% (w/v) was added by 10 mL H2O and homogenized. Na+K+tartrate 1% was taken about 3 mL and added by 15 mg CuSO4**.**5H2O 0.5% (w/v). The samples were prepared and put into the test tube and added by 5.5 mL second reagent, followed by the addition of 0.5 mL Folin. The absorbance was read at 650 nm by spectrophotometer. The standard curve employed BSA at the concentrations of 0; 0.1; 0.2; 0.4; 0.6; 0.8; and 1.0 mg mL-1.

**Chemical Quality**

**Protein Content, (**[**AOAC, 2005**](#_ENREF_1)**) .** The protein content in the samples was analyzed by Kjeldahl method. Sample (1 g) was placed in a 100 mL Kjeldahl flask. The protein content was calculated using the following formula:

 Protein content (% wb) = 6.25 x % Nitrogen

**Water Content (**[**AOAC, 2005**](#_ENREF_1)**).** Sample (2 g) was placed in an aluminum dish and dried in oven at 105 °C for 15 h. The calculation was using the following formula:

 Water content (%wb) = $\frac{initial sample-dried samples}{initial sample} x 100\%$

**Fat Content (**[**AOAC, 2005**](#_ENREF_1)**).** Sample (2 g) was weighed and placed in a Soxhlet extraction apparatus. The fat content was calculated using the following formula:

 Fat content (%wb) = $\frac{extractable fat weight}{weight of fat sample} x 100\%$

**Ash Content (**[**AOAC, 2005**](#_ENREF_1)**).** The samples were heated in a kiln at 400 °C temperature to obtain constant weight. The ash content was obtained using following formula:

 Ash content (% wb) = $\frac{ash weight}{The weight of the sample} x 100\%$

**Carbohydrate Content (**[**AOAC, 2005**](#_ENREF_1)**).** Carbohydrate content was calculated using the following formula:

 Carbohydrate content (%) = 100% - (water % + ash % + protein % + fat %)

**Amino Acid Analysis (**[**Osthoff *et al.*, 2002**](#_ENREF_17)**).** The amino acid composition was determined using high-performance liquid chromatography (HPLC). The samples were hydrolyzed with acid based on Osthoff *et al*. (2002). The composition of nitrogen in total protein was determined using Kjeldahl method. The concentration of amino acids in the sample was calculated using the following formula:

 AA Concentration (ppm) = $\frac{broad peak samples}{lstandard peak area x 1000}$

**Physical Properties**

**Water activity (aw) (**[**AOAC, 2005**](#_ENREF_1)**).** The water activity of the product was determined using aw meter (Novasina) . Prior to analysis, the device was calibrated using saturated NaCl solution (aw around 0.7509). The samples were put into aw meter. The aw value was detectable when the device was in completed mode.

**Texture analysis (**[**Buriti *et al.*, 2007**](#_ENREF_5)**).** The test was performed using LFRA texture analyzer, applied using Texture Expert, windows 1.20. The samples were uniformly sized, then placed on the instrument. The gel hardness of products was determined from the maximum force (peak value) at the first compression.

**pH value (**[**AOAC, 2005**](#_ENREF_1)**).** The pH value was measured *in duplo* according to direct-probe method. The pH was measured using pH meter (Hanna Instruments, USA), first calibrated using pH 4 and pH 7 buffer solutions.

**Yield (**[**Sani *et al.*, 2013**](#_ENREF_19)**).** The yield was calculated using the following formula:

Yield (%) = $\frac{Initial production (weight of milk used)}{final production (weight curd formed)}$

**Microbiological Analysis (**[**ISO, 1996**](#_ENREF_11)**)**

Themicrobiological test was started with sample dilution. Sample (25 g) was incorporated to 225 mL Buffer Peptone Water (BPW) then diluted to obtain 10-3 dilution for detection of coliform using Violet Red Bile Agar (VRBA), and also molds and yeasts using Potato Dextrose Agar (PDA).

**Organoleptic Test (**[**Arief *et al.*, 2014**](#_ENREF_2)**)**

The determination of hedonic quality test based on the principle evaluation of panelists.. All of panelists tasked to record resulting ago scored into the form provided. Sensory evaluation of hedonic quality test using a scale of 1 to 5. Fourty untrained panelists were used and provide an explanation and guidance for hedonic quality tests. Hedonic quality test such as aroma, flavor, texture and color.

**Statistical Analysis**

The completely randomized factorial design (CRFD) with three replicates was used. The first factor was the heat temperature (70, 80, and 90 °C) and the second factor was the papain concentration (0.2, 0.3, and 0.4%). The data were processed using analysis of variance (ANOVA). The significant differences between the means were determined by Duncan multiple range test (P<0.05). The papain and amino acids were identified using exploratory descriptive analysis, while the organoleptic test data were analyzed using non-parametric statistical test Kruskal-Wallis (Steel and Torrie 1995).

**RESULTS AND DISCUSSION**

**Molecular weight of Papain**

SDS PAGE profiles showed that there was a band at each line. Figure 1 demonstrates that papain obtained has similar molecular weight (19.17 kDa) to enzyme from commercial. This finding is in accordance with previous study. [Monti *et al.* (2000)](#_ENREF_15) reported that the molecular weight of purified fresh papaya latex was 21 kDa, detected in a single band.

**Papain Concentration**

The results showed that papain concentration obtained was higher than commercial papain from Merck and Paya (Table 1). The purification and dialysis used in this research may contribute to this result, suggesting that the high quality enzyme was successfully prepared by the methods. However, high levels of protein may result from non-enzyme proteins extracted by ammonium sulfate. The enzyme from dialysis still contained non-enzyme proteins, although it was free from non-protein contaminants ([Putri *et al.*, 2013](#_ENREF_18)).

**Chemical Characteristics of Dangke**

 Table 2 demonstrates the chemical characteristics of dangke (protein, moisture, fat, ash and carbohydrates). No significant interaction between heat temperature and papain concentration (P>0.05) was observed, but processing temperature significantly affected protein content (P<0.05).

 Table 2 showed that protein content increased at the heat temperature of 80 °C, but decreased at 90 °C. This indicated that the excessive heat in milk could affect the dangke production. However, pasteurization of fresh milk is still needed to reduce the pathogenic and spoilage bacteria and spoilage bacteria that also affect dangke quality. Winarno (1993) argued that heating treatment could promote denaturation protein, causing the change in its structure. Denaturation decreased solubility and enhanced its viscosity, called as coagulation.

Papain concentration significantly affected protein and carbohydrate content (P <0.05), as presented in Table 2. The increase could be seen from the concentration of 0.2% to 0.3%. This might be caused by the addition of papain consequently increases its activity. [Arreneuz & Pardede (2013)](#_ENREF_3) stated that the optimum coagulation could be achieved at the proper enzyme activity, and this condition was obtained with the sufficient enzyme for the reaction and media for the activity. The addition of papain as biocatalyst influences protein content as papain is a protease. Protease degrades protein by hydrolyzing peptide bonds.

 Lactose is a disaccharide consisting of glucose and galactose ([Arreneuz & Pardede, 2013](#_ENREF_3)). Carbohydrate content was at the lowest level for S2 and S3, which was contributed by high water content in dangke. This was in accordance with [Fox *et al.* (2004)](#_ENREF_9) that carbohydrate content was inversely related to moisture content. The lower water content was attributed to the higher milk sugar (carbohydrate). Lactose found in milk is in the real solution phase with only 20% solubility at room temperature. Thus, levels of lactose or carbohydrate in dangke depends on the water content ([Winarno, 1993](#_ENREF_21)).

Amino acids are components of proteins, and consist of carboxylic groups (-COOH) and an amino group, Amino acids are distinguished by their side chains (R). Two types of amino acid are essential amino acid and non-essential ([Buwono & Si, 2000](#_ENREF_6)). Amino acid profile was analyzed using HPLC and presented in Table 3.

 Glutamic acid is the dominant amino acid in dangke treated with different heating temperature and papain concentration. The highest amino acids content, both essential and non-essential, was obtained at 90 °C heat temperature and 0.4% papain concentration. This is due to the addition of papain, which amino acids is known asprotein-building component. [Bresnick (2004)](#_ENREF_4) stated that more than 50 amino acids were contained in papaya latex. Milk as main ingredient of dangke is also main source protein. [Kustyawati & Tobing (2012)](#_ENREF_12) argued that the cow milk contained amino acids 1.13%.

**Physical Characteristics of Dangke**

 Table 4 exhibited influences of heat temperature and papain concentration on physical properties of dangke. Combination of the treatments had no effects on aw, pH and hardness (P>0.05), but showed significant different in yield (P<0.05). The lowest yield was observed at 90 °C heat temperature and 0.2% papain concentration. High heating temperature might be less effective as optimum temperature for its activity was 60-70 °C. Besides, low papain concentration would lead to low syneresis. This finding is in line with [Gaman *et al.* (1994)](#_ENREF_10). that the decline of papain activity was about 20% at 70 °C, pH 7.0, and heat temperature for 30 min. Papain is more desirable compared to other proteolytics such as bromelain and ficin since it has heating stabile properties, wider pH range, and highe purity. [Arreneuz & Pardede (2013)](#_ENREF_3) explained that in exceed enzyme concentration, the substrate availability was insufficient for enzyme activity.

Significant effects on dangke texture (Table 4) were observed as result of the treatments. The increasing enzyme concentration resulted in higher hardness, which might be caused by the cross-linking or gel matrix induced by papain. [Zusfahair *et al.* (2014)](#_ENREF_23) stated that protease degraded peptide bonds of aromatic amino acids such as phenylalanine, leucine and tyrosine. This is referred to proteolysis. Meanwhile, lipase induced degradation of milk fat to promote formation of a distinctive flavor, texture and chemical composition of a cheese. This reaction is usually called as lipolysis.

**Microbiological Analysis**

The microbiological profile of dangke is presented in Table 5. Interaction of heat temperatures and papain concentration was not significantly different (P>0.05), but significant effect of heating temperatures and papain concentration was observed on total coliform in dangke (P<0.05).

[Commission (2005)](#_ENREF_7) determined that maximum threshold for coliform contamination was 2 log cfu / g. Our result indicated that the contamination was under the threshold. The coliform is non-heat resistant, in accordance with ([Fitoni *et al.* (2013)](#_ENREF_8)) that heating treatment at more than 60 °C for 15 min could provide lethal effect to coliform bacteria such as *E. coli*. Besides, papain potentially had antibacterial properties due to presence of such components as *flavonoids, alkaloids, tannins, triterpenoids, seroid and saphonin* ([Miskiyah *et al.*, 2011](#_ENREF_14)).

Total number of molds and yeasts exceeded the maximum threshold in dairy products. The maximum limit of molds and yeasts contamination in dairy products (solid and semi-solid) was 10 cfu/g (USDA, 2001). High water activity might cause increase in total molds and yeasts. Most of yeasts could grow better at the sufficient water supply (Bryden, 2007).

**Organoleptic Quality**

Table 6 exhibits organoleptic evaluation (flavor, aroma, color and texture) of dangke. The heat temperature and papain concentration showed significant effects on the flavor (P<0.05). The score from panelists on the heat temperature treatment was 3.61-4.05, which was perceived as tasteless. The papain treatment also promoted tasteless flavor. This might be due to the absence of ripening process on dangke, while the bitter taste might be from papain.

Texture is a palpation or touch-associated sense. The organoleptic test on dangke texture was in a moderately soft. The soft texture of the dangke resulted from high water content. This finding is in accordance with [Yerlikaya & Karagozlu (2011)](#_ENREF_22) that texture is one of the consumer ratings for determining the food product quality and as direct observable parameter. Sensory attributes also reflected the consumer acceptance. Texture of cheese was influenced by several components such as water , protein and fat. The hardness is defined as the force of senses required to press the meal between the teeth, mouth and palate, which the positive absolute peak is obtained from the first suppresion.

**CONCLUSION**

The concentration of papain extracted from papaya latex was 401.67 mg/100 g, which was almost equivalent to the commercial papain of Merck (360.63 mg/100g), and these enzymes had similar molecular weight of 19.17 kDa. The best dangke was processed at 80 °C and added with 0.3% of papain in term of chemical properties and hedonic evaluation. In addition, high yield and hardness of dangke were obtained at 70 °C and papain concentration 0.4%. Microbiological quality of dangke processed at 90 °C and papain concentration 0.3% was under threshold of coliform, mold, and yeast contamination.

**REFERENCE**

**AOAC.** 2005. Official methods of analysis of AOAC International. AOAC International.

**Arief, I., T. Suryati, D. Afiyah, & D. Wardhani.** 2014. Physicochemical and organoleptic of beef sausages with teak leaf extract (Tectona grandis) addition as preservative and natural dye. International Food Research Journal21(5): 2033-2042.

**Arreneuz, S., & B. E. Pardede.** 2013. Pemanfaatan enzim papain dari getah buah pepaya (*Carica papaya* L) Dalam pembuatan keju cottage menggunakan bakteri *Lactobacillus bulgaricus*. Jurnal Kimia Khatulistiwa2(3).

**Bresnick, S.,** 2004. Intisari Kimia Organik. Jakarta: Penerbit Hiprokrates.

**Buriti, F. C., H. R. Cardarelli, T. M. Filisetti, & S. M. Saad.** 2007. Synbiotic potential of fresh cream cheese supplemented with inulin and Lactobacillus paracasei in co-culture with Streptococcus thermophilus. Food chemistry104(4): 1605-1610.

**Buwono, I. I. D., & M. Si.** 2000. Kebutuhan asam amino esensial dalam ransum ikan. Kanisius.

**Commission, E.,** 2005. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. J. Eur. Union L 338: 1–26.

**Fitoni, C. N., M. T. Asri, & M. T. Hidayat.** 2013. Pengaruh pemanasan filtrat rimpang kunyit (*Curcuma llonga*) terhadap pertumbuhan koloni bakteri *Coliform* secara in vitro. Jurnal Mahasiswa Teknologi Pendidikan2(3).

**Fox, P. F., P. L. McSweeney, T. M. Cogan, & T. P. Guinee.** 2004. Cheese: chemistry, physics and microbiology: general aspects. Academic Press.

**Gaman, P., K. Sherrington, & M. Gardjito.** 1994. Ilmu Pangan: Pengantar Ilmu Pangan, Nutrisi dan Mikrobiologi. Gadjah Mada University Press.

**[ISO] International Organization For Standardization.** (1996). Microbiology of food and animal feeding stuffs- General rules for microbiological examinations. ISO 7218:1996(E)

**Kustyawati, M. E., & D. Tobing.** 2012. Profil asam lemak dan asam amino susu kambing segar dan terfermentasi [Fatty acid and amino acid profile of fresh and fermented goat milk]. Jurnal Teknologi Dan Industri Pangan23(1): 47.

**Marfiyanti, F. V. K., S. M. Sayuthi, A. N. m. Al-Baarri, & A. M. Legowo.** 2013. Karakteristik dangke dari susu dengan waktu inkubasi berbeda pasca perendaman dalam larutan laktoferin. Jurnal Aplikasi Teknologi Pangan2(3).

**Miskiyah, S., Usmiati, & Mulyorini.** 2011. Pengaruh Enzim Proteolitik dengan Bakteri Asam Laktat Probiotik terhadap. JITV 16(4): 304-311.

**Monti, R., C. A. Basilio, H. C. Trevisan, & J. Contiero.** 2000. Purification of papain from fresh latex of Carica papaya. Brazilian Archives of Biology and Technology43(5): 501-507.

**Nitsawang, S., R. Hatti-Kaul, & P. Kanasawud.** 2006. Purification of papain from carica papaya latex: aqueous two-phase extraction versus two-step salt precipitation. Enzyme and Microbial technology39(5): 1103-1107.

**Osthoff, G., A. Hugo, & H. Venter.** 2002. Study of the Changes in Protein Fractions and Amino Acids of an Unfermented South African Dried Sausage.

**Putri, R. A., A. Kusrijadi, & A. Suryatna.** 2013. Kajian penggunaan amonium sulfat pada pengendapan enzim protease (papain) dari buah pepaya sebagai koagulan dalam produksi keju cottage. Jurnal Sains dan Teknologi Kimia4(2).

**Sani, R. N., F. C. Nisa, R. D. Andriani, & J. M. Maligan.** 2013. Analisis rendemen dan skrining fitokimia ekstrak etanol mikroalga laut Tetraselmis chuii [in press april 2014]. Jurnal Pangan dan Agroindustri2(2): 121-126.

**Syah, S. P., C. Sumantri, I. I. Arief, & E. Taufik.** 2016. Isolation and identification of indigenous lactic acid bacteria by sequencing the 16S rRNA from dangke, a traditional cheese from Enrekang, South Sulawesi. Pakistan Journal of Nutrition : in press.

**Winarno, F.,** 1993. Pangan, gizi, teknologi dan konsumen. Gramedia Pustaka Utama. Jakarta: 165-166.

**Yerlikaya, O., & C. Karagozlu.** 2011. Effects of ripening period on textural and sensory properties of capper cheeses. Journal of Animal and Veterinary Advances10(9): 1171-1176.

**Zusfahair, Z., D. R. Ningsih, & F. N. Habibah.** 2014. Karakterisasi papain dari daun pepaya (carica papaya l. Characterization of papain from *Carica papaya* L. LEAVES. Molekul9(1): 44-55.

Table 1 Difference of papain concentration with several source using spectrophotometer (650 nm)

|  |  |  |
| --- | --- | --- |
| No | Types of Papain enzymes | Papain concentration (mg/100g) |
| 1.2.3.4. | Commercial Papain PayaaCommercial Papain MerckPapain from this study1% Dilution of papain form the studyb | 7.14360.63401.6720.55 |

a Commercially available papain. b Papain used in dangke production

Table 2 The results of chemical characteristics of dangke on different temperatures and papain concentrations

|  |  |  |  |
| --- | --- | --- | --- |
| Variables | Heating temperature | Papain concentration | Mean±SD |
| K1 | K2 | K3 |
| -----------------%bb---------------- |  |
| Protein | S1 | 14.36±1.44 | 16.32±0.75 | 16.24±1.31 | 15.64±1.16ab |
| S2 | 14.80±1.41 | 16.86±1.44 | 17.10±1.04 | 16.26±1.29a |
| S3 | 11.88±1.58 | 16.05±1.17 | 14.73±2.79 | 14.22±1.84b |
| Mean±SD | 13.68±1.47b | 16.41±1.12a | 16.03±1.71a |  |
| Water | S1 | 60.46±3.05 | 59.78±2.79 | 59.69±1.80 | 61.05±2.77 |
| S2 | 60.74±1.88 | 58.75±0.41 | 60.57±1.77 | 59.66±2.07 |
| S3 | 61.96±3.37 | 60.77±3.01 | 58.53±4.30 | 59.60±2.62 |
| Mean±SD | 59.98±2.54 | 60.02±1.35 | 60.42±3.56 |  |
| Fat | S1 | 12.62±2.28 | 13.76±3.78 | 15.62±6.90 | 14.00±4.32 |
| S2 | 13.30±0.46 | 15.19±2.05 | 13.55±1.67 | 14.01±1.39 |
| S3 | 10.98±1.16 | 12.09±2.25 | 17.51±8.27 | 13.53±3.89 |
| Mean±SD | 12.30±1.30 | 13.68±2.68 | 15.56±5.61 |   |
| Ash | S1 | 2.15±0.25 | 2.32±0.14 | 2.19±0.08 | 2.22±0.15 |
| S2 | 2.24±0.34 | 2.31±0.21 | 2.36±0.17 | 2.30±0.24 |
| S3 | 2.08±0.07 | 2.21±0.16 | 2.08±0.25 | 2.12±0.16 |
| Mean±SD | 2.15±0.22 | 2.28±0.17 | 2.21±0.16 |  |
| Carbohydrate | S1 | 10.39±2.85 | 7.80±6.79 | 6.24±5.75 | 8.14±5.13 |
| S2 | 9.86±3.19 | 5.88±2.51 | 6.86±3.82 | 7.53±3.17 |
| S3 | 13.08±1.62 | 8.86±1.51 | 7.13±1.05 | 9.69±1.39 |
| Mean±SD | 11.11±2.55a | 7.51±3.60ab | 6.74±3.54b |  |

S1 : Temperature 70 °C , S2 : Temperature 80 °C, S3 : Temperature 90 °C, K1: concentration 0.2%, K2: concentration 0.3%, K3: concentration 0.4%. a,b) Values with superscript letters behind the different figures in the same column indicate significant differences (P<0.05).

Table Total amino acids of dangke treated with different temperatures and papain concentrations

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Amino acid | S1K1 | S1K2 | S1K3 | S2K1 | S2K2 | S2K3 | S3K1 | S3K2 | S3K3 |
| ------------------------% W/W-------------------------- |
| Aspartic acid | 1.64 | 1.47 | 1.48 | 1.52 | 1.45 | 1.66 | 1.09 | 1.38 | 2.73 |
| Glutamic acid | 5.90 | 5.43 | 4.55 | 5.53 | 5.33 | 5.96 | 3.72 | 4.84 | 8.20 |
| Serine | 1.13 | 1.05 | 1.12 | 1.06 | 1.04 | 1.24 | 0.76 | 0.96 | 2.08 |
| Glycine | 0.34 | 0.34 | 0.36 | 0.33 | 0.31 | 0.36 | 0.21 | 0.27 | 0.69 |
| Alanine | 0.65 | 0.59 | 0.64 | 0.60 | 0.57 | 0.66 | 0.42 | 0.53 | 1.17 |
| Arginine | 0.72 | 0.67 | 0.70 | 0.67 | 0.63 | 0.72 | 0.44 | 0.56 | 1.33 |
| Tyrosine | 1.09 | 1.02 | 1.05 | 1.04 | 1.01 | 1.13 | 0.71 | 0.90 | 1.85 |
| *Total non-essential amino acids* | 11.47 | 10.57 | 9.9 | 10.75 | 10.34 | 11.73 | 7.35 | 9.44 | 18.05 |
| Histidine | 2.44 | 0.45 | 0.51 | 0.47 | 0.43 | 0.48 | 0.30 | 0.37 | 0. 89 |
| Threonine | 0.71 | 0.63 | 0.82 | 0.67 | 0.59 | 0.73 | 0.47 | 0.60 | 1.63 |
| Methionine | 0.79 | 0.69 | 0.52 | 0.76 | 0.71 | 0.83 | 0.50 | 0.64 | 0.95 |
| Valine | 1.27 | 1.15 | 1.24 | 1.19 | 1.13 | 1.26 | 0.82 | 1.06 | 2.23 |
| Phenylalanine | 1.23 | 1.14 | 1.00 | 1.16 | 1.13 | 1.23 | 0.80 | 1.03 | 1.80 |
| I-leucine | 1.12 | 0.99 | 1.01 | 1.03 | 0.97 | 1.09 | 0.73 | 0.93 | 1.82 |
| Leucine | 1.96 | 1.79 | 1.94 | 1.83 | 1.78 | 1.94 | 1.26 | 1.62 | 3.44 |
| Lycine | 1.44 | 1.44 | 1.42 | 1.43 | 1.41 | 1.57 | 0.94 | 1.21 | 2.54 |
|  *Total essential amino acids* | 10.96 | 8.28 | 8.46 | 8.54 | 8.15 | 9.13 | 5.82 | 7.46 | 14.41 |
| Total amino acid | 22.43 | 18.84 | 18.36 | 19.28 | 18.49 | 20.87 | 13.16 | 16.89 | 33.35 |

S1K1 : Temperature 70 °C and concentration 0.2%, S1K2 : Temperature 70 °C and concentration 0.3%, S1K3 : Temperature 70 °C and concentration 0.4%, S2K1 : Temperature 80 °C and concentration 0.2%, S2K2 : Temperature 80 °C and concentration 0.3%, S2K3 : Temperature 80 °C and concentration 0.4%, S3K1 : Temperature 90 °C and concentration 0.2%, S3K2 : Temperature 90 °C and concentration 0.3%, S3K3 : Temperature 90 °C and concentration 0.4%

Table 4 Results of physical characteristics of dangke on different heating temperatures and concentrations of papain

|  |  |  |  |
| --- | --- | --- | --- |
| Variables | Heating temperature | Papain concentration | Mean±SD |
| K1 | K2 | K3 |  |
| aw | S1 | 0.86±0.01 | 0.87±0.01 | 0.87±0.01 | 0.87±0.01 |
| S2 | 0.87±0.00 | 0.87±0.01 | 0.87±0.01 | 0.87±0.01 |
| S3 | 0.87±0.01 | 0.87±0.01 | 0.87±0.01 | 0.87±0.01 |
| Mean±SD | 0.87±0.00 | 0.87±0.01 | 0.87±0.01 |  |
| pH | S1 | 6.62±0.06 | 6.62±0.12 | 6.58±0.12 | 6.61±0.10 |
| S2 | 6.63±0.09 | 6.62±0.09 | 6.61±0.08 | 6.62±0.09 |
| S3 | 6.58±0.16 | 6.57±0.16 | 6.52±0.19 | 6.56±0.17 |
| Mean±SD | 6.61±0.10 | 6.60±0.12 | 6.57±0.13 |  |
| Yield (%) | S1 | 15.54±1.01a | 15.77±1.18a | 16.96±1.62a | 16.09±1.27a |
| S2 | 16.79±2.91a | 15.66±2.31a | 15.23±1.66a | 15.89±2.29a |
| S3 | 10.29±0.55b | 15.93±1.01a | 16.10±1.79a | 14.11±1.12b |
| Mean±SD | 14.21±1.49b | 15.79±1.50a | 16.10±1.69a |  |
| Hardness (gf) | S1 | 600.9±150.8 | 954.7±297.1 | 829.0±63.9 | 794.8±170.6ab |
| S2 | 770.8±106.9 | 752.0±18.5 | 1020.6±60.6 | 847.8±62.0a |
| S3 | 435.8±42.4 | 719.1±32.8 | 887.1±132.3 | 680.7±69.1b |
| Mean±SD | 602.5±100.0b | 808.6±116.1a | 912.2±85.6a |  |

S1 : Temperature 70 °C , S2 : Temperature 80 °C, S3 : Temperature 90 °C, K1: concentration 0.2%, K2: concentration 0.3%, K3: concentration 0.4%. a,b) Values with superscript letters behind the different figures in the same column indicate significant differences (P<0.05).

Table 5 Mean of coliforms, molds and yeasts of dangke as result of different heating temperatures and concentrations of papain

|  |  |  |  |
| --- | --- | --- | --- |
| Variables | Papain concentration | Heat temperature | Mean±SD |
| S1 | S2 | S3 |
| --------------------- Log cfu/g ----------------- |  |
| Coliform | K1 | 2.36±0.53 | 2.56±0.47 | 1.93±0.10 | 2.28±0.55a |
| K2 | 2.53±0.49 | 2.32±0.82 | 1.49±0.61 | 2.11±0.18ab |
| K3 | 1.73±0.24 | 1.67±0.92 | 1.38±0.42 | 1.59±0.34b |
| Mean±SD | 2.21±0.40a | 2.18±0.33a | 1.60±0.11b |   |
| Moids and yeast | K1 | 1.84±0.46 | 2.45±0.40 | 2.31±1.02 | 2.20±0.55 |
| K2 | 1.76±0.83 | 1.94±0.63 | 0.91±1.21 | 1.54±0.51 |
| K3 | 1.11±0.91 | 1.03±0.79 | 1.97±0.63 | 1.37±0.12 |
| Mean±SD | 1.57±0.33 | 1.81±0.49 | 1.73±0.55 |   |

S1 : Temperature 70 °C , S2 : Temperature 80 °C, S3 : Temperature 90 °C, K1: Concentration 0.2%, K2: Concentration 0.3%,K3: Concentration 0.4%. a,b) Values with superscript letters behind the different figures in the same column indicate significant differences (P<0.05).

Table 6 Hedonic quality test results of Dangke due to different heating temperatures and papain concentrations

|  |  |  |  |
| --- | --- | --- | --- |
| Variables | Heat temperature | Papain concentration | Mean±SD |
| K1 | K2 | K3 |
| Flavor | S1 | 4.03±0.41 | 4.06±0.41 | 4.07±0.45 | 4.05±0.43a |
| S2 | 4.10±0.48 | 4.06±0.52 | 3.97±0.56 | 4.04±0.52a |
| S3 | 3.13±0.34 | 3.80±0.48 | 3.90±0.60 | 3.61±0.48b |
| Mean±SD | 3.75±0.41 | 3.98±0.47 | 3.98±0.54 |  |
| Color | S1 | 4.00±0.69 | 3.86±0.77 | 3.73±0.69 | 3.63±1.01 |
| S2 | 3.96±0.72 | 3.73±0.78 | 3.83±0.75 | 3.58±0.99 |
| S3 | 3.46±0.51 | 3.83±0.79 | 3.93±0.87 | 3.59±0.80 |
| Mean±SD | 3.81±0.64 | 3.81±0.78 | 3.83±0.77 |  |
| Aroma | S1 | 3.73±0.90 | 3.66±1.06 | 3.48±1.07 | 3.63±1.01 |
| S2 | 3.73±0.87 | 3.58±1.07 | 3.41±1.04 | 3.58±0.99 |
| S3 | 3.50±0.51 | 3.41±1.07 | 3.86±1.07 | 3.59±0.80 |
| Mean±SD | 3.65±0.76 | 3.55±1.07 | 3.59±0.98 |   |
| Texture | S1 | 3.50±0.97 | 3.33±0.88 | 3.33±0.76 | 3.39±0.87 |
| S2 | 3.36±0.96 | 2.97±0.85 | 3.00±0.87 | 3.11±0.89 |
| S3 | 2.76±0.57 | 3.47±0.89 | 3.47±0.89 | 3.23±0.79 |
| Mean±SD | 3.21±0.84 | 3.26±0.88 | 3.27±0.84 |  |

Taste : 1 (Very sour).2 (sour).3 (sourish).4 (Tasteless).5 (Very tasteless). Color :1 (Yellow.2 (yelowish).3 (whitish).4 (white).5 (very white). Aroma : 1 (very rancid). 2 (putrid). 3 (less putrid). 4 (special milk). 5 (not putrid). Texture: 1 (Very hard. Not padded) 2 (hard).3 (Paddish).4 (Soft, padded).5 (very padded).a,b) Values with superscript letters behind the different figures in the same column indicate significant differences (P<0.05).

225 kDa

150 kDa

100 kDa

75 kDa

50 kDa

35 kDa

25 kDa

19.17 kDa

 **M a b c**

Figure 1 Results of enzyme papain electrophoresis . (M) protein marker, (a) papain enzyme of this study, (b) Merck papain enzyme, (c) the enzyme papain dilutions of 1% BM = 19.17 kDa.