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PHYSICO-CHEMICAL, AND SENSORY PROPERTIES OF SOY BASED GOUDA CHEESE ANALOG MADE FROM DIFFERENT CONCENTRATION OF FAT, SODIUM CITRATE AND VARIOUS CHEESE STARTER CULTURES

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Abstract

Gouda cheese analog (GCA) was made using soy protein isolate (SPI), skim milk powder (SMP), fat (palm fat and butter fat), and water (W) at optimal ratio of SPI : SMP : F : W = 14 : 6 : 20 : 60. The effects of butter fat, sodium citrate, and cheese starter culture on the sensory properties of ripened product were assessed by preference test, hedonic test, and the texture profile analysis (TPA) of GCA. The free fatty acids, water-soluble nitrogen, and reduction in pH value of progel were also measured. The use of 100% butter fat (BF) produced strong Gouda flavor. It could be due to the fatty acids content in BF; in contrast, product with 100% palm fat (PF) produced tasteless GCA. It might be due to fatty acids content in PF, middle, and long chain fatty acids. Single cheese starter culture could not develop Gouda flavor during ripening. The use of mixed fat (50% BF and 50% PF) and mixed cheese starter culture together with *Brevibacterium linens* developed a suitable characteristic flavor of Gouda product during ripening. The addition of 0.5% sodium citrate could improve the flavor; nevertheless, it reduced the stability of texture.

Abstract

Sifat-sifat Fisik Kimiawi dan Sensori Keju Gouda Analog Berbasis Kedelai yang Terbuat dari Variasi Konsentrasi Lemak dan Sodium Sitrat dan Variasi Starter Culture. Gouda cheese analogue (GCA) diproduksi dengan menggunakan soya protein isolate (SPI) tepung susu skim (SMP) lemak (F) yaitu lemak nabati dan lemak *butter* dan air (W) dengan perbandingan optimal antara SPI : SMP : F : W = 14 : 6 : 20 : 60. Pengaruh lemak *butter*, sodium citrate, kultur starter keju pada sifat-sifat sensoris produk keju diuji dengan uji kesukaan, hedonic test, dan analisis profil tekstur (TPA) dari GCA. Asam lemak bebas, nitrogen terlarut air, penurunan nilai pH progel juga diukur. Penggunaan 100% lemak *butter* (BF) menghasilkan aroma gouda yang kuat. Ini disebabkan kandungan asam lemak dalam lemak *butter*, sebaliknya produk dengan 100% lemak nabati (PF) menghasilkan gouda yang hambar. Ini mungkin disebabkan oleh kandungan asam lemak dalam PF yang berupa rantai menengah dan rantai panjang. Kultur starter keju yang tunggal tidak dapat mengembangkan flavor gouda selama pemeraman. Penggunaan lemak campuran (50% BF dan 50% PF) dan kultur starter keju yang campuran bersama sama dengan *Brevibacterium linens* mengembangkan aroma karakteristik dari gouda selama pemeraman. Penambahan 0.5% sodium citrate dapat memperbaiki aroma namun hal ini mereduksi stabiltas tekstur produk.

Keywords: butter fat, citrate, flavor, GCA, soy protein isolate

1. Introduction

Soy protein has been widely used as packaging material, such as *soy protein like sheets* [1], and also in wood preservative substance [2] besides its application in food. This paper discusses the use of soy protein in food industry.

Our previous study [3] found that satisfactory texture properties of Gouda analogue made from soy protein was produced by heating and suitable formulation as follows: SPI : SMP : PF : W = 14 : 6 : 20 : 60, and the vegetable fat (palm fat) was used to reduce or to substitute animal fat. However, Gouda cheese flavor was not developed. We found that palm fat provides good texture property of Gouda analogue, even though it provides no development of Gouda cheese flavor. In this research butter fat (BF) was used to improve the flavor of GCA. Volatile fatty acids play an important role in developing the cheese aroma, as reported by Engelsa *et al*. [4]. Other compounds, such as fat, lactose and amino acids also contribute to aroma development.

Mahdy *et al*. [5] reported that the use of soy protein isolated in cheddar cheese improved the sensory properties of cheese after 5 months ripening and gave no bitter-off flavor. Thus, they concluded that soy protein could be used to improve the quality of cheese, and the addition of 5% soy protein isolate could be recommended to improve the texture of cheddar type soy supplemented cheese. Sodium citrate in dairy product or in dairy analogue has been used in soy bean low fat spread [6] mozzarella cheese analogue [7]. The sodium citrate increased flow ability of the spread and decreased oiling off finished product [6] in mozzarella; cheese analogue sodium citrate has no effect on texture profile analysis of product with regard to fracturability and hardness [7]. In our present study, the experiment was carried out to improve the flavor of ripened soy protein isolate based GCA. The purpose of this experiment is to study the effect of butter fat (BF), sodium citrate, and various cheese cultures on sensory properties of ripened Gouda cheese analogue.

2. Methods

Soy protein isolate SUPRO 760 (SPI) was obtained from Protein Technology International St. Louis, MO USA, through its subsidiary in Frankfurt, Germany; skim milk powder was obtained from Schwarzdwaldmilch GmbH, Offenburg, Germany; butter fat was obtained from Institute of Food Technology, Hohennheim University, Stuttgart, Germany; and fractionated, hydrogenated and deodorized palm fat (Palmowar F 35 melting point 35 °C) was obtained from Aarhus Olie, Denmark. Single cheese starter culture (*Leuconostoc mesenteroides*, *Lactococcus lactis* ssp *lactis* biovar *diacetylactis* and mixed cheese culture (Probat M2) consisting of *Leuconostoc mesenteroides*, *Lactococcus lactis* ssp *lactis* biovar *diacetylactis, L. lactis* ssp.*lactis,* and *L. lactis* ssp *cremoris* were obtained from Wiesby laboratory, Niebuell, Germany; *Leuconostoc paramesenteroides* R.08, *Lactococcus lactis* ssp *diacetylactis* R.22, *Enterococcus faecalis* ssp *liquefaciens* R.32, *Lactobacillus casei* ssp *casei* R 35, and *Lactobacillus cremoris* R 48 were obtained from Prof. Hosono's laboratory, Shinshu University, Japan; red smear culture (*Brevibacterium linens*) was obtained from Dr Drewes GmbH Laboratory, Seesen, Germany; and sodium citrate was obtained from BK Ladenburg, Germany.

Standard processing procedure was conducted to prepare Gouda analogue based on soy protein isolate, conducted in previous study shown in Figure 1 [3]. To study the factors and their effect on sensory properties of GCA, butter fat, sodium citrate, and cheese cultures were used as dependent variables.

The level of butter fat: (0, 50, 75, 100% wet basis); the level of sodium citrate: (0, 0.5, 1.0, 1.5% wet basis); the concentration of butter fat (BF) and Palm fat (PF) used in this experiment was 50%; effect of cheese culture (six various cultures): A) *Leuconostoc mesenteroides*; B) *Lactococcus lactis* ssp *lactis* biovar *diacetylactis*; C) a mixed cheese culture consisting of culture A, culture B, *Lactococcus lactis* ssp *lactis* and *L. lactis* ssp

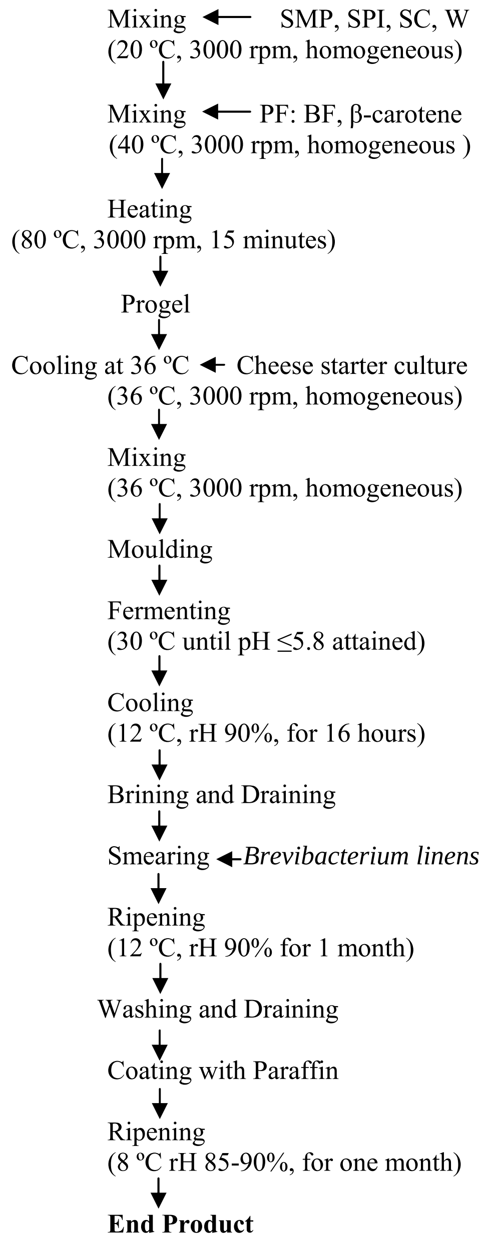

Figure 1. Manufacture of GCA [3]

cremoris; D) a mixed culture consisting *Leuconostoc paramesenteroides* R.08, *Lactococcus lactis* ssp *diacetylactis* R.22, *Enterococcus faecalis* ssp *liquefaciens* R.32, and *Lactobacillus casei* ssp *casei* R.35; E) a mixed culture consisting of *Leuconoistoc paramesenteroides* R.08, *Lactococcus lactis* ssp. *diacetylactis* R.22, *Enterococcus faecalis* ssp *diacetylactis* R32, and *Lactobacillus cremoris* R 48. 0.5%-wet basis of sodium citrate was used in each formula of Gouda analogue manufactured. Gouda analogue was prepared at 2500 g in every experiment, and were carried out in triplicate.

Texture Evaluation and Chemical Analysis. Texture Profile analysis (TPA) was determinate according to Yang and Taranto [8]. Total nitrogen content was determined by Kjeldhal procedure, and water soluble nitrogen was analyzed according to Kuchro and Fox [9]. The pH value progel during incubation was measured by pH meter (192 WTW with glass electrode Ingold Weilheim, Germany). Free fatty acids of Gouda analogue was estimated by Godinko and Fox [10].

Sensory Evaluation: a) Preference test. The aim of the experiment with various BF was to determine the simultaneous effect of BF treatment in improving the intensity of the taste and flavor of ripened GCA and also its acceptability. The procedure of preference test using hedonic scale was described by Jellineck [11]; and Hedonic scale to evaluate the acceptability of ripened GCA as described by Fliedner and Wilhelmi [12].

Chemical Analyses: Free fatty acids. Evaluation of the activities of lipolytic enzyme in product was done as described by Godinco and Fox [10]. 1.5 g blended sample and 5 mL destilated water (45 °C) were placed in centrifuge glass. After added with 5 mL mix solution of 0.5 M H3PO4 and H2SO4 (1:1), it was then centrifuged (3000 rpm/min = 1300 x g) for 25 minutes, then the organic phase was removed and mixed with 20 mL diethylether. 10 mL of this solution, 5 drops of blue bromothymol, and 10 mL neutralized ethanol 95% were placed in Erlenmeyer to titriert with 0.01 N alcohol-NaOH. The used total volume of 0.01 N alcohol-NaOH was equivalent with free fatty acid in product.

Water soluble nitrogen. Test to determine the water soluble nitrogen of product as indicator for the activity of proteolitic enzyme in product was carried out acoording to Kuchroo and Fox [9]. 3g of the grated sample and 45 mL warm distillate water in erlenmeyer were extracted in water bad rotary shaker (40 °C) for 90 minutes, then in centrifugal at 3000 x g x 30 min and filtered with glass woll. The filtrate is analyzed then with Kjeldahl-method.

Texture profile analysis [8] of the textural properties of GCA was measured with Instron Universal testing machine (Instron Corp, Canton, MA). An Instronmodel TM with a CCM compression load cell was used to measure the TPA. A rectangle plate with a diameter of 50 mm was attached to the moving crosshead. The speed of the crosshead was set 20 mm/min in both upward and downward directions. The recording chart speed was 50 mm/min. Six cubes of sample with side of 25 mm were obtained from ripened GCA using a sharp knife. Samples were equilibrated to 20 ± 1 °C and tested at the same temperature. The deformation was set of 80%, meaning only 20 mm sample compressed. From these tests, six textural parameters were evaluated as shown in Table 2.

Statistical Analysis. The experiment was designed according to fixed constant model [13], and the results were expressed as mean ± standard deviation. Means were analyzed by one-way analysis of variance (ANOVA) using SAS computer software package (SAS Institute Inc. Cary, NC, USA).

3. Results and Discussion

Effect of butter fat. Butter fat (BF) resulted in positive effect on taste and flavor of the ripened GCA. The rank and rank sum of ripened GCA with various BF are shown in Table 1. It is evident that the substitution of Palm Fat with BF up to 100% increased the Gouda flavor development significantly as shown by the most preferred by panelists as compared to others, due to its strong Gouda flavor. On the other hand, the Gouda analogue with 0% BF was the least preferred and had flat and tasteless flavor. The difference between both products was significant (p = 0.01). Furthermore, substitution of PF to BF, up to 50%, was found to be the second preferred by panelists and there was no significant different as compared to 100% level of BF there. The panelists noticed that GCA with 50% level of BF had a mild taste and an appropriate aroma. It has an advantage because people under stress prefer fat foods (27). The GCA having 50% BF and 50% PF can be offered to the public as an alternative healthy food.

Table 1. Rank and Rank Sum of GCA Prepared with SPI: SMP: Water =14:6:60 Formulation and Butter Fat Indicated, Cheese Culture C was Used. It Consist of *Leuconostoc mesenteroides*, *Lactococcus lactis* ssp *lactis* biovar *diacetylactis*, *Lactococcus lactis* ssp *lactis* and *L. lactis* ssp.*cremoris*

Butter wet basis (%)	n	Flavor intensity	
		Rank [a)]	Hedonic scale
00.0	31	4	97**
50.00	31	3	71
75.00	31	2	82
100.00	31	1	60**

[a)] 1 = strong flavor, 4 = weak flavor
** significant ($p<0.01$)
SPI= Soy protein isolate, SMP= skim milk powder.

The effects of BF on free fatty acids level in GCA are presented in Figure 2. A steady increase of free fatty acids content was observed during ripening in all GCAs. Free fatty acids of GCA with 100% level of BF and 50% level of BF had no significant difference during ripening.

On 60th day of ripening, the free fatty acids of GCA with 50% level of BF showed slightly higher free fatty acid (2.86 mL 0.01N KOH/g product as compared to 100% level of BF (2.80 mL 0.01N KOH).

Effects of sodium citrate. The use of 5% sodium citrate in GCA gave a positive effect to flavor, but not to texture. The percentage of "like" preference of hedonic scale for various sensory attributes appearance, texture, flavor and overall acceptability of Gouda analogue containing 0% sodium citrate (control) and 0.5% sodium citrate are presented in Figure 3.

The use of 05% sodium citrate reduced the elasticity value of ripened GCA significantly. The sensory properties confirmed and validated the results of TPA by Yang and Taranto [8] as shown in Table 2.

Six parameters of TPA (fracturability, hardness, cohesiveness, elasticity, gumminess and chewiness) were obtained from measuring by Yang and Taranto method [8]. The use of 0.5% sodium citrate in ripened

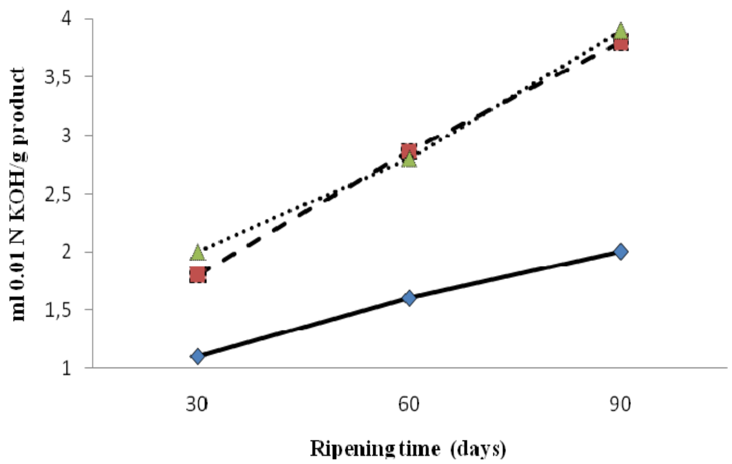

Figure 2. Free Fatty Acids in GCA. All Products Prepared with SPI, SMP, F and W = 14:6:60 Formulation with Butter Fat. Mix Cheese Starter Cultures C was Used. It consists of *Leuconostoc mesenteroides, Lactococcus lactis* ssp *lactis* biovar *diacetylactis, L. lactis* ssp *lactis, L. lactis* ssp *cremoris*) and for Ripening Process *Brevibacterium linens* Culturewas Used. 0% PF (♦), 50% BF (■), 100% BF (▲)

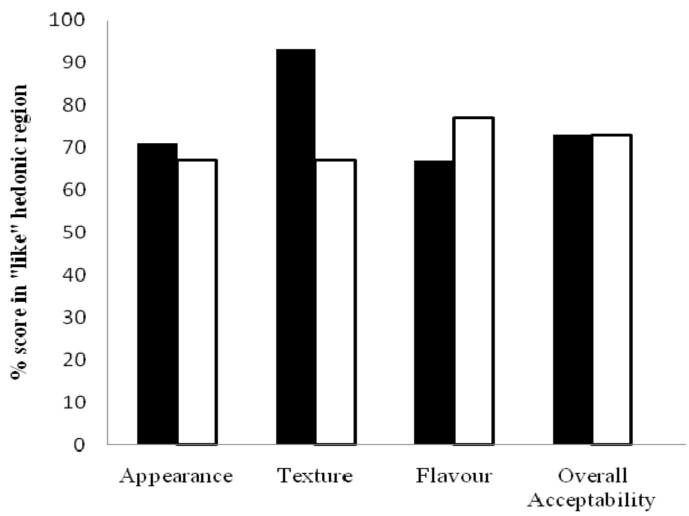

Figure 3. Acceptability of GCA Containing Sodium Citrate. Panelists n= 31, all Products Prepare with SPI: SMP :PF:BF: W = 14: 6: 10:10:60 Formulation. Mixed Cheese Culture C Consisted of *Leuconostoc mesenteroides*, *Lactococcus lactis* ssp *lactis* biovar. *diacetylactis, L. lactisssplactis, L. lactis* ssp *cremoris*, and Ripening Cheese Culture *Brevibacterium linens* were Used. 0% Sodium citrate (■),0.5% Sodium Citrate (□)

Table 2. Texture Profile Analysis (TPA) of Ripening Product Prepared with Ratio from SPI: SMP: BF: PF: Water = 14: 6:10:10:6 and Sodium Citrate Indicate [a)]Cheese starter culture C was used. It consist of *Leuconostoc mesenteroides*, *Lactococcus lactis* ssp *lactis* biovar *diacetylactis*, *Lactococcus lactis* ssp *lactis* and *L. lactis* ssp *cremoris C*

Sodium Citrate (%w/w)	Fracturability (Newton=N)	Hardness (N)	Cohesiveness	Elasticity (cm)	Gumminess (N)	Chewiness (N-cm)
0.0	69.50±3.95a	257.8±16.93a	0.055±0.006a	0.315±0.08a	14.23±2.18a	4.49±0.818a
0.5	45.68±3.06b	249.4±11.67a	0.051±0.004ab	0.205±0.02b	12.83±0.97a	2.63±0.301b
1.0	35.58±1.99b	170.2±07.02b	0.045±0.002b	0.180±0.02b	07.75±0.24b	1.39±0.182c

a) Each value is mean±SD (n=6) mean in the same column with different letters are significantly different (p=0.05)

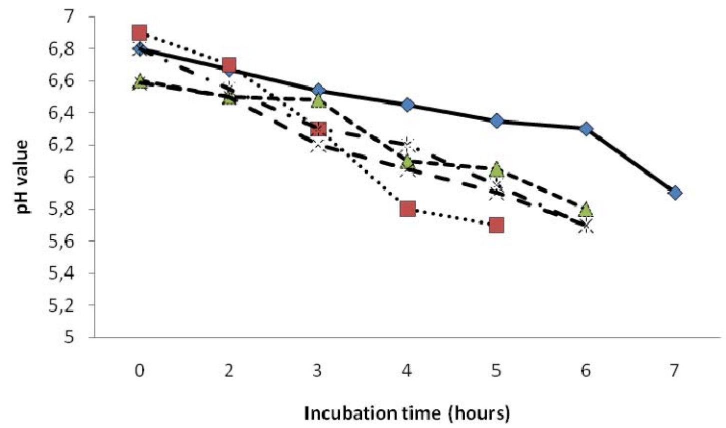

Figure 4. Reduction pH Value of Progel with Various Cultures. All Products Prepared with SPI:SMP;PF:BF:W=14:6:10:10:60 Formulation and 0,5% Sodium Citrate, Various Culture A) *Leuconostoc mesenteroides*, B) *Lactococcus lactis* ssp *lactis* biovar *diacetylactis*, C) a Mixed Cheese Culture Consisting of Culture A, Culture B, *Lactococcus lactis* ssp *lactis* and *L lactis* ssp *cremoris,* D) a Mixed Culture Consisting *Leuconostoc paramesenteroides* R.08, *Lactococcus lactis* ssp diacetylactis R22, *Enterococcus faecalis* ssp liquefaciens R 32, *Lactobacillus casei* ssp casei R 35; E) a Mixed Culture Consisting of *Leuconoistoc paramesenteroides* R 08, *Lactococcus lactis* ssp. diacetylactis R 22, *Enterococcus faecalis* ssp diacetylactis R32, dan *Lactobacillus cremoris* R 48. Culture A (♦),Culture B (■),Culture C (▲),Culture D (X), Culture E (†)

GCA decreased the value of all parameters of TPA as shown by significantly lower fracturabiliy, elasticity and chewiness of Gouda analogue than control. However the hardness of ripened GCA with 0.5% sodium citrate had no significant difference with control (240 ± 11.67N and 257.8 ± 16.93N, respectively). Hence, 0.5% sodium citrate had negative effect on TPA of the ripened soy protein based GCA. It is interesting to study the use of sodium citrate less than 0.5% to improve both flavor and TPA of the ripened GCA.

The Effect of Cheese culture. The effect of cheese culture on the acidification process of progel is presented in Figure 4. Acidification of progel was faster using culture B, D, and E as compared to culture A and C: 4 to 6 hours to reach pH 5.8 or lower. Culture D or E of *dadih cultures* was relative rapid in acid formation due to capability to adapt the new media.

On the other hand, culture A reached pH 5.8 after more than 7 hours. The acidification process of progel with culture C was moderate: 6 hours to reach pH 5.8. Short time of acidification process was really needed to get optimum hardness of gel. After attaining the pH 5.8 in the progel, it was transferred into cool room to form a stable gel. Although the acidification process of culture D or E was shorter to reach pH 5.8 of progel, these culture were not appropriate for GCA, because GCA with culture D or E had distinct beany flavor; Culture C was the best starter culture for GCA. The implementation of various starter culture in soy based GCA was not always appropriate; it was not easy to create soy based GCA starter culture. On the other hand, the positive effect of using soy protein isolate in this product did not produced waste.

The usage of soy protein preparate like SPI in cheese product seems to be appropriate. To strengthen this argument about the usage of SPI, we could refer to the research of Jana and Mandal [28]. That research indicated that the addition of milk protein concentrate in the manufacturing of mozzarella cheese increases the yield of product up to 6.31% compare to control [28].

The Hedonic scale of colour/appearance, texture flavor and overall acceptability of ripened GCA are clearly affected by various cultures used as shown in Figure 5. This result provides information that the GCA with culture C was accepted, as shown by 80% panellists scored "like" by hedonic scale. On the other hand, all various sensory attributes and overall acceptability of the Gouda analogue with culture A and B were not accepted by panelists with the overall acceptability 45% and 70%, respectively.

The GCA with culture C (mixed cheese cultures) might have more flavor substances in comparison to single culture A and B. These responses were in agreement with water soluble nitrogen (WSN) of tested Gouda analogue (Figure 5).

Steady increase in WSN was observed during ripening period for all three Gouda cheese analogs, and the GCA with mixed cheese culture (culture C) showed highest content of WSN (Figure 6). It could be suggested that the mixed cheese culture C during ripening possibly produced various proteolytic enzymes to accelerate protein-hydrolysis and positively contribute to sensory properties.

Flavor of cheese is an important sensory characteristic and together with appearance and texture play important role in determining product quality [14]. Banks *et al.* [15] reported that fat content in cheese product might responsible for mouth feel. Protein and fat composition in cheese, particularly its derivative compounds produced by microbial enzymes from cheese starter

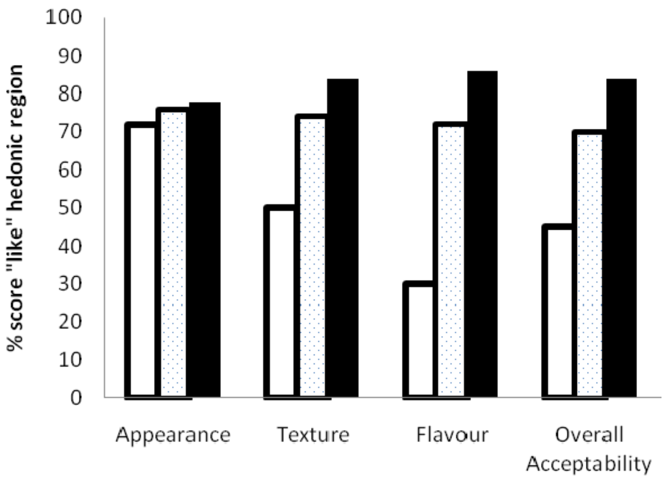

Figure 5. Acceptability of GCA with Various Cheese Starter Cultures. A) ***Leuconostoc mesenteroides*, B) *Lactococcus lactis* ssp *lactis* biovar *diacetylactis*, C) A Mixed Cheese Culture Consisting of Culture A, Culture B, and *Lactococcus lactis* ssp *lactis* and *L lactis* ssp *cremoris,* All Products Prepared with SPI:SMP; PF:BF:W = 14:6:10:10:60 Formulation and 0.5% Sodium Citrate. Culture A (□), Culture B (□), Culture C (■)**

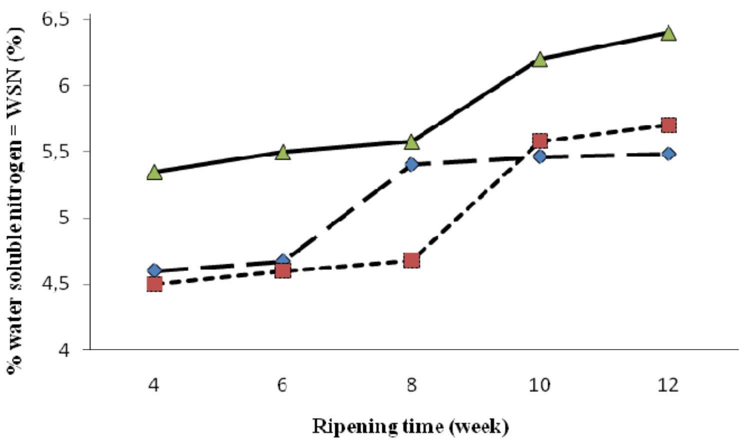

Figure 6. Water Soluble Nitrogen (WSN) of Product with Various Type of Cheese Culture. A) *Leuconostoc mesenteroides*, B) *Lactococcus lactis* ssp *lactis* biovar *diacetylactis*, C) a Mixed Cheese Culture Consisting of Culture A, Culture B, and *Lactococcus lactis* ssp *lactis* and *L lactis* ssp *cremoris,* All Products Prepared with SPI:SMP; PF:BF:W = 14:6:10:10:60 Formulation and 0,5% Sodium Citrate. Culture A (♦), Culture B (■), Culture C (▲)

culture or other lactic acid bacteria during ripening are potential contributor to develop aroma. We suggest that the different fat composition in GCA clearly influences the taste and flavor of ripened GCA. Palm fat in Camembert cheese-like product [16] and in cholesterol free Camembert cheese [17] had been used successfully. However, the use of 100% PF in GCA resulted in flat or tasteless flavor. Therefore, we hypothesized that the use of BF in Gouda analogue could improve the taste and the flavor of product.

Our results proved that the addition of 50% BF improved the flavor of the ripened Gouda analogue. The free fatty acid of the GCA with 50% BF had almost the same quantity in all period to that with 100% BF, and cheese cultures play important role developing the flavor of GCA by producing compatible enzymes to available substrate such as short, middle, and long chain fatty acids. Therefore, the taste and the flavor of GCA with 50% BF was mild and not too strong. The free fatty acids of GCA with 50% of BF contain not only short chain free fatty acids but more quantity of middle chain free fatty acids. Furthermore, it has been reported that flavor detection threshold for short chain fatty acids e.g. C4 is relative lower as compared to for long chain fatty acids e.g. C18:1 [18]. That means a little quantity of short chain free fatty acids in GCA is noticeable and caused an intensive flavor. On the contrary, a more quantity of middle and long chain free fatty acids could not develop the flavor.

The use of sodium citrate in dairy products had already been introduced e.g. Mozzarella analogue [7] or in spread cheese analogue [6]. However, the results in those studies had either positive effect or no effect. In our study, the addition 0.5% sodium citrate improved the taste and flavor of ripened GCA. This Gouda analogue was not sour and had good taste, due to some flavor substances such as different fatty acids, diacetyl, and acetoin. The last two compounds are produced from citrate. Sodium citrate in sour milieu is released and the free citrate compound is transported inside cells via citrate permease. Citrate is then cleaved to acetate and oxaloacetate by citrate lyase and oxaloacetate is decarboxylated to pyruvate by decarboxylase α-Acetolactate synthase, and then condenses two pyruvate molecules to form CO_2 and α-Acetolactate. α-Acetolactate is unstable and then decarboxylation may be oxidative, yielding diacetyl or non-oxidative forming acetoin [19]. Furthermore, Boumerdasi *et al.* [19] reported that the production of diacetyl and acetoin by *Lactococcus lactis* ssp *lactis* can be stimulated and optimized in the determined sodium citrate concentration. Even in special type of cheese fermentation, sodium citrate has a function to eye formation [20]. Nevertheless, the use of 0.5% sodium citrate in this study impaired the texture of the ripened Gouda cheese analogue; it might be due to the role of 0.5% sodium citrate in GCA together with the mix cheese cultures in releasing citric compound and hence, produced more acetoin diacetyl and CO_2. The last compound led to high pressure in ripened Gouda analogue; consequently, the texture was very crumbly. We assumed that the use of sodium citrate less than 0.5% might improve both the sensory properties and TPA of GCA.

However, factors influencing flavor development were not only on BF and sodium citrate but also cheese starter cultures and smear culture. According to Fox *et al*. in Gunasekaran and Mehmet [21], in cheese there are five substances as ripening agents: coagulant chymosin or suitable proteinase; milk, some indigenous enzyme; starter cultures; host of enzyme released upon cell death and lysis; and secondary microbes producing secondary metabolites such as butyric acid and propionic, Exogenous enzyme proteinase, peptidase, and lipase to accelerate cheese ripening. Our study showed that only a mixed cheese cultures developed an appropriate Gouda cheese flavor. The presence of citrate stimulates the production of flavor substance by *Leuconostoc mesenteroides* culture and is occurred only at less than pH 6.0 [22]. Hoecker and Hammer 1994 in [22] showed that for high diacetyl production by pure culture of *Leuconostoc mesenteroides*, a heterofermentative lactic acid bacterium, an acidic environment is needed. Furthermore, our study showed that the GCA with mixed cheese starter cultures contained highest WSN in during ripening. Previous findings revealed that volatile fatty acids, amines, sulphur compounds, acetate, and higher fatty acids serve as precursor flavor in cheese product [23]. *Brevibacterium linens* has long been recognized as an important dairy microorganism [24]. Cheese starter cultures, together with *Brevibacterium linens,* create symbiotically in producing flavor substances. *Brevibacterium linens* is capable to produce lipolyitic, esterolytic, and proteolytic enzymes [24], even though it had distinct lipolytic activity in Gouda cheese [25]. During ripening, the lactic acid, which has been used by microbes, particularly yeast, leads to pH increase that facilitates the growth of *Brevibacaterium linens* [26]. Even some *Brevibacterium linens* metabolite strongly the lactic acids to other substances [15]. *Brevibacterium linens* as a smear culture should be noted as an important contributor to develop flavor. Our preliminary study showed that product without smear culture had strong beany flavor. These results show that the use of mixed cheese starter cultures (culture C) and *Bervibacterium linens* in soy protein based GCA provide a synergistic condition to develop Gouda cheese flavor.

4. Conclusions

Palm fat, butter fat and mixed cheese starter cultures showed synergetic effect on chemical and physical properties of Gouda cheese analog. Taken together, butter fat, mixed cheese starter cultures, smear culture of *Brevibacterium linens*, and sodium citrate play important role in flavor development of ripened soy-protein based GCA.

Acknowledgement

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