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A Novel Time/Temperature Approach to Sous Vide Cooking of Beef Muscle

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Abstract Sous vide treatment is encountering a renewed interest among chefs and catering operators, but an important concern is that they had been joined by those who were not aware resulting in foods held at time/temperature combination that may be not appropriate by a qualitative point of view. In this study, beef *semitendinosus* muscles were sous vide cooked by applying two different time/temperature treatments, a typical low temperature–long time (LT-LT) condition realized by cooking 36 h at 75 °C (SV75) and an innovative high temperature–short time (HT-ST) one for 2 h at 100 °C (SV100). Data were compared to traditionally boiled meat, and changes in pasteurization values, weight loss, texture, color, vitamins of B group as well as volatile compounds profile were evaluated. HT-ST treatment proved to achieve a pasteurization value sufficient to exclude *Clostridium perfringens* risk, while on the contrary, LT-LT may be a sous vide cooking approach that could be subjected to this microbiological hazard. Total weight loss of SV100 resulted significantly lower compared to SV75. SV75 samples showed the lowest shear force and hardness, being also less red than the other two samples. In addition, vitamin B_3 retention was very similar for both sous vide methods, while LT-LT condition allowed a higher retention of B_{12}. Finally, volatile compounds of beef muscles cooked by means of LT-LT and HT-ST sous vide conditions showed lower accumulation of off-flavor such as hexanal or 3-octanone in comparison to traditional boiling technique and better preserved the volatile profile of raw meat. Sous vide cooking at HT-ST condition used in this study could represent a feasible alternative to low-temperature treatment allowing to obtain comparable or better qualitative standards except for vitamin B_{12} retention and hardness.

Keywords Beef · Sous vide · Texture · Color · Vitamins · Volatile compounds

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Introduction

The sous vide cooking technique can be considered as a variant of cook and chill catering technology receiving great attention both from chefs and researchers in the last 10 years (Baldwin 2012; Rinaldi et al. 2013). This preparation technique was reported to give more benefits (increased tenderness, improved color retention, texture, and flavor) than conventionally cooked foodstuffs because of the mild process and the absence of oxygen in the pack (Baldwin 2012). It consists in cooking of raw materials (e.g., meat, fish, and vegetables) at product-specific temperatures and time in vacuum pouches, followed by rapid cooling and storage at refrigerated temperature (Baldwin 2012).

Regarding meat products, sous vide is the typical cooking method used for low temperature–long time treatments (LT-LT), commonly realized in the temperature and time ranges of 65–90 °C and 2–48 h, respectively (Vaudagna et al. 2002). The major disadvantage of LT-LT sous vide treatment is precisely represented by the great time required to obtain microbiological safety and inactivation of vegetative cells as consequence. This fact obligates food operators to prepare cooked meats several days before serving and to store them until consumption under refrigerated condition. Sous vide literature about microbiological safety has mainly focused on botulism (Hyytiä-Trees et al. 2000), but there are other

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potential hazards that have received less attention: the main food safety risks of LT-LT sous vide cooking are *Clostridium perfringens* and bacteria that form heat stable toxins such as *Bacillus cereus* and *Staphylococcus aureus*. In particular, *C. perfringens* was reported to be the pathogen best adapted to growth during processing of sous vide meat (Willardsen et al. 1978) due to improper cooling or temperature maintenance, and several actions were tested as chemical preservative addition to extend their shelf life (García and Heredia 2001).

From a qualitatively point of view, many studies showed that low temperature–long time treatments cause an increase in tenderness for different kinds of meat such as beef (James and Yang 2012; Vaudagna et al. 2002; García-Segovia et al. 2007), bulls (Christensen et al. 2013), pork (Sánchez del Pulgar et al. 2012), and lamb (Roldán et al. 2013). Regarding sous vide cooking technology, Vaudagna et al. (2002) reported an increase in cooking weight loss and a decreased in shear force values for beef muscles as the sous vide treatment temperature was raised from 50 to 65 °C, while processing times (90–360 min) did not have a significant effect on these variables. García-Segovia et al. (2007) studied the effect of different sous vide temperature and time combinations (60–80 °C and 15–60 min) on beef, observing a toughness decrease with increasing temperature as well as a more intense reddish and a less intense brownish-green color compared to traditional cooking.

From a nutritional point of view, sous vide cooking method was widely reported to guarantee a higher retention of vitamins compared to traditional cooking methods (Creed 1995). Only few studies deal with vitamins B_3 and B_{12} retention after sous vide cooking of meat. Lassen et al. (2002) reported no significant differences in vitamins B_1, B_2, and B_6 retention between traditional and sous vide cooking of pork loin, stating that the internal temperature of the meat is probably the single most important factor influencing the extent of these vitamin retention and suggesting the importance of studying time–temperature combination (Lassen et al. 2002).

In this framework, the application of new sous vide time/temperature combinations for meat cooking deserves considerable attention as the final product may present organoleptic, safety, and nutritional characteristics that are deeply different. Díaz et al. (2008) working on spoilage during the refrigerated storage of sous vide cooked pork loin reported that cooking at 70 °C for 2 h effectively pasteurized the vacuum-packed cuts, but they suffered considerable sensory deterioration during refrigerated storage due to residual enzymatic activity of the muscle proteases. The authors only suggested increasing cooking temperature up to 90 °C to prevent deteriorating of sensorial attributes during storage. To the best knowledge of the authors, no studies deal with sous vide cooking of beef muscles with a temperature higher than 80 °C and its effect on product quality and yield. Thus, the aim of this study was to compare the effect of an innovative HT-ST time–temperature combination (2 h at 100 °C) with a LT-LT approach (36 h at 75 °C) and the traditional boiling method (2 h at 100 °C) on sous vide-cooked beef muscles of semitendinosus by means of hygienical, physico-chemical, and nutritional parameter evaluation.

Materials and Methods

Sample Preparation and Cooking Treatments

Ten beef muscles (semitendinosus) (700±50 g) were obtained from a local supermarket at 36–48 h from slaughter and analyzed within 12 h. Muscles were totally trimmed of surface fat and cut into blocks measuring 120 mm×120 mm×40 mm.

Proximate composition (water 74.8 %, protein 22 %, fat 2.6 %, and ash 0.8 %) of each fresh muscle (R) was immediately confirmed in triplicate using AOAC methods (2002).

Sous vide (SV) samples were prepared vacuum packing each block and removing the air in vacuum bags (oriented polyamide/polypropylene (PP) 15/65, thickness 80 μm, Orved, Musile di Piave, Italy) with a packaging machine (Dito Electrolux, Stockholm, Sweden) and cooking them at 100 °C for 2 h high temperature–short time (HT-ST) treatment (named SV100 in the text) and at 75 °C for 36 h low temperature–long time (LT-LT) treatment (named SV75 in the text) in a water bath (JULABO Labortechnik GmbH, Seelbach, Germany). After rapid chilling (easyChill, Zanussi, Italy), samples were stored at 4 °C for 1 day and then reheated for 20 min in a water bath (JULABO Labortechnik GmbH, Seelbach, Germany) at 70 °C, as usually done in restaurants and caterings. Boiled samples (B) were prepared cooking blocks at 100 °C for 2 h in the same water bath without any packaging and without salt. Three cooking trials were performed for each condition.

Heat Penetration Curve, Pasteurization Values, and Degree of Cooking Determination

Time–temperature profiles at muscle centers, considered as the slowest heating point, were recorded by means of electronic data loggers (Ebro Electronic GmbH Ingolstadt, Germany) with an acquisition interval of 1 min and data points were then collected in an Excel® worksheet.

The pasteurization units and the degree of cooking, expressed in terms of cook value, at the thermal center were obtained from the integration of the experimental heat penetration curve:

$$F^{z}_{\mathrm{Tref}}/C^{z}_{\mathrm{Tref}} = \int_{0}^{t} 10^{(T-T_{\mathrm{ref}})/z} dt$$

where t is time (min), T_{ref} is the reference temperature set equal to 90 °C for pasteurization units and to 100 °C for cook value, and z is temperature increase that induces a tenfold increase of the reaction rate of the chemical reaction taken as reference. A z value of 7.7 °C (*C. perfringens*) was used for pasteurization value (Byrne et al. 2006) and of 33 °C for cook value (Vittadini et al. 2005).

Weight Loss and Water Content Determination

Total weight loss was calculated as the percent weight difference between the raw and cooked meat piece referred to the weight of the raw one. The weight of the boiled (B) and sous vide samples (SV100 and SV75) was measured after 30 min from the end of the cooking or reheating cycle, respectively. Samples rested on an open rack at room temperature and were then immediately analyzed for color and texture.

Water loss was calculated as the percent difference between water content of raw and cooked samples determined according to the official method (AOAC 2002) referred to the raw one. Briefly, 3–4 g of raw and treated homogenized sample (as triplicate) was dried in a convection oven at 105 °C for at least 16 h until reaching constant weight according to AOAC (2002).

Color

Color determination of raw and cooked samples was carried out using a Minolta Colorimeter (CM 2600d, Minolta Co., Osaka Japan) equipped with a standard illuminant D65. The assessments were carried out on five preselected locations at the surface of each sample. L* (lightness, black = 0, white = 100), a* (redness >0, greenness <0), b* (yellowness, b* >0, blue <0), C (chroma, 0 at the center of the color sphere), and hue° (hue angle, red = 0°, yellow = 90°, green = 180°, blue = 270°) were quantified on each samples using a 10° position of the standard observer.

Texture

Texture of the raw and cooked samples was analyzed by Warner–Bratzler shear force (WBSF) analysis using a TA.XT2 Texture Analyzer (Stable Micro Systems, Goldalming, UK). From each raw and cooked muscle block, four samples of parallelepiped geometry (30×60×30 mm) with muscle fibers running parallel to the major dimension were obtained. More than ten samples were obtained and analyzed for each cooking trial. Muscle fibers were placed perpendicular to the Warner–Bratzler blade, and shearing was carried out with a 25-kg compression cell at a velocity of 5 mm/s. The maximum force of the force/distance peak (WBSF) was taken as a measure of hardness (tenderness) of the sample using the application software provided (Texture Expert for Windows, version 1.22).

Texture profile analysis (TPA) tests were also performed on cooked samples. From the center of each cooked muscle block, four cubic samples (20×20×20 mm) were extracted and subjected to TPA test using a cylindrical aluminum probe (35 mm diameter) at a crosshead speed of 2 mm/s to compress samples to 50 % of their original height. The textural parameters considered were hardness (HD, peak force of the first compression cycle, in N), cohesiveness (CO, ratio of positive force area during the second compression to that during the first compression area, dimensionless), springiness (SP, ratio of the time duration of force input during the second compression to that during the first compression, dimensionless), and chewiness (CH, hardness × cohesiveness × springiness, in N) (Bourne 1978).

Volatile Compound Analysis

The volatile fraction of raw and cooked meat was extracted using dynamic headspace solid phase microextraction technique as follows: 1 g of finely sliced samples was placed in a 30-ml glass vial sealed with Black Viton septa (Supelco, Bellefonte, PA, USA) and extracted for 45 min at 30 °C (water bath) as per Rinaldi et al. (2013). Toluene (250 μg/kg) was added as internal standard. Each sample was analyzed in duplicate.

For the analyses, a silica fiber was used coated with 50/30 μm of divinylbenzene–Carboxen–polydimethylsiloxane(DVB/Carboxen/PDMS) (Supelco, Bellefonte, PA, USA). Before the analysis, the fiber was conditioned by insertion into the GC-MS injector at 220 °C for 2 min. GC-MS analyses were performed using an Agilent Technologies 6890N gas chromatograph coupled to an Agilent Technologies 5973 mass spectrometer, resolving compounds on a SUPELCOWAX 10 capillary column (Supelco, 30 m×0.25 mm, f.t. 0.25 μm) (Supelco, Bellefonte, PA, USA) with an electron impact ionization and a full scan acquisition mode (from 40 m/z to 500 m/z). The main volatile compounds of the aromatic profiles were identified by comparison of the mass spectra obtained from the analyses of the samples with the reference mass spectra libraries (WILEY275, NBS75K): a match quality of 98 % minimum was used as a criterion for the identification.

Vitamins B_{12} and B_3 Determination

Determination of cobalamin (B_{12}) and niacin (B_3/PP) was carried out as follows. Briefly, after addition of 30–40 ml of water to 10 g of sample, a slurry was prepared by homogenization by means of an Ultra-Turrax homogenizer at 9,500 rpm for 5 min. Afterwards, the volume was adjusted at 50 ml by addition of water, then the slurry was transferred in a

sonication bath and treated for 10–15 min. The solution was then filtered on paper (Whatman No. 40) and then through a 0.45-mm Whatman acrodisc filter before the analysis. A LC-QDUO system (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used equipped with a Jupiter C18 column (250×4.6 mm, 5 μm) (Phenomenex, Castel Maggiore, Bologna, Italy).

Vitamin B_{12} was separated under isocratic conditions using as mobile phase 0.1 % HCOOH and CH_3CN 15:85 *v/v* at a flow rate of 1 ml/min. The MRM analysis was performed monitoring the transition 1355→1209 using a positive ionization mode.

Vitamin B_3 was separated using 0.1 % HCOOH (eluent A) and CH_3OH (eluent B) as mobile phase; the flow was set at 1 ml/min. The linear gradient moves from 20 % eluent B to 50 % eluent B in 15 min, followed by a reconditioning step (5 min). The quantification was performed under SIM conditions by monitoring the molecular ion 123 *m/z*.

The percentage of nutrient retention after cooking was calculated by using the following equation (Bognar and Piekarski 2000):

$$\%\ \text{retention} = \frac{\text{nutrient content}/100\ \text{g cooked meat}}{\text{nutrient content}/100\ \text{g raw meat}} \times \frac{\text{meat weight (g) after cooking}}{\text{meat weight (g) before cooking}} \times 100$$

Statistical Analysis

SPSS statistical software (Version 20.0, SPSS Inc., Chicago, IL) was used to perform one-way analysis of variance among samples from different treatments. The least significant difference at a 95 % confidence level ($p<0.05$) was performed to further identify differences among groups.

Results and Discussion

Pasteurization and Cook Values

Pasteurizing and cook values calculated from experimental time–temperature profiles are reported in Table 1. B and SV100 showed very similar pasteurizing values, as they presented the same thermal treatment and significantly higher compared to SV75 despite its very long cooking time. All the considered cooking treatments achieved a sufficient inactivation of pathogens from beef meat but required a refrigerated storage as *Clostridium botulinum* spores were not inactivated. Other authors (Roldán et al. 2013) reported an effective reduction of microbial population for sous vide-cooked meat products subjected to low-temperature long time treatments. Regarding the inactivation of *C. perfringens* spores, LT-LT treatment (SV75) did not achieve an adequate number of decimal reduction (Table 1) if a *D* value of 30.8 min was considered (Byrne et al. 2006), confirming the potential safety risk with reference to sporigen pathogen for this culinary technique.

SV75 showed higher cook value with respect both to B and SV100. Cook value is a measure of the cumulative heat impact of a complex time/temperature history on a food quality attribute; thus, a cook value increase corresponds to a higher degree of cooking. Experimentally calculated cook values were further used to explain observed changes in physicochemical properties.

Table 1 Slowing heating point cook, pasteurizing values, surface cook values, and number (*n*) of decimal reductions of cooked beef samples

	B	SV75	SV100
T–t condition	100 °C—2 h	75 °C—36 h	100 °C—2 h
$F_{90}^{7.7}$	1,342.9±21.0a	23.8±1.7b	1,339.3±20.5a
C_0	85.6±11.5a	372.0±21.3b	83.8±13.6a
C_{0sup}	120.7±8.5b	377.5±35.2a	124.0±9.4b
n (*C. perfringens*)	43.6	0.8	43.5

Values are expressed as mean ± SD ($n=3$). Means in rows followed by different letters differed significantly ($p<0.05$)

B boiled, *SV75* sous vide at 75 °C, *SV100* sous vide at 100 °C, $F_{90}^{7.7}$ pasteurizing effect with T_{ref} of 90 °C and *z* of 7.7 °C, *C0* cooking effect at core with T_{ref} of 100 °C and *z* of 33 °C, *C0sup* cooking effect at surface with T_{ref} of 100 °C and *z* of 33 °C

Weight Loss and Water Loss

Water content, weight loss, and water loss for both raw and cooked samples are reported in Fig. 1.

Raw samples presented a water content of about 75 %, which was significantly higher compared to cooked meat, as expected. Among cooked samples, B presented the lowest values, while no significant differences were observed between SV100 and SV75. Same results were obtained for percentage water losses: B presented the highest value while other samples presented no significant differences among them. The package prevented water removal from meat samples as it served as a physical barrier and it held moisture inside the bag preventing also the discharge of liquid due to limited space.

Regarding total weight loss, SV75 presented the highest value (38.9 %), while on the contrary, SV100 presented the lowest (35.8 %). Despite no differences were observed for

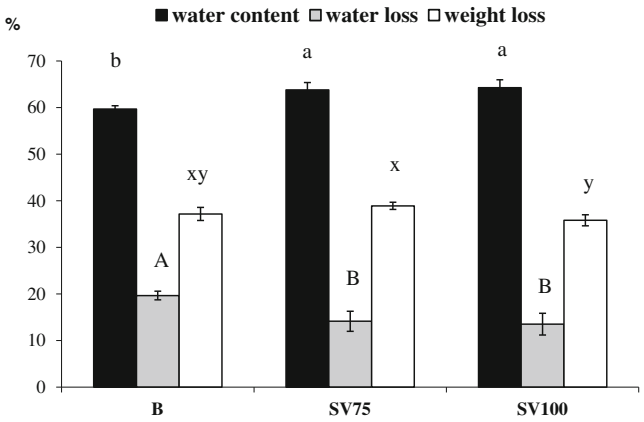

Fig. 1 Weight loss and water content of cooked samples. *Error bars* represent ±1 SD, (n=3). *Bars of histograms with different letters* are significantly different (p<0.05). *B* boiled, *SV75* sous vide at 75 °C, *SV100* sous vide at 100 °C

water content and water loss between SV75 and SV100, the first presented a significantly higher total weight loss showing a greater effect of time than temperature on the release of other water-soluble components, such as soluble protein, and collagen solubilization, as previously found by Sánchez del Pulgar et al. (2012) on pork cheeks.

Color

Colorimetric data of meat samples are reported in Table 2. All the samples were lighter compared to raw meat but L* did not significantly differ among cooked samples. García-Segovia and coauthors (2007) did not found significant differences for L* values among sous vide- and atmospheric pressure-cooked beef. Regarding a* values, no significant differences were observed between SV100 and B. On the other hand, SV75 showed significantly lower value that the other two samples. The intensity of the a* parameter of cooked meat is inversely related to the degree of denaturation of myoglobin, a process which takes place between 55 and 65 °C although continues until 75 or 80 °C (King and Whyte 2006). This result is also in agreement with experimentally calculated cook values, which were significantly higher in SV75 compared to SV100 and B, as shown Table 1, and probably in relation with higher myoglobin degradation as cooking values increased. No significant differences were observed for b* values, with a total ranges in mean b* values of about 0.5 units, which represents a data of little relevance for meat internal color as previously stated by Yancey et al. (2011).

Regarding color saturation (C), SV75 showed lower value compared to SV100 and B, although not significantly. C was not only reported to be related to the concentration of myoglobin but also to its degree of denaturation as it is higher with greater concentrations of myoglobin and at a lower rate of denatured myoglobin (Ledward 1992). Thus, this result was in accordance with calculated cook values (Table 1), as previously stated also for a* values that were found to significantly differ instead.

Finally, hue angle (H°), which is affected by the chemical state of the myoglobin as it was generally related to a great content of metmyoglobin giving browner color (Hunt et al. 1999), resulted higher in SV75 compared to both B and SV100 and inversely related to a* values, as previously reported by Sánchez del Pulgar et al. (2012).

Table 2 Color indices (L*, a*, b*, C, and Hue°) and textural parameters for raw and cooked beef samples

	R	B	SV75	SV100
Color				
L*	41.6±1.9	56.3±3.5a	54.3±3.9 b	54.7±5.5 b
a*	10.2±1.2	5.7±0.8a	4.0±0.7 b	5.7±0.6a
b*	10.5±1.2	11.2±1.4a	10.9±1.3a	11.4 ±1.5a
C	14.8±2.2	12.7±3.3a	11.6±2.6 a	12.9±2.1a
Hue	45.1±3.7	61.0±5.5b	69.9±3.7a	62.8±5.0 b
Warner–Bratzler				
WBSF (N)	50.1±3.8	64.8±4.0a	50.7±3.4b	65.3±5.0a
TPA				
HD (N)	1.34±0.12	12.85±1.42a	3.88±0.28c	9.95±0.58 b
CO	0.46±0.05	0.33±0.05a	0.38±0.04a	0.38±0.03a
SP	0.92±0.04	0.45±0.05a	0.42±0.05a	0.41±0.05a
CH (N)	0.58±0.10	1.88±0.51a	0.63±0.16b	1.53±0.32a

Values are expressed as mean ± SD (n=10). Means in rows followed by different letters differed significantly (p<0.05). Statistical significance of raw sample was not considered

R raw, *B* boiled, *SV75* sous vide at 75 °C, *SV100* sous vide at 100 °C, *HD* hardness, *CO* cohesiveness, *SP* springiness, *CH* chewiness, *WBSF* Warner–Bratzler shear force

Texture

The raw material was characterized by a peak shear force of about 50 N (Table 2) in agreement with literature data about raw beef muscles (L'Hirondelle and Martin 1975). B- and SV100-cooked samples presented higher Warner–Bratzler shear force (Table 2) compared to raw. On the other hand, SV75 exhibited comparable values to those of uncooked meat. Among treatment, values of SV75 were significantly lower compared to both B and SV100 probably due to its lower end point temperature (about 75 vs. about 99 °C) as well as its higher water content (Fig. 1). Vaudagna et al. (2002) suggested that heat in conjunction with the moist in-pack environment solubilizes the connective tissue leading to meat tenderization, also contrasting the denaturation of myofibrillar proteins, which leads to meat toughening in LT-LT in sous vide-cooked beef increasing cooking time. On the contrary, SV100 hardness values presented no significant differences compared to B probably because moisture into pack was not

able to efficiently prevent protein denaturation at high temperature.

A TPA test was also carried out in this study as it was reported to be useful for predicting sensory texture of cooked meat (Ruiz de Huidobro et al. 2005), and the results are reported in Table 2. Unlike WB, TPA hardness (HD) values were different among all the cooked samples. B presented a significantly higher hardness compared to SV100, while SV75 showed the lowest value. Roldán et al. (2013) reported a significant lower hardness values for loin lamb samples sous vide cooked at 60, 70, and 80 °C for 24 h compared to 8 and 16 h due to an extensive disintegration of the perimysium around muscle bundle. García-Segovia et al. (2007) observed by microscopy that in LT-LT sous vide-cooked beef muscle, connective tissue (endomysium) structures were more diffused, and myofiber–sarcoplasma was not as compact as atmospheric pressure cooking in relation with greater collagen solubilization and gel formation with a smaller degree of aggregation. This effect could be more marked under LT-LT condition in comparison with HT-ST ones. Cohesiveness and springiness did not significantly differ among samples while chewiness was significantly higher for B and SV100 in accordance with hardness (Table 2).

Vitamins B_3 and B_{12} Retention

Vitamins B_3 and B_{12} content in raw samples was about 5.96 mg/100 g fresh weight and 3.90 μg/100 g fresh weight, respectively; obtained values are in agreement with published data on raw beef muscles (Lombardi-Boccia et al. 2004). Percentage retentions of vitamins B_{12} and B_3 after cooking are reported in Fig. 2. Regarding B and SV100, these samples presented a significantly lower retention of vitamin B_{12}, which was generally retained to be relatively stable to both atmospheric oxygen and heat under most food processing operations (Lešková et al. 2006) compared to SV75. Vitamin B_{12} is also water soluble and loss during heat treatment of unpacked food as in B samples mainly due to leaching in boiling water and being also higher, increasing the quantity of water used for cooking (Lešková et al. 2006). Otherwise, the higher loss shown by SV100 samples in comparison with SV75 was probably due to the lower cooking temperature (75 vs. 100 °C) for the latter. Thus, the feasibility of sous vide cooking at high temperature for short time could be limited with respect to vitamin B_{12} retention.

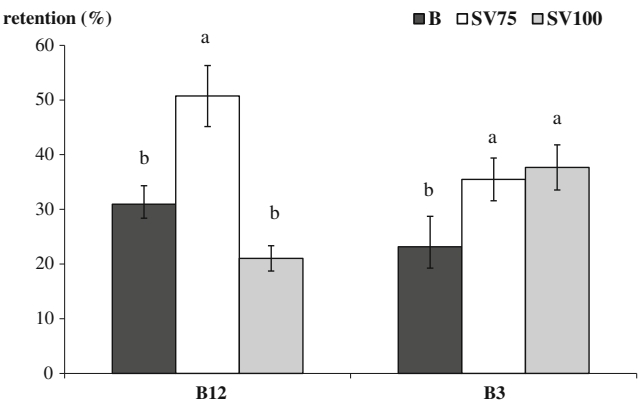

Fig. 2 Vitamins B_3 and B_{12} percentage retention in beef muscles after cooking. *Error bars* represent ±1 SD, (n=3). *Bars of histograms with different letters* are significantly different (p<0.05). *B* boiled, *SV75* sous vide at 75 °C, *SV100* sous vide at 100 °C

On the contrary, vitamin B_3 (niacin) retention did not show significant differences between SV100 and SV75 with both values significantly higher than B (Fig. 2). Niacin is the most stable water-soluble vitamin while leaching is usually the primary pathway of its loss during food preparation (Eitenmiller and Landen 1995). Thus, plastic packaging in SV75 and SV100 could have prevented niacin loss into cooking water allowing a better retention than traditional boiling procedure (B).

Volatile Compounds

It is generally accepted that the aroma of meat is mainly developed upon heating due to lipid peroxidation, Maillard and Strecker reaction, and thiamine degradation (Resconi et al. 2013). Among this transformation, lipid peroxidation is also responsible for the formation of unpleasant rancid flavors during storage and/or cooking. Accordingly, a triphasic fiber characterized by a good recovery towards carbonyl compounds was used, and a total of 16 gas chromatographic signals were identified in all the considered samples. The identification is reported in Table 3 along with the calculated retention index and the retention index from the literature. A number of minor compounds have been also recorded, although univocal identification was not possible.

Volatile levels detected in raw and in sous vide-cooked meat are comparable being 31.6, 30.9, and 28.9 μg/kg in raw meat, SV75 and SV100, respectively; on the contrary, their total content is significantly higher in boiled beef muscle (42.6 μg/kg).

Considering the volatile profiles obtained for B, SV75, and SV100 in comparison to raw meat, it can be noticed that sample treated under sous vide condition better preserve the volatile profile, avoiding the accumulation of off-flavor such as hexanal or 3-octanone. It is important to notice that volatiles can be released differently from raw and cooked meat; thus, flavor perception is strongly influenced by the texture. In boiled or mild cooked meat, volatiles can actually be entrapped into gelatinized proteins, resulting in a lower release into the headspace (Brewer and Vega 1995).

In this work, carbonyl compounds such as aldehydes and ketones have been found to be the most abundant compounds in cooked beef (Fig. 3a, b). This is in agreement with the literature since lipid-derived volatiles were found to dominate

Table 3 Volatiles found in raw and cooked beef muscles

Volatile (μg/kg)	LRI_{calc}	LRI_{lit}	R		B		SV75		SV100	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Hexanal	1,080	1,085[a]	2.58	0.05	17.59	0.20	4.71	0.05	3.75	0.04
Limonene	1,193	1,193[a]	1.68	0.20	2.40	0.18	2.17	0.04	1.57	0.12
3-Octanone	1,271	1,285[b]	8.05	0.25	11.02	0.22	8.70	0.17	9.83	0.20
4-Methylthio-2-butanone	1,302	1,317[b]	3.15	0.06	2.08	0.02	8.03	0.09	7.88	0.10
2-Heptenal	1,325	1,333[a]	0.60	0.04	0.42	0.02	0.76	0.03	0.60	0.03
2-Hexenyl acetate	1,332	1,327[b]	0.89	0.02	0.37	0.02	0.81	0.03	0.84	0.04
3-Nonenal	1,339	1,345[b]	0.71	0.05	0.30	0.00	0.56	0.01	0.15	0.00
1-Hexanol	1,359	1,357[a]	3.14	0.10	0.67	0.01	0.67	0.01	0.32	0.01
1-Octen-3-ol	1,455	1,454[a]	0.35	0.01	2.46	0.05	0.67	0.01	0.60	0.01
2-Ethyl-1-hexanol	1,494	1,493[c]	0.32	0.01	0.42	0.01	0.57	0.01	0.66	0.02
2-Nonenal	1,507	1,502[c]	0.60	0.02	0.49	0.01	0.40	0.00	0.19	0.00
Methylfurfurylthiol	1,538	1,527[b]	0.72	0.01	2.81	0.07	1.64	0.04	1.18	0.03
1-Octanol	1,562	1,561[a]	0.31	0.02	0.55	0.00	0.23	0.00	0.21	0.00
2-Octen-1-ol	1,623	1,618[b]	0.16	0.01	0.21	0.01	0.07	0.00	0.07	0.00
γ-Butyrolactone	1,650	1,648[a]	0.33	0.01	0.28	0.01	0.37	0.01	0.45	0.01
1-Nonanol	1,669	1,668[c]	0.71	0.05	0.20	0.00	0.16	0.00	0.14	0.00

Data are given as mean concentration and SD (n=3) and expressed as microgram per kilogram

[a] Linear Retention Index according to Cirlini et al. (2012)

[b] Linear Retention Index according to the NIST database http://www.nist.gov

[c] Linear Retention Index according to Dall'Asta et al. (2011)

R raw, *B* boiled, *SV75* sous vide at 75 °C, *SV100* sous vide at 100 °C

quantitatively others in boiled or treated meat under mild temperature conditions in particular ruminant meat (Drumm and Spanier 1991). In this context, it is important to underline that an oxygen-free packaging cannot completely avoid oxidation as traces of oxygen are actually present even immediately after the product is vacuum-packed, and no commercial material is entirely impervious to oxygen (Gill 1996).

Aldehydes, ketones, and esters are the main substances deriving from lipid oxidation in meat, although linear alcohols and hydrocarbons such as alkanes and alkenes may be formed at a lower extent. Among them, ketones are represented at similar levels in raw meat (8.0 μg/kg) as well as in cooked beef muscles being about 11.0 μg/kg in boiled meat, 8.7 μg/kg in SV75, and 9.8 μg/kg in SV100. Boiled beef muscle is mainly characterized by aldehydes (18.9 μg/kg) than sous vide-treated samples (about 6.7 and 5.4 μg/kg in SV75 and SV100, respectively). 3-Octanone, with a typical herbal note, occurs at comparable levels in raw meat and sous vide-treated sample being, on the contrary, slightly higher in boiled muscle. SV75 and SV100 are strongly characterized by 4-methylthio-2-butanone that is significantly lower in B and in raw meat: this compound is characterized by a typical roast note.

Boiled beef muscle is mainly characterized by hexanal, as reported in Fig. 3b; this compound is usually responsible of a typical green note, but at higher amount, it is also responsible for rancidity and warmed over flavor, especially when boiled beef is stored in the refrigerator and reheated (Kerler and Grosch 1996). SV75 and SV100 are, on the contrary, less characterized by this compound in comparison to other lipid-derived volatiles. Alcohols such as 1-hexanol and 1-octen-3-ol are the most abundant volatiles in raw beef muscles. Both compounds went lost upon cooking, probably on account of their strong hydrophilic behavior.

None of the volatiles reported in Table 3 can be ascribed to thiamine degradation: this is in agreement with the literature since this precursor seems to be more important in pork than in meats from other species, such as ruminants (Meinert et al. 2009). In particular, concentration of thiamine is the lowest in beef muscle (0.08–0.11 mg/100 g) than in other animal meat (Resconi et al. 2013). In addition, when cooking treatment is performed at mild temperature conditions, the vitamin is retained in the meat juice expelled but not thermally degraded (Lassen et al. 2002).

Maillard reaction followed by Strecker degradation usually leads to the formation of pyrazines that are predominant in roasted and fried meat and sulfur compounds, the latter being fundamental for meat aroma on account of their very low threshold. Sulfur containing compounds are represented by 4-methylthio-2-butanone and methylfurfurylthiol (Table 3),

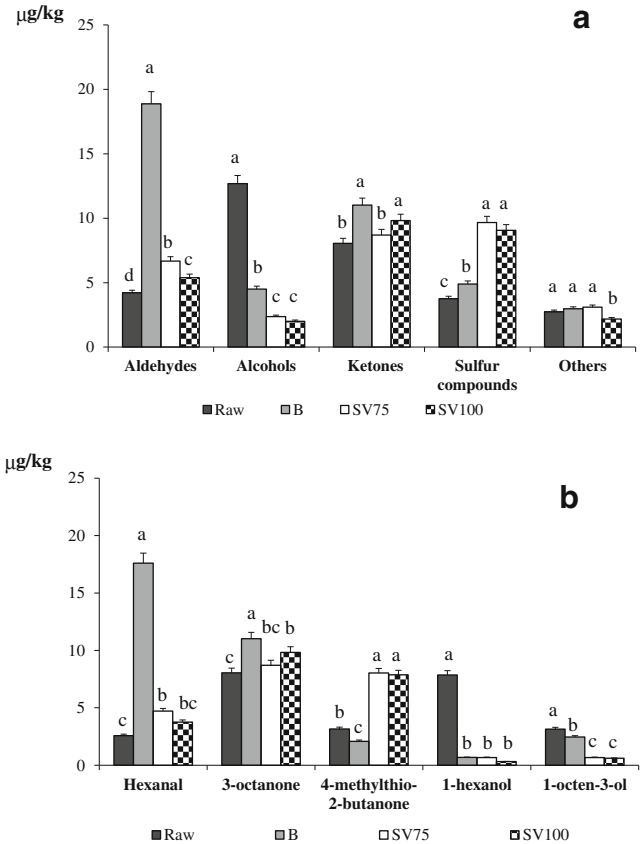

Fig. 3 Volatile groups (**a**) and main compounds (**b**) found in raw and cooked beef muscles. Data are expressed as micrograms per kilogram and corrected for water loss upon cooking. *Error bars* represent ±1 SD (n=3). *Bars of histograms with different letters* are significantly different (p<0.05). *B* boiled, *SV75* sous vide at 75 °C, *SV100* sous vide at 100 °C

both with a typical earthy and roasted note and both deriving from cysteine and methionine through the Strecker reaction. The formation of methylfurfurylthiol occurs at low level in raw meat and at higher concentration in cooked beef muscles (2.8, 1.6, and 1.2 μg/kg in B, SV75, and SV100, respectively), while the occurrence of 4-methylthio-2-butanone seems to be favored by sous vide treatment. Although cooked meat is usually strongly characterized by sulfur compounds, only two of them have been identified and found at reasonable levels within this study. Since their formation is temperature dependent, this behavior can be explained on the basis of the mild treatment applied herein. In addition, their detection is commonly difficult and requires specific conditions such as specific SPME fibers, since they occur in very low concentrations, are very reactive and may degrade easily.

Among compounds found at a comparable level in both raw and cooked samples, limonene clearly derived from the animal diet based on green forage (Vasta and Priolo 2006). Terpenes are usually stored into the adipose tissue of the animal and then released unchanged upon cooking. Similarly, γ-butyrolactone is known to be higher when the animals are finished on pasture or grass (Sivadier et al. 2008).

Conclusions

High temperature–short time (SV100) treatment demonstrated to be a good alternative to traditional low temperature–long time (SV75) one as it allowed achieving a sufficient reduction of *C. perfringens*, the most important pathogen in this kind of products. By a qualitative point of view, SV100 presented the lowest weight loss but significantly higher shear force and TPA hardness than SV75, although this latter value is very low in comparison with boiling probably due to the different cooking condition that have affected meet structure. On the other hand, SV100 was redder than SV75 with values comparable to B. Vitamin B_3 retention after cooking strengthened the feasibility of HT-ST treatment in sous vide cooking of meat with no differences in comparison to LT-LT procedure. On the contrary, vitamin B_{12} was better retained in SV75 probably due to the lower cooking temperature. Finally, samples treated under sous vide conditions, both LT-LT (SV75) and HT-ST (SV100), better preserved the volatile profile, avoiding the accumulation of off-flavor such as hexanal or 3-octanone in comparison to traditional boiling technique and allowed for a lower loss of volatiles compared to raw meat.

In conclusion, sous vide cooking at high temperature for short time could represent a feasible alternative to low-temperature treatment and allows obtaining a higher safety level and comparable or better qualitative standards with only the exception of vitamin B_{12} retention and hardness. These findings should be enforced by the sensorial evaluation of the proposed cooking procedure that could be also important to assess consumer acceptance for sous vide-cooked beef.

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