TECHNOLOGICAL AND SENSORY PROPERTIES OF HAMBURGERS ENRICHED WITH CALCIUM STUDY OF THE *IN VITRO* BIOAVAILABILITY

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ABSTRACT

Hamburgers were supplemented with three calcium salts (calcium gluconate CG, calcium lactate CL and calcium citrate-malate CCM). They were added in sufficient amount to that 100 g of hamburger gives 20 or 30% of the Ca RDA (1000 mg). Their technological and sensory properties were studied. CG 30% gave the worst sensory properties and it was discarded. Bioavailability of calcium depends on the type of salt used and the highest value was obtained with CCM (14.5%). For that, this salt is proposed as the most adequate for the enrichment of fresh meat products.

- Keywords: bioavailability, calcium salts, hamburgers -

INTRODUCTION

Meat and meat products are important to the human diet; they contain proteins with all nine essential amino acids of high biological value, accounting for 40% of total amino acids. They are an excellent source of bioactive compounds, including vitamins (B-complex), iron, zinc, phosphorus (FERNÁNDEZ *et al.*, 2005; WEISS *et al.*, 2010).

The interest on the human health and the actual consumer tendencies, who prefer more nutritious food, have stimulated interest in developing meat products with bioactive compounds with attractive physiological activities (GRIGUELMO *et al.*, 1999; CENGIZ and GOKOGLU, 2005; DECKER and PARK, 2010). Greater emphasis has been placed on strategies involving the addition of bioactive compounds with recognized health benefits, such as proteins, fibre, polyphenols, unsaturated fatty acids, probiotics or minerals (ROBERFROID, 2002; SAIGA *et al.*, 2003; CACERES *et al.*, 2006; ARHIARA, 2006; JIMÉNEZ-COLMENERO *et al.*, 2006; DECKER and PARK 2010; ALONSO *et al.*, 2010; ZHANG *et al.*, 2010; KHAN *et al.*, 2011). Dietary minerals are essential for various physiological functions and they have been associated with the prevention of several diseases (MENÉNDEZ-CARREÑO *et al.*, 2008; DECKER and PARK, 2010; ZHANG *et al.*, 2010). Calcium (Ca) is one of the most important. It gives structural integrity to mineralized tissue preventing osteoporosis and contributing to the "bone health". Although interest in Ca primarily derives from this role, it also plays other essential physiological roles in arterial hypertension, cellular function, skeletal muscle contraction, blood coagulation and enzymatic reactions as a co-factor (PRINCE *et al.*, 2006; STRAUB, 2007, ADLURI *et al.*, 2010). Health authorities have recommended a Ca daily allowance (RDA) of 1000 mg for adults aged 19-50 (Institute of Medicine, IOM, 2004) or 800 mg (Directive 2008/100/EC), without concern for age.

Milk and dairy products account for much of the Ca in the human diet, (75% of Ca intake, aprox); only 16% comes from fish and vegetables and a 6-7% from mineral water (GUÉGUEN and POINTILLART, 2000; CHAROENKIATKIU *et al.*, 2008). Since meat and meat products are poor source of Ca, supplementing them with Ca salts could be a good option to increase its intake particularly those in which the consumption of milk can be a health problem. Previous studies have examined the addition of Ca to meat products, but mainly for reducing sodium levels (GIMENO *et al.*, 1998, 1999) and the final Ca content was not sufficient to consider them as *a source* of this mineral. Studies previously performed in our laboratory (CÁCERES *et al.*, 2006), it was reported that calcium could be successfully added to both cooked and dry-fermented sausages,

but until now, fresh meat products have been not assayed.

In this way, the present work deals with the manufacture of fresh meat products (hamburgers) enriched with calcium. For that, three different calcium salts were assayed: Ca gluconate (CG), Ca lactate (CL) and Ca citrate-malate (CCM). They are permitted in food (Regulation EC No 1907/2006) and characterized because of their high bioavailability (KORSTANJE and HOEK, 2001). The technological and sensory properties of the hamburgers manufactured were studied and finally, a study of the Ca bioavailability have been performed, using an *in vitro* static method to simulate the passage through the intestinal cell-membrane (GLAHN *et al.*, 2002; PERALES *et al.*, 2005; SHIOWATANA *et al.*, 2006).

MATERIAL AND METHODS

Hamburger manufacture

Beef meat was obtained from a local abattoir and chopped in a grinder using a 3 mm plate (Grinder C10, Falsf Co., Spain).The Ca salts (Panreac, Castellar del Vallés, Spain) were added separately to the ground meat in sufficient amount to give a final Ca content of 20 or 30% of the RDA (1000 mg/day) (IOM, 2004). These calculations took into account the calcium content of the molecules and the purity of the Ca salts, which was >98% based on the anhydrous formula (Table 1). The Ca salts were homogeneously distributed into the ground meat in a mixer (Mainca, Pamplona, España). Then, hamburgers were moulded into plates (10 cm diameter, 1 cm height) and kept under refrigeration (2 °C) until analysis, less than 24 h.

Seven batches were manufactured: a control batch without Ca and 6 batches added with CG, CL or CCM at two concentrations (20 and 30% RDA). According to the type and Ca salt amount, the batches were named as CG20, CG30, CL20,

Table 1

Calcium salt amount added to raw hamburgers to provide 20 or 30% of the RDA	
Batch	**g/100 g**
Control	0
CG20	2.24
CG30	3.36
CL20	1.54
CL30	2.31
CCM20	0.93
CCM30	1.40

CL30, CCM20 and CCM30. The hamburgers manufacture was made in triplicate.

Hamburgers were cooked on an electric grill preheated to 180 °C. They were placed for 2 min on each side, sufficient time to achieve a temperature of 60 °C in the inner of the hamburger and a good final degree of doneness (THORNBERG, 2005). Temperature was controlled using a digital thermometer (Testo Mod 735, Barcelona, Spain).

Physico-chemical analyses

Water activity was determined with a Decagon CX1 dew point hygrometer (Decagon Devices, Pullman, WA, USA). The pH was measured using a Crison 2001 pH meter using a glass electrode and according to the AOAC (2011). Water-holding capacity (WHC) was tested according to ZAMORANO and GAMBARUTO (1997). For that, a sample of 0.1 g was placed on a filter paper (Whatman No. 2), sandwiched between translucent plastic plates, and pressed for 1 min. The meat area and liquid area on the filter paper were measured with a planimeter. The following formulae were applied for WHC:

$$WHC= [\text{area of liquid (cm}^2) - \text{area of meat (cm}^2)] \times [9.47/\text{moisture in sample (mg)}] * 100$$

The measurements were made in quintuplicate and the final result was the average value.

Colour analysis

Colour was measured at room temperature on the surface of raw hamburgers, using a Chroma Meter CR-200 colorimeter (Minolta Co., Osaka, Japan) according to the Space colour CIE L*a*b* system and calibrated with a rose tile (L* 44.88, a* 25.99, b* 6.67). A D-65 illumination source was used. L*, a*, b*, Hue angle (tonality) and Saturation index (vivacity) were estimated according to ARTÉS and MÍNGUEZ, (2002). For each batch, twenty five measurements were taken.

Texture analysis

Textural properties were determined using a texturometer Stable Micro System Mod. TA.XT 2i/25 (Surrey, UK). Texture Profile Analysis (TPA) was performed on central cores of cooked hamburgers which were compressed twice to 50% of their original height. A cylindrical probe (2.5 cm diameter) of aluminium was used for the assay. The following parameters were determined: Hardness (N), Springiness (cm), Cohesiveness (ratio), Adhesiveness (N s), Gumminess (N) and Chewiness (N cm) (BOURNE, 1978). Shear force (N) and Work of shearing (N s) were estimated using a Warner-Bratzler blade. In both tests, the samples were 1 cm high and 2.5 cm in diameter; the crosshead speed was 2 mm/s. All determinations were carried out in quintuplicate for each batch.

Sensory analyses

The taste panel consisted of forty untrained assessors selected according to their eating habits, acquaintance with the product to be analyzed and sensitivity, as well as the reproducibility of their evaluations.

First, an Anchored Descriptive Analysis was performed in which the assessors evaluated the similarity between the external appearance of the enriched raw hamburgers and the control batch. This test was performed under a D-65 illumination source using a 5-point descriptive scale, in which the value of 3 points corresponded to the control batch. The value of 1 point meant *much worse than the reference*; 5 points, *much better than the reference;* 2 and 4 points were intermediate values. Three series were prepared: Control-CG20; Control-CL20-CL30 and Control-CCM20-CCM30. The series were presented to the panellists with 30 min of difference to avoid subjectivities.

After this test, a Hedonic Test was performed with cooked hamburgers. In this case the taste panel consisted by 15 trained panellists. They were in individual booths constructed according to ISO DP 6658 (ISO, 1985), under white fluorescent light. The assessors evaluated different attributes (odour, colour, texture, taste and overall acceptability) using a 10 cm non-structured scale (0 = extremely dislike and 10 = extremely like). Two sessions per day were carried out with an interval of at least 1 h between them to avoid panellist fatigue. Unsalted crackers and room-temperature water were provided to clean the palate between samples. The hamburgers were cooked and served in transparent Petri dishes. In each session three randomly selected hamburgers were served.

Bioavailability

Calcium bioavailability was studied using a static *in vitro* test that simulates the gastric and intestinal phases of the digestion process according to the methodology of SHIOWATANA *et al.*, (2006). All enzymes were from Sigma-Aldrich (Steinheim, Germany) and the reactives from Panreac (Barcelona, Spain). The samples were 10 g of cooked hamburgers which were homogenised (Polytron®, Littau-Luzerne, Switzerland) with 50 ml of 0.2 M phosphate buffer. The pH was adjusted to 2.0 with 5 N HCl. To simulate the gastric phase, 0.33 ml of suspension of pepsin (0.16 g pepsin (EC 232-629-3) per ml 0.1 N HCl) was added. Then, the samples were incubated in a shaker (130 rpm) at 37 °C for 2 h (Thermo Scientific MaxQ4000, Iowa, USA).

To simulate the intestinal phase, 20 g of the gastric digest was mixed with 5 ml of pancreatin-bile conjugate, [0.2 g pancreatin from porcine pancreas (EC 232-468-9) and 1.25 g porcine bile extract (EC 232-369-0) in 50 ml 1 M

NaHCO$_3$]. The pH was adjusted to 7-7.5 with 2 M NaHCO$_3$ and the total volume of the digest was measured.

Distilled water (25 ml) and the same volume of 2 M NaHCO$_3$ determined before were added inside to a cellulose dialysis tube with a molecular weight cut-off of 12000-14000 Da and a diameter of 25 mm (Sigma Aldrich, Steinheim, Germany). The tube was introduced into a flask containing other 20 g aliquots of the gastric digest and it was incubated at 37 °C in a shaker (130 rpm) until the pH reached a value of 5. Then, 5 ml of pancreatin-bile conjugate was added to the gastric phase and it was incubated again during 2 h at 37 °C.

After the dialysis process, the dialyzed calcium (inside the dialysis tube) and the non-dialyzed calcium (outside the dialysis tube) were determined. This last one, beside with the calcium in the solid delivery, corresponds to the calcium that would be eliminated. The difference between the amount of calcium detected in these two phases and that determined in the gastric phase was considered as the calcium remaining in the solid residue (solid delivery) and would be eliminated with the feces. It was calculated by difference.

The percentage of bioavailability was expressed as follows:

Bioavailability (%) = [Dialyzed Ca (mg)/ Ca sample (mg)] *100

Calcium determination

Calcium levels were determined according to IKEM *et al.*, (2002). For microwave digestion, 1.0 g of each sample was subjected to an acid digestion with 6 ml of HNO$_3$ and 2 ml of H$_2$O$_2$ (Suprapure, Merck) in a microwave digestion system and diluted to 10 ml with deionised water (Milli-Q, Millipore). Blank digestion was carried out in the same way. Digestion conditions for the microwave system were the following: 2 min - 250 W, 2 min - 0 W, 6 min - 250 W, 5 min - 400 W and 8 min - 550 W. A Perkin Elmer DV 3300 inductively coupled plasma-optical emission spectrometry (ICP-OES) was used to analyze the calcium level in the digested samples. Bovine muscle BCR No 184 was used as reference (CÁCERES *et al.*, 2006). Experimental values obtained were in the range of the certificate value with its uncertainty. All determinations were carried out in duplicate.

Statistical analyses

Results were statistically analysed by two-way ANOVA, which factors were represented by type and concentration of calcium salt. Values of p<0.05 were considered to be significant. The effect of the panellists in the sensorial responses was removed by rescaling all the scores given by each assessor from 0, which represents the minimum score used by a given assessor, to 10, which is the maximum score used by that assessor. After rescaling, the effect of the assessors in the sensorial responses was analysed by a two factor analysis of variance according to a randomised balanced block experimental design. The factor represented the type and percentage of calcium salt, while the block variable had 15 levels representing each of the panellists that collaborated in the trials. The F test showed that the effect of the panellists was not significant *(P > 0.05)*. In consequence, the final model considered of only 1 factor (one-way ANOVA) representing the calcium salt content.

Statistical analyses were performed using the Statgraphics Centurion XVI.I (Statistical Graphics Corporation, Herndon, VA, USA).

RESULTS AND DISCUSSION

Physico-chemical parameters

Table 2 showed the results of the a$_w$ and pH of the batches manufactured. The a$_w$ of the reminded batches ranged between 0.945 and 0.989 and no significant differences were observed between control and calcium added batches. CL batches showed the lower values. The lower pH values corresponded to the CG20, CL20 and CL30 batches, which showed a pH of 5.70, 5.55 and 5.50, respectively. They differ significantly to the reminder batches. According to the FDA (FDA, 2014) these values assure the safety of these meat products.

GC30 values are not showed due to its technological attitude like it was explained in the following section (Colour parameters).

WHC showed similar values in all the batches and ranged between 36.0-39.0%. The addition of whichever calcium salts supposed no significant changes in the WHC (p>0.05).

The values of all these parameters are according to those reported for several authors in raw

Table 2

Batch	a$_w$	pH
Control	0.966±0.016 [AB]	5.80±0.02 [B]
CG20	0.958±0.027 [AB]	5.70±0.05 [C]
CL20	0.954±0.030 [B]	5.55±0.02 [D]
CL30	0.945±0.031 [B]	5.50±0.10 [D]
CCM20	0.989±0.037 [A]	5.86±0.04 [AB]
CCM30	0.955±0.013 [AB]	5.92±0.06 [A]

Effect of added calcium salts on water activity (a$_w$) and pH on of raw hamburgers

or ground meat (LAWRIE, 1998; LEE *et al.*, 1998; POULANNE and HALONEN, 2010).

Colour analysis

The worse results were obtained with the CG batches. The CG crystallized in contact with the water of the meat and formed visible small white crystals that appeared homogeneously distributed in the hamburger. These crystals gave an external appearance different to that usually expected by the consumers in a commercial hamburger. It was especially striking at the maximum concentrations (CG30) because the crystals were larger and numerous. For that, it was considered that this batch didn't have enough quality and it was discarded for this and the following tests. Batch with CG20 also had crystals, but they were smaller, as white dots. Therefore, it was considered adequate for continuing the experiment, although its visual appearance would be compromised.

Colour parameters (L*, a* and b*) were determined in raw hamburgers (Table 3). The differences observed were related to the type of salt added. Lightness was very similar in all the batches without significant differences (p<0.05). However, it can be observed as the L* parameter was slightly higher in batches manufactured with CL, while, the lower values corresponded to those manufactured with CCM. It is important to remember that CL batches were those that showed the lower pH values. The relationship between color and pH is widely accepted (MANCINI and HUNT, 2005) and several authors (KIM *et al.*, 2006; MANCINI and RAMANATHAN, 2008; NAIR *et al.*, 2014) have reported specifically the effects of lactate on meat color. These authors proposed that lactate plays an indirect role in color stability by generating NADH, which is subsequently used to maintain reduced forms of myoglobin, increasing in this way, the stability of meat colour.

The values of redness (a*) and yellowness (b*) were similar between the control and calcium added batches. In relation to the redness, the lower values were observed in the CCM batches and the same occurs in the yellowness (b*). The higher value was recorded in the batch CL20 but it seems to be an inconsistent data. It was described that the haem pigment contents are mainly related to a* and, consequently, it is considered as the most important parameter in meat, while redox state influenced b* (MANCINI and HUNT, 2005). This parameter reach less importance because colours represented (blue and yellow) are not typical or intuitively related to meat. According to these authors, and giving greater relevance to the a* parameter, it can be concluded that the colour is very similar in all the batches, although CL20 and CCM30 batches were the only ones that showed any difference. Hue Angle and Saturation Index behaved in a similar way and these batches showed again, the only differences in relation to the remainder ones. The colour lecture of hamburgers did not reflect the presence of the small white crystals due to their size. It is important to take into account, firstly, that the colorimeter project the light from the probe, which has 1 cm of diameter and secondly, that the crystals had a much smaller size. For that, the colorimeter read the colour of the meat and thus the data of CG20 and control batches (without calcium) were similar, without significant differences.

The colour of cooked samples (data not shown) was very similar in all batches without exceptions. It has been described that during heat treatment, the meat colour changes from red to grey-brown (Maillard reaction) and increase

Table 3

Effect of added calcium on colour properties of raw hamburgers					
Batch	**L***	**a***	**b***	**Hue Angle**	**Saturation Index**
Control	44,45±1,96[AB]	21,72±1,15[AB]	8,37±0,88[BC]	20,62±1,85[BC]	23,36±1,23[AB]
CG20	44,63±6,64[AB]	22,60±4,96[AB]	9,20±3,21[AB]	21,79±4,88[BC]	24,49±4,78[AB]
CL20	45,68±4,35[A]	23,36±3,46[A]	10,76±2,53[A]	24,74±3,81[A]	25,72±3,95[A]
CL30	46,60±7,54[A]	21,39±3,44[AB]	9,30±3,42[AB]	23,96±6,22[AB]	22,36±4,18[BC]
CCM20	43,64±5,72[AB]	20,34±3,36[BC]	7,34±1,91[BC]	19,12±3,58[C]	21,73±3,65[BC]
CCM30	41,32±5,43[B]	19,60±3,77[C]	7,04±2,81[C]	19,10±6,67[C]	20,88±4.14[C]
Data are mean ± S.D.					
Values sharing the same letters are not significantly different (p > 0.05)					

the opacity when the internal meat temperature is between 45 °C and 67 °C (PAKULA and STAMMINGER, 2012). In this way, TORNBERG (2005) reported that the increase in meat opacity is related to the myosin desnaturation, which starts at about 35 °C. Above 50 °C, the myosin molecules are completely coagulated and the meat appears opaque.

Textural analyses

Textural parameters were determined in cooked hamburgers (Table 4). It was observed that in both CL and CCM batches, the higher the calcium amount, the greater the hardness was. This is probably because calcium, which is a divalent cation, establishes bonds between meat proteins, mainly with myosin. This favors the formation of a stronger network which leads to the highest firmness (DAMODARAN, 2008). According to TORNBERG (2005) it could be also due to the relationships stabilized between calcium and

meat proteins, partially denatured by the cooking. This last one favors the formation of a more compact network that increases the hardness.

Springiness was quite similar in all the calcium enriched batches and shown higher values to batch control. According to BELITZ et al., (2009), the increase of the ionic strength leads the extraction to the surface of the particles of minced meat yielding sticky exudates. During heating, the proteins interact between them, yielding a structure consist on a protein gel that can modify the springiness of the cooked products. (TORNBERG, 2005).

Adhesiveness and cohesiveness parameters did not change significantly (p>0.05). Gumminess and chewiness behaved as the hardness because they are secondary parameters dependent on it. So, the lower values corresponded to the CG which was the batch which showed the lower harness

The results obtained in the shear test showed the same tendency than in the TPA and so,

Table 4

Batch	Hardness (N)	Adhesiveness (N s)	Springiness (cm)	Cohesiveness (ratio)	Gumminess (N)	Chewiness (N cm)
Control	27,58±3,58 [B]	-0,03±0,004 [A]	0,51±0,01 [C]	0,60±0,01 [A]	16,52±1,95 [B]	8,25±0,97 [C]
CG20	18,16±5,86 [C]	-0,03±0,019 [A]	0,54±0,04 [BC]	0,52±0,06 [B]	10,19±3,67 [C]	4,99±2,07 [B]
CL20	35,07±6,34 [A]	-0,03±0,015 [A]	0,59±0,04 [A]	0,62±0,04 [A]	21,64±4,28 [A]	12,30±2,60 [A]
CL30	38,44±5,59 [A]	-0,04±0,021 [A]	0,57±0,02 [AB]	0,60±0,06 [A]	22,41±3,34 [A]	13,81±1,89 [A]
CCM20	27,37±6,08 [B]	-0,03±0,011 [A]	0,55±0,05 [BC]	0,61±0,03 [A]	16,75±3,73 [B]	9,12±1,45 [B]
CCM30	38,74±8,15 [A]	-0,04±0,021 [A]	0,56±0,04 [ABC]	0,60±0,04 [A]	23,30±6,08 [A]	12,04±1,57 [A]

Effect of added calcium salts on textural properties of experimental cooked hamburgers[ß]

[ß] Data are mean ± S.D.

Values sharing the same lower case letters are not significantly different (p > 0.05)

Table 5

Effect of added calcium salts on the sensory properties of raw (Anchored Descriptive Analisys) and cooked hamburgers (Hedonic Test)ß

Batch	Anchored Descriptive Analysis	Hedonic Test		
		Texture	Taste	Overall Acceptability
Control	3.00[*]	6.06±1.40 [AB]	6.13±1.33 [A]	6.22±1,15 [AB]
CG20	1.87±0.21 [D]	6.24±1.80 [AB]	4.94±2,44 [AB]	5.18±2,05 [B]
CL20	2.40±0.35 [B]	7.42±3,23 [A]	5.13±2,30 [AB]	5.87±2,10 [A]
CL30	3.53±0.29 [A]	6.06±2,22 [AB]	4.56±2,30 [B]	5.33±1.97 [B]
CCM20	2.67±0.40 [B]	5.79±1.78 [B]	5.62±1.91 [AB]	5,38±1.99 [B]
CCM30	2.20±0.26 [B]	5.90±1.30 [AB]	5.60±1.33 [AB]	5.80±1.35 [AB]

[ß] Data are mean ± S.D.

Values sharing the same uppercase letters are not significantly different (p > 0.05).

[*] Reference

the principal differences were an increase of the Work of Shearing related with the calcium amount added, independently to the salt added (data not shown).

Sensory analyses

Table 5 shows the results of the Anchored Descriptive Analysis, performed with raw hamburgers. Control batch was the reference and it was awarded a score of 3 in the 5 points scale used. The panellists evaluated the Visual Appearance of enriched hamburgers in comparison to the control.

The worse results were obtained with the CG20 batch, mainly due to the presence of small white crystals (see Colour section). In this case, the number and the size of the crystal were lower than in the CG30, but they were sufficient to modify negatively the appearance of the hamburgers.

CL30 batch was the best evaluated, even more than the reference (p<0.05). This result is according to the instrumental colour measurement (Table 3) in which this batch was the one that had the greatest the higher L* and Hue Angle.

Table 5 also shows the results of the Hedonic Test performed with cooked hamburgers. Odour and colour were well-evaluated in all the cases obtaining similar values in all batches (p>0.05) (data not shown). The texture achieves punctuations higher than 6 except the batches enriched with CCM20 and CCM30 which achieved scores of 5.79±1.78 and 5.90±1.30, respectively. Significant differences were observed only between CL20 and CCM20, but, in general terms it can be observed a great similarity between the enriched batched and the control batch.

However, the presence of calcium salts influenced negatively on the taste and so, independently of the type of salt or the amount added, all the enriched batches achieved punctuation lowers than the control batch.

Significant differences were observed between control and CL30 batches; the panellists described this last batch as *slightly more acidic*.

The overall acceptability values behaved similar than the taste ones and, consequently, the taste seemed to be the most influential param-

eter. So, the punctuations obtained by the acceptability were very similar in all the enriched batches, although it was observed that the lowest values were those for batches CG20 and CL30. The control batch reached the best score although the difference was not significant with CL20 and CCM30 batches. In any case none of the batches were discarded because all of them exceeded the value of 5 points.

Bioavailability

In order to facilitate the calcium analysis, it was decided to perform the bioavailability study using the batches manufactured with the higher concentration: CL30 and CCM30. The CG batches were discarded for this study because of the low sensory quality. Table 6 shows the results obtained. The bioavailability was determined in the cooked hamburgers.

The calcium amount determined was slightly higher than the expected, a 20% approximately. This increase could be due to the loose of water during the cooking as it has been reported by PAN and SHING (2001). These authors established that the water losses during ground beef cooking (60 ºC, 2 min) is closed to 15-20%. The final calcium amount determined was sufficient to give the calcium levels proposed in our objective (30% RDA).

In the gastric phase was detected all the calcium presented in the cooked hamburgers.

The dialyzed calcium was close two fold higher in the hamburgers enriched with CCM30 than those with CL30. The solid deliveries calculated were 171.29 mg in the CL batch and 129.12 mg in the CCM one. This difference represents a calcium loss close to 47% and 34%, respectively. It could be due to that the CCM interferes with proteins during the cooking and contributes, as it has been described above, to the formation of a more compact protein network that could difficult the activity of pancreatin-bile complex, and to favor the retention of a higher calcium amount.

Applying the formula (see Material and Methods section), the bioavailability percentage was 7.73% for CL and 14.55% for CCM.

The variability in calcium bioavailability is

Table 6

Calcium content in the different phases of the in vitro digestion and calcium bioavailability (%) from cooked hamburgers [ß]					
Calcium Salt	Sample*	Gastric Phase Ca*	Non Dialyzed Ca*	Dialyzed Ca*	Bioavailability (%)
CL30	373.65±37.36	363.95±36.53	163.82±26.14	28.84±2.20	7.73
CCM30	361.85±25.33	382.6±23.00	200.82±12.01	52.66±3.08	14.55

[ß] Data are mean ± S.D.
*mg Ca/100 g hamburger
Bioavailability (%) = [Dialyzed Ca (mg) / Ca sample (mg)]*100

consistent with numerous studies. One of the first bioavailability assays performed (MORRISEY and FLYNN, 1972) reported that the calcium absorption from cow's milk ranged from 21 to 45% in healthy human adults. RECKER *et al.*, (1988) described that the absorption of calcium carbonate from enriched whole milk, chocolate milk, yogurt, imitation milk and cheese ranged between 21-26% in postmenopausal women. KRUGER *et al.*, (2003) concluded that calcium bioavailability was similar in milk fortified with calcium carbonate or milk calcium. In a study performed by SITTIKULWITIT *et al.*, (2004) with different calcium salts and milk powder, bioavailable calcium ranged between 28.5 and 58.7%. Other authors determined calcium absorption from infant milk formula of 39% (NELSON *et al.*, 1996) and 29% from enriched orange juices (GONELLI *et al.*, 2007). CILLA *et al.*, (2011) reported calcium efficiency uptake values from 10.47-19.82% for different milk-based fruit beverages and PERALES *et al.*, (2005) found calcium bioavailability to be 5.0-31% for infant and adapted milks.

The differences reported between the values of calcium dialyzable have be also attributed to the methodology applied, particularly with regard to the amount and activity degree of the enzymes used, the pH values and incubation times during the gastric and intestinal phases. In this way, VAN der HEE *et al.*, (2009) reported that calcium absorption from food depends on numerous factors, causing a range broadly from 15 to 44%. So, bioavailability described in this paper is according to the data collected by other authors. Furthermore, our results showed that CCM would be the most bioavailable salt from hamburgers, which suggests that is the most appropriate for the enrichment of fresh meat products.

SITTIKULWITIT *et al.*, (2004) reported that the dialysis rate of calcium (calcium bioavailable) in milk powder is 28%. Taking into account that 200 ml of milk would give 240 mg of calcium, the bioavailable amount would correspond to 67.2 mg. According to our data, if 100 g of hamburger enriched with CCM at the concentration of 30% IDR leaves bioavailable 52.66 mg (Table 6), one commercial hamburger of 120 g, would leave 63.2 mg calcium, a similar quantity to that reported for milk.

CONCLUSIONS

CCM and CL can be adequate for enriching fresh meat products (hamburgers). They can be added in sufficient amount to give a theoretical calcium level of 30% of RDA. GC crystallizes and it is not recommended for its use. The calcium bioavailability from CCM enriched hamburgers was the highest, close to 15%. This allows that one hamburger of 120 g enriched with CCM 30% RDA, would leave bioavailable 63.2 mg calcium, a similar calcium amount to 200 ml of milk.

Consequently, CCM is proposed as the most adequate salt and the hamburger manufactured with it could be considered as a *source of* calcium, according to the Regulation N° 1907/2006 of the European Parliament.

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