



## Effect of genetic type and casein haplotype on antioxidant activity of yogurts during storage

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### ABSTRACT

The aim of this work was to investigate the antioxidant activity of yogurt made from the milk of 2 breeds—Italian Brown and Italian Holstein—characterized by different casein haplotypes ( $\alpha_{S1}$ -,  $\beta$ -, and  $\kappa$ -caseins) during storage up to 15 d. The casein haplotype was determined by isoelectric focusing; antioxidant activity of yogurt was measured using 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid). The statistical analysis showed a significant effect of the studied factors. Antioxidant activity increased during storage of both yogurt types, but yogurt produced with Italian Brown milk showed higher antioxidant activity than those produced with Italian Holstein milk. A high scavenging activity was present in yogurts with the allelic combination of *BB-A<sup>2</sup>A<sup>2</sup>-BB*. The results of this study suggest that the genetic type and the haplotype make a significant contribution in the production of yogurts with high nutraceutical value.

**Key words:** yogurt, genetic type, casein haplotype, antioxidant activity

### INTRODUCTION

Interest in yogurt production is based on 3 fundamental aspects: (1) to create a particularly interesting nutraceutical product; (2) to transform milk without residue from processing; and (3) to reclaim and obtain a product without any risk of failure. Consumer interest in the consumption of yogurt has undergone a profound change, from being a product consumed by a few people to becoming a worldwide phenomenon, as consumers become more aware of the association between yogurt and welfare and disease prevention. Yogurt is now considered a functional food; it is a healthy food due to the high digestibility and bioavailability of its protein, energy, and calcium. In addition, the microbial fermentative activities (e.g., proteolysis) of yogurt lead to the modification of the allergenic properties of milk (Lourens-Hattingh and Viljoen, 2001) and to the release

of several of bioactive peptides encrypted within the native sequence of the proteins (Gobbetti et al., 2004).

Previous research has shown that some qualities of yogurt, such as its antioxidative, antithrombotic, antimicrobial, immunomodulatory, ion binding, opioid antagonistic activities, or angiotensin-converting enzyme inhibitory qualities, have beneficial effects on bodily functions in humans (Pattorn et al., 2012). Many authors have shown that the antioxidant activity of whey and CN proteins in yogurt could be related to their high tendency to chelate metals (Tong et al., 2000; Rival et al., 2001) and to their ability to donate electrons and atoms (Colbert and Decker, 1991).

Milk proteins are prone to genetic variations; De Noni et al. (2009) demonstrated that genetic polymorphism may determine the type of bioactive peptides released from milk proteins. The relationships between genetic polymorphism and milk composition have been studied in depth by several authors (Ng-Kwai-Hang, 1998; Di Stasio and Mariani, 2000). In many studies, the effects of CN haplotypes on milk production were evaluated considering a single locus (Ng-Kwai-Hang et al., 1984; Aleandri et al., 1990; Bovenhuis et al., 1992). However, studies on the effects of individual loci are rather contradictory and, as shown in previous research, their influence on milk production could be due to the cumulative effect of different CN loci on chromosome 6 (Braunschweig et al., 2000). Grosclaude (1988) have suggested studying whole combinations of alleles rather than single alleles due to the tight genetic linkage among CN loci. Currently, it is believed that a better estimation of effects is obtained when considering the whole CN cluster instead of the single CN loci within a single breed (Gambacorta et al., 1994, 2005; Boettcher et al., 2004; Secchiari et al., 2009). The aim of this work was to evaluate the effect of genetic type and CN haplotype on the antioxidant capacity of yogurt during storage.

### MATERIALS AND METHODS

#### Samples

This study was conducted on an intensive farm, consisting of more than 350 Italian Holstein and Italian

Received June 21, 2012.

Accepted February 10, 2013.

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Brown cattle, in the countryside of Potenza, southern Italy. Before starting the test, about 200 animals in lactation were identified by isoelectric focusing (IEF) to define their haplotypes. Haplotypes were formed by the combination of the individual allelic loci aggregated by the  $\alpha_{S1}$ -,  $\beta$ -, and  $\kappa$ -CN. After definition of individual phenotypes and separation by genetic type (GT), the cows were grouped by haplotype to obtain more consistent milk, which is needed to manufacture yogurt. Yogurt was obtained with a specific yogurt starter culture consisting of a mixture of 2 species of lactic acid bacteria (LAB)—*Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*—and stored at 4°C for 3, 6, 9, and 15 d.

### Chemicals and Apparatus

The chemical compound 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, sodium phosphate, sodium hydroxide, phosphoric acid, acetic acid, 2-mercaptoethanol, urea, *N,N,N',N'*-tetramethylethylenediamine, ammonium persulfate, and sodium acetate were purchased from Sigma-Aldrich (Milan, Italy). Acrylamide, bis-acrylamide, ampholine buffer were purchased from GE Healthcare Amersham Bioscience (Buckinghamshire, UK). Coomassie Brilliant Blue G250 was purchased from Bio-Rad (Richmond, CA). The LAB, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*, were purchased from Insaio s.r.l. (Liscate, Milan, Italy). The spectrophotometer used was a UV-VIS Spectrophotometer 1204 (Shimadzu, Kyoto, Japan). The apparatus for IEF used was a Multiphor II Electrophoresis System (Pharmacia LKB, Sweden).

### Sample Preparation for IEF

Individual milk samples, kept at 4°C, were defatted by centrifugation ( $3,000 \times g$  for 30 min at 4°C); the fat layer was solidified at  $-20^\circ\text{C}$  for 20 min and removed. Casein was prepared by isoelectric precipitation at pH 4.6 with 10% (vol/vol) acid acetic and 1 M sodium acetate at room temperature. After centrifugation at  $3,000 \times g$  for 10 min at 4°C, the CN pellet was washed twice with distilled water and stored at  $-20^\circ\text{C}$ . The whole CN was dissolved in 9 M urea and 1% 2-mercaptoethanol for IEF analysis, according to Aschaffenburg and Drewry (1959).

### Genetic Variants of CN and Whey Proteins by IEF

The genetic variants of the different CN by IEF were determined according to the method of Trieu-Cuot and Gripon (1981). The IEF analysis was performed

on polyacrylamide gel (5% acrylamide and 0.15% bis-acrylamide) with a thickness of 1 mm and 2% carrier ampholytes to create a gradient of pH 2.5 to 10. The gel was prefocused at a constant value of 0.35 W/mL of gel and at the maximum limit of 1,200 V. The gel was stained in Coomassie Brilliant Blue G-250 according to Blakesley and Boezi (1977). Haplotype frequencies were determined by the number of each haplotype divided by the total number of haplotypes [ $\% = (n_{i, \text{haplotype}}/n_{\text{tot, haplotype}}) \times 100$ ]. Haplotypes are presented as  $\alpha_{S1}$ -,  $\beta$ -, and  $\kappa$ -CN.

### Yogurt Manufacture

After heat treatment at 95°C for 15 min followed by cooling to 45°C, whole milk samples were inoculated at the same time with 1% (vol/vol) *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Fermentation was carried out at 45°C; each fermentation process was monitored systematically by recording the pH values at intervals of 30 min until the pH value reached  $4.6 \pm 0.1$ . Yogurts were immediately cooled after fermentation at 4°C and stored for 3, 6, 9, and 15 d.

### Preparation of Water-Soluble Extracts of Milk and Yogurt

Milk and yogurt samples were centrifuged at  $5,000 \times g$  at 4°C for 20 min. The supernatant was separately filtered through a membrane filter (0.45  $\mu\text{m}$ ) and was used to measure the antioxidant activity.

### Antioxidant Activity of ABTS Radical Scavenging Assay

A modification of the original method of Re et al. (1999) was applied to assess the scavenging capacity of yogurt samples in a reaction with the ABTS radical cation (ABTS<sup>+</sup>), generated by oxidation of ABTS diammonium salt stock solution with potassium persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>). Stock solutions of ABTS (7 mM) and potassium persulfate (140 mM) were prepared in water, and ABTS<sup>+</sup> radical solution was produced by reacting 10 mL of the ABTS stock solution with 175  $\mu\text{L}$  of potassium persulfate solution. The mixture was left in the dark at room temperature for 12 to 16 h before use. For the evaluation of antioxidant capacity, the ABTS<sup>+</sup> solution was diluted with ethanol (96%) to obtain the absorbance of  $0.700 \pm 0.020$  at 734 nm. Two milliliters of ABTS<sup>+</sup> solution was mixed with 100  $\mu\text{L}$  of the water-soluble extracts of samples in a cuvette and the decrease in the absorbance was measured after 30 min. The reagent blank was prepared by adding 100  $\mu\text{L}$  of ethanol instead of the sample. Antioxidant activity

was expressed as a percentage inhibition ( $I$ ) of ABTS<sup>+</sup> radical and calculated by the equation

$$I(\%) = \left( \frac{A_{734}^{Control} - A_{734}^{Extract}}{A_{734}^{Control}} \right) \times 100.$$

### Statistical Analysis

Data were analyzed according to the following linear model (SAS Institute, 1996):

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

where  $y_{ijk}$  is the observation;  $\mu$  is the overall mean;  $\alpha_i$  is the fixed effect of the  $i$ th GT ( $i = 1, 2$ );  $\beta_j$  is the fixed effect of the  $j$ th haplotype ( $j = 1, 2, 3, 4, 5, 6, 7$ );  $(\alpha\beta)_{ij}$  is the interaction of GT  $\times$  haplotype; and  $\varepsilon_{ijk}$  is the random error. Before setting the values, expressed as a percentage, they were subjected to angular transformation. Student's  $t$ -test was used to for all variable comparisons and correlation between parameters was determined by Pearson correlation analysis.

## RESULTS AND DISCUSSION

### Haplotype Frequencies

Seven different CN haplotypes were identified by isoelectric focusing. The different allelic combinations of loci  $\alpha_{S1}$ -,  $\beta$ -, and  $\kappa$ -CN and their frequencies are reported in Table 1. Alleles  $BB-A^2A^1-AA$  and  $BB-A^2A^2-BB$  (20.83%) and  $BB-A^2B-AB$  (16.67%) were frequent, whereas  $BB-A^2A^2-AA$ ,  $BB-A^2A^2-AB$ , and  $BB-A^2A^2-BB$  showed the lowest frequency (8.34%). All haplotypes were present in the Italian Brown population, whereas haplotypes  $CC-A^2A^2-BB$  and  $BB-A^2B-AB$  were absent in the Italian Holstein population. The selection boost carried out only for specific aspects related to some phenotypic characteristics of milk (e.g., fat and protein yield) led to a reduction of the variability of allelic combinations.

### Antioxidant Activity

The decolorization of the ABTS radical cation (ABTS<sup>•+</sup>) was used to assess the antioxidant activity of yogurt samples. The ABTS assay is one of the most widely used methods for the screening of antioxidant activity as it measures the scavenging activity of several natural products and is applicable to both hydrophilic and lipophilic antioxidant systems (Re et al., 1999). The ABTS values were expressed in terms of inhibition percentage ( $I$ , %), which is reported in Table 2. The

antioxidant activity was measured on both milks, aggregates for single haplotypes, and on obtained yogurts. The results of the study on milk ABTS showed very low variability and statistical significance, so the results were omitted. This could be related to the presence of large peptides and proteins not hydrolyzed, which have low antioxidant activity (Hernández-Ledesma et al., 2005a).

The role of bacteria fermentation on the production of different bioactive peptides and the relationship between antioxidant activity and concentration of low molecular weight peptides has been reported in many studies (Kudoh et al., 2001; Virtanen et al., 2007; Gomez-Ruiz et al., 2008). The antioxidant capacity is also conditioned by the heat treatment undergone by the milk for the manufacture of the yogurt (95°C for 15 min) because the denaturation of proteins exposed initially buried reactive sites (Galleher et al., 2005).

The antioxidant activity of the yogurt was evaluated during storage at 4°C and up to 15 d (Table 2). Although the shelf life of yogurt is at least 30 d, in a previous study (E. Gambacorta, I. Intaglietta, A. Simonetti and A. Perna, unpublished data) we observed that the ABTS values increased up to 15 d, and storage to 30 d resulted in very small variation with no detectable statistically significant differences. This finding was similar to that of Shori and Baba (2011), who reported a similar trend in the antioxidant activity of yogurt during refrigerated storage up to 14 d. All yogurt samples showed antioxidant activity at formation (1 d). At 1 d, the average ABTS value was 52.38%, with a variation of 45.98 to 57.98%; the antioxidant activity gradually increased during refrigerated storage (1–15 d; Table 2). The ABTS radical scavenging activity reached its highest value at 15 d ( $I = 70.97\%$ ), with an increase of about 37% over the initial value ( $I = 52.38\%$ ). Our results are in agreement with those reported by Gupta et al. (2009), who observed an increase in antioxidant activity as ripening proceeded in Cheddar cheese samples, reaching a maximum value during the fourth month. The relationship between radical scavengers' activity and proteolysis was previ-

**Table 1.** Frequencies of the  $\alpha_{S1}$ -,  $\beta$ -, and  $\kappa$ -CN haplotypes in Italian Brown and Italian Holstein cows

Haplotype ( $\alpha_{S1}$ -CN, $\beta$ -CN, $\kappa$ -CN)	Frequency (%)
$BB, A^2A^1, AA$	20.83
$BB, A^2A^2, BB$	20.83
$BB, A^2B, AB$	16.67
$BB, A^2A^1, AB$	12.50
$CC, A^2A^2, BB$	12.50
$BB, A^2A^2, AB$	8.33
$BB, A^2A^2, AA$	8.33

**Table 2.** Radical-scavenging activity of yogurts during storage at 4°C for up to 15 d<sup>1</sup>

Item	Days									
	1		3		6		9		15	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Genetic type										
Italian Brown	53.09 <sup>a</sup>	4.78	58.44 <sup>a</sup>	3.63	61.16 <sup>a</sup>	1.88	69.35 <sup>a</sup>	4.90	70.50 <sup>a</sup>	3.47
Italian Holstein	51.67 <sup>b</sup>	3.88	56.16 <sup>b</sup>	2.64	61.49 <sup>b</sup>	1.77	64.84 <sup>b</sup>	3.53	71.43 <sup>b</sup>	3.49
Haplotype ( $\alpha_{SI}$ -CN, $\beta$ -CN, $\kappa$ -CN)										
<i>BB, A<sup>2</sup>A<sup>1</sup>, AA</i>	54.52 <sup>a</sup>	1.60	56.95 <sup>a</sup>	2.11	59.57 <sup>a</sup>	1.20	64.83 <sup>a</sup>	3.36	64.26 <sup>a</sup>	0.56
<i>BB, A<sup>2</sup>A<sup>1</sup>, AB</i>	51.31 <sup>b</sup>	1.55	57.74 <sup>b</sup>	1.88	60.27 <sup>b</sup>	1.10	69.01 <sup>b</sup>	3.70	70.36 <sup>b</sup>	0.79
<i>BB, A<sup>2</sup>A<sup>2</sup>, AA</i>	57.98 <sup>c</sup>	2.03	61.45 <sup>c</sup>	1.94	63.14 <sup>e</sup>	0.69	74.87 <sup>c</sup>	4.06	71.15 <sup>c</sup>	0.65
<i>BB, A<sup>2</sup>A<sup>2</sup>, AB</i>	45.98 <sup>d</sup>	0.96	51.30 <sup>d</sup>	0.96	59.51 <sup>a</sup>	1.20	64.68 <sup>a</sup>	1.43	70.70 <sup>bc</sup>	1.00
<i>BB, A<sup>2</sup>A<sup>2</sup>, BB</i>	48.65 <sup>e</sup>	1.11	57.00 <sup>a</sup>	1.97	62.56 <sup>c</sup>	0.93	62.70 <sup>d</sup>	3.19	73.18 <sup>d</sup>	1.67
<i>BB, A<sup>2</sup>B, AB</i>	50.74 <sup>b</sup>	1.10	58.26 <sup>be</sup>	1.90	61.32 <sup>d</sup>	1.70	66.33 <sup>e</sup>	1.94	71.28 <sup>c</sup>	2.08
<i>CC, A<sup>2</sup>A<sup>2</sup>, BB</i>	57.46 <sup>c</sup>	1.58	58.40 <sup>e</sup>	1.96	62.90 <sup>e</sup>	0.64	67.23 <sup>f</sup>	3.51	75.83 <sup>e</sup>	1.44
Overall	52.38	4.40	57.30	3.36	61.32	1.82	67.09	4.82	70.97	3.50

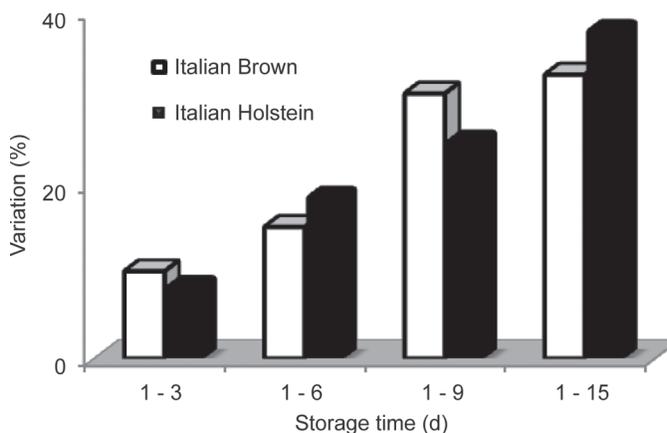
<sup>a-f</sup>Means within a column with different superscripts differ ( $P < 0.01$ ).

<sup>1</sup>Measured using 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and expressed as percentage inhibition ( $I$ ) of the ABTS<sup>+</sup> radical.

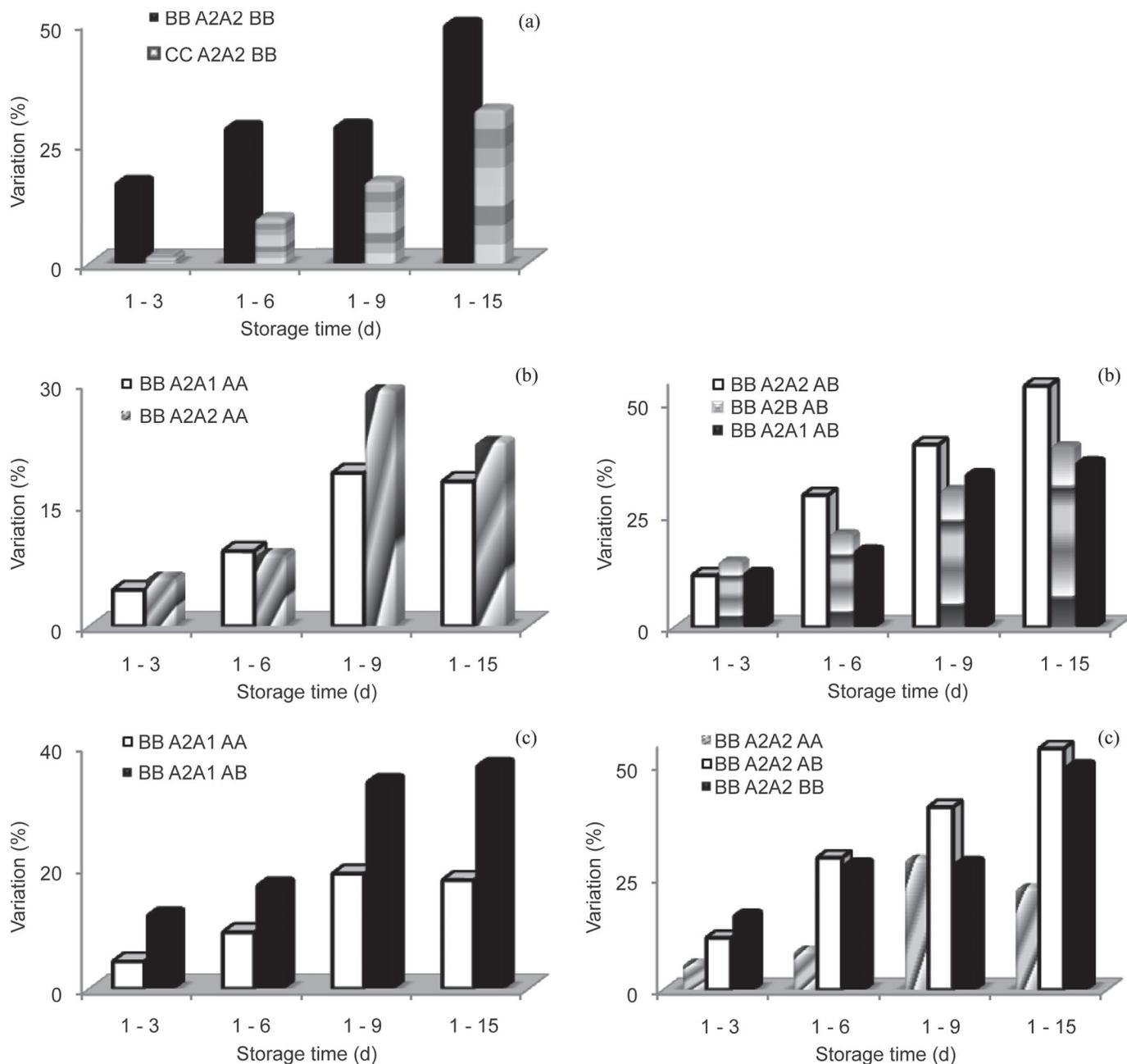
ously reported in several studies (Kudoh et al., 2001; Ryhanen et al., 2001; Hernández-Ledesma et al., 2005b; Gupta et al., 2009). Antioxidant activity attributed to yogurt is also influenced by the fermentation and post-acidification during storage that determine production of organic acids (Correia et al., 2004) characterized by biological activity. Statistical analysis by 2-way ANOVA showed a statistically significant effect of the GT and haplotype ( $P < 0.001$ ). Differences observed by  $t$ -test for the mean ABTS values are presented in Table 2. Comparing different GT, the differences between the ABTS average values were statistically significant ( $P < 0.05$ ) in all studied levels. Italian Brown yogurts showed the highest antioxidant activity compared with Italian Holstein yogurts ( $P < 0.05$ ). Chiang and Chang (2005) highlighted the positive correlation between protein content and antioxidant activity. In fact, Italian Brown presents higher protein content than the Italian Holstein (3.79 vs. 3.24%, respectively) and CN nitrogen (2.75 vs. 2.28%, respectively; results not shown) in particular, as reported by De Marchi et al. (2007). Previous research reported that CN represent the primary substrate for proteolysis operated by bacterial starter and a reserve for a wide variety of bioactive peptides (Meisel, 1998).

The percentage change of antioxidant activity encountered at different stages and compared with the initial value gives an indication of the progress of the process at different intervals: 1 to 3, 1 to 6, 1 to 9, and 1 to 15 d (Figure 1 and Figure 2). In general, the antioxidant capacity increased, Italian Holstein yogurt showed a trend of steady increase and the Italian Brown yogurt showed a much more marked variation in the third interval (Figure 1). These findings suggest that antioxidant activity, despite being dependent on the degree

of proteolysis, is strongly influenced by strain-specific characteristics of LAB (Kudoh et al., 2001; Ryhanen et al., 2001; Hernández-Ledesma et al., 2005b; Virtanen et al., 2007; Gupta et al., 2009). Hajirostamloo (2010) reported that the concentration of angiotensin-converting-enzyme (ACE)-inhibitory peptides depends on a balance between their formation and further breakdown into inactive peptides and amino acids that, in turn, depends on storage time and conditions. Conversely, the variation of antioxidant activity is linked to the possible aggregation of peptide processes that occur during the enzymatic hydrolysis of whey protein and CN, with formation of macro-aggregates that reduce the antioxidant capacity (Adt et al., 2011).



**Figure 1.** Percentage variation of antioxidant activity in Italian Brown and Italian Holstein yogurt during storage for 15 d, measured using 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and expressed as percentage inhibition ( $I$ ) of the ABTS<sup>+</sup> radical.



**Figure 2.** Percentage variation of antioxidant activity, measured using 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and expressed as percentage inhibition ( $I$ ) of the  $ABTS^{\bullet+}$  radical in yogurt with different CN haplotypes during 15-d storage: (a) same phenotypes for  $\beta$ - and  $\kappa$ -CN; (b) same phenotypes for  $\alpha_{S1}$ - and  $\kappa$ -CN; (c) same phenotypes for  $\alpha_{S1}$ - and  $\beta$ -CN. Haplotypes are expressed as the alleles for  $\alpha_{S1}$ -CN ( $BB$  or  $CC$ ),  $\beta$ -CN ( $A^2A^2$ ,  $A^2A^1$ ,  $A^2B$ ), and  $\kappa$ -CN ( $AA$ ,  $BB$ , and  $AB$ ).

Antioxidant activity is influenced by the CN haplotype. The degree of CN heterogeneity may influence the release of peptides formed by proteolysis (Minervini et al., 2003) because of the primary structure, original conformation, and proportion of CN (Ng-Kwai Hang and Grosclaude, 1992). This is due to the presence in the sequence of a specific protein known as the stra-

tegic zone that has biological activities (Fiat and Jollés, 1989). The ABTS values of samples with different haplotypes are shown in Table 2. Antioxidant activity showed different values as a function of the haplotype linked to the release of bioactive peptides. At 1 d, the increasing sequence of radical scavenging of the yogurt with different haplotypes was  $CC-A^2A^2-BB > BB-A^2A^2-$

$AA > BB-A^2A^1-AA > BB-A^2A^1-AB > BB-A^2B-AB > BB-A^2A^2-BB > BB-A^2A^2-AB$ . After 15 d, yogurt with the haplotype  $CC-A^2A^2-BB$  showed the highest radical scavenging activity (75.83%), whereas yogurt with the haplotype  $BB-A^2A^1-AA$  showed the lowest value (64.26%; Table 2). The different antioxidant activity observed among yogurts with different haplotypes could be due to the specific amino acid sequence of the milk protein variants. Hernández-Ledesma et al. (2004) showed that the formation of an angiotensin-I-converting enzyme is peptide specific to the bovine  $A^1$  variant and is not present in other  $\beta$ -CN alleles. Jinsmaa and Yoshikawa (1999) have reported that the release of  $\beta$ -casomorphin 7 does not occur in presence of Pro in position 67 of the  $A^2$  variant of  $\beta$ -CN. Hernández-Ledesma et al. (2005b) have demonstrated that peptides released from the  $A$  variant of  $\beta$ -LG are small (3 kDa) and are mainly responsible for antioxidant activity compared with the  $AB$  variant of  $\beta$ -LG. The haplotype gives an indication of phenotypic expression coded by well-defined allelic combinations. The effect of an allele combination cannot be regarded as a mere summation of the effects of individual alleles, but as an expression of the interaction between them. This means that a single allele causes different results depending on the combination that contributes to the formation of the haplotype.

Genetic changes may alter the properties of individual proteins, affect the state of aggregation of the micellar system, and interfere with the action of proteolytic enzymes. Interactions that are established at the level of closely associated loci influence the effect of single alleles with regard to antioxidant activity. The analysis of results showed that the effect of the allelic combination  $BB$  rather than  $CC$  of  $\alpha_{S1}$ -CN, in the context of the haplotype  $A^2A^2$   $\beta$ -CN and  $BB$   $\kappa$ -CN, always presents an expression of the highest antioxidant activity in all intervals (Figure 2). The allelic combination  $A^2A^2$   $\beta$ -CN, in the context of the haplotype  $BB$   $\alpha_{S1}$  and  $AB$   $\kappa$ -CN, resulted in an increasingly high antioxidant activity, except in the first interval (1–3 d), whereas the combination  $A^2A^1$   $\beta$ -CN resulted in lower antioxidant activity. The combination  $A^2B$   $\beta$ -CN positioned itself to a radical scavenger intermediate level, except during the third interval (1–9 d; Figure 2). The location of the combination  $A^2A^2$   $\beta$ -CN, compared with  $A^2A^1$ , was also preserved in the context of the haplotype  $BB-AA$   $\alpha_{S1}$ - $\kappa$ -CN. The allelic combination  $AA$   $\kappa$ -CN, in the context of  $BB$   $\alpha_{S1}$ - and  $A^2A^1$   $\beta$ -CN, was expressed with a lower antioxidant activity in all intervals than  $AB$   $\kappa$ -CN. No change was observed when 2 combinations of  $\kappa$ -CN were inserted in the context of  $BB$   $\alpha_{S1}$ - and  $A^2A^2$   $\beta$ -CN. The effect of the allelic combination  $BB$   $\kappa$ -CN in the context of  $BB$   $\alpha_{S1}$ - and  $A^2A^2$   $\beta$ -CN was

highest in the first interval (1–3 d), whereas the other 2 allelic combination expressions remained intermediate in the subsequent intervals (Figure 2). The analysis of the results showed that the combination of  $BB$   $\kappa$ -CN was expressed at higher values than those of  $AA$   $\kappa$ -CN. Ng-Kwai-Hang et al. (1984) reported that the  $BB$   $\kappa$ -CN had a significant effect on milk production, protein, fat content, and technological properties. Although the best result was conditioned by heterozygous  $AB$   $\kappa$ -CN, this could be a heterotic effect.

## CONCLUSIONS

The formation of biologically active peptides is strongly influenced by CN polymorphism, which was shown by the significant difference between different haplotypes in yogurt. Genetic type influenced the concentration of milk proteins, in particular on CN that are the main substrate of fermentation in starter bacteria for yogurt production, which resulted in the formation of biopeptides. Few studies have been done on the effect of casein haplotype on antioxidant activity of yogurts; therefore, the knowledge on this subject is not complete. More studies are therefore needed to confirm these findings and to understand the effects of milk protein polymorphism on the antioxidant activity of yogurt and evaluate the possible nutritional implications for human health.

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