



## Sensory quality of roasted apple cv. Reineta affected by processing and storage conditions

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**Keywords:** Microbiology; sensory analysis; time storage; temperature storage

### 1 SUMMARY

The Designation of Origin (PDO) ‘Manzana Reineta del Bierzo’ realizes a very rigorous process of selection rejected apples can be used to obtain products that have greater added value as roasted apples. The changes in roasted apples cv. Reineta elaborated by procedures H1, H2, H3 and H4 and then packed in a commercial film and stored at temperatures of 3 °C and 20 °C degrees for a storage period of up to 140 d were studied following a factorial statistical pattern (4×2×10). Microbiological and sensory analyses were performed every 7 d until the end of the storage period. Each sample was made up of 146 fruit for each type of elaboration, storage temperature and storage time. Means comparison was based on the Tukey test. A PCA without rotation was performed on the mean sensory scores. No differences between the newly elaborated roasted apples and the roasted apples stored at 3 °C were found until the 42<sup>nd</sup> day of storage. Differences ( $p < 0.001$ ) in some visual attributes and in all olfactory-gustatory attributes were found between storage times whereas no differences were found between the type of processing conducted and acid taste or alcoholic flavour. The H4 was the only type of elaboration technique that kept the original properties of roasted apple cv. Reineta that were stored at 20 °C temperature for a 14 d period.

### 2 INTRODUCTION

‘Reineta’ is one of the two Spanish apples protected throughout the Community as a Protected Designation of Origin (PDO) ‘Manzana Reineta del Bierzo’ (The Commission of the European Communities, 2001). As a by-product of the selection process that the PDO demands, great quantities of unsuitable fruits are produced. Some of these fruits are apples that show only some deformity and are therefore used for the production of juice, but these apples could also be used to obtain products of greater added value such as roasted apple.

Roasted apple, which is done in coal or electric ovens, is a well-known dessert in Spanish homes. There are no references to any studies

that are based on the comparison of different types of ovens for roasting apples, or the adaptation of these ovens to the production of roasted apples, or their influence on the characteristics of the final product. There is little information concerning the impact of heat treatments on the sensory properties of apples. Universidad de León (2009) recommended that the proper procedure for roasting the apple cv. Reineta should be: roasting the apple, packing the apple in a flexible bag and finally finishing the process by pasteurizing the apple. This procedure is named *sous vide* (Juneja and Snyder, 2007). It is based on using an electric oven for a short period of time to produce apples with an external roasted aspect; subsequently the apples

are vacuum packed and pasteurized. These apples show similar aspects to those apples that have been roasted in a conventional oven.

Microwave heat is an attractive alternative to conventional heating methods because the electromagnetic wave that penetrates the surface is converted into thermal energy within the material. High speed start-up, selective energy absorption, instantaneous electric control, non-pollution, high energy efficiency, and high product quality are several advantages of microwave heating. Therefore, this technology is used in many industrial applications (Cha-um *et al.*, 2011).

### 3 MATERIALS AND METHODS

The experiment was carried out using a factorial statistical pattern ( $4 \times 2 \times 10$ ). The first factor was the type of processing used to roast the apples (H1, H2, H3, and H4). The second factor was the storage temperature ( $T_3$ , and  $T_{20}$ ). The third factor was storage time (7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 d) for the sensory analyses; however, the microbiological test continued until the 140<sup>th</sup> day.

**3.1. Fruit material:** Apple (*Malus domestica* Borkh) cv. 'Reinette du Canada' grown in "El Bierzo" region, province of Leon (Spain) and stored for 60 d in a controlled atmosphere U.L.O. (Ultra Low Oxygen) (2 %  $O_2$ , 1 %  $CO_2$ , 95 % R.H., 2 °C) was transported to the University of León to be processed.

**3.2. Roasted apple preparation and packaging:** Fruit of uniform size (75-80 mm diameter) were allowed to ripen at 20 °C for 12 days; then the apples were washed, disinfected with chlorine solution 2% (w/v) containing sodium hypochlorite (chlorine active 40 g  $L^{-1}$ ), and rinsed by pulverization with water. Finally, they were dried individually using filter paper. The central cores of the fruit were carved out, to remove the calyx and seeds, preserving the apple's peduncle (Marcelo *et al.*, 2010). Groups of six randomly chosen apples were then roasted using the four different roasting methods. A multifunction oven that had a microwave oven and electric system combination was used (model 801 042 ES, Siemens, Co., Madrid, Spain) capable of generating 0.8 kW power at 2450 MHz, lower and upper heating elements (2.5 kW power) and grill (2.0 kW power).

Storing roasted apple cv. Reineta in non-refrigerated conditions, which still allows the apple to be suitable for consumption, is very important, even if it is only for a few days, because it greatly reduces the cost of the distribution process and postharvest losses.

The objective of this study was to apply microbiological and sensory analyses to characterize the changes in roasted apples cv. Reineta that were processed by four different types and then packed in a commercial film and stored at two different temperatures for a storage period of up to 140 days.

H1: The apples were heated in a microwave oven at 0.8 kW for 10 min. Afterwards, the fruits were cooled. When the internal temperature of the fruit dropped to 40 °C, the apples (180-200 g) were then individually packed in flexible plastic bags LORE 90 COOK (90  $\mu m$  thick) (Cryovac Grace Packaging Corp. Barcelona, Spain). The permeability data of the plastic film at 23 °C were the following: water vapour transmission rate 2.6 g  $m^{-2} d^{-1}$ ;  $O_2$  transmission rate 4 ml  $m^{-2} d^{-1} kPa^{-1}$ ;  $CO_2$  transmission rate 15 ml  $m^{-2} d^{-1} kPa^{-1}$ . They were sealed using a compensated vacuum machine (Workshops Ramón, S.L. Barcelona, Spain) with a pressure of -0.1 MPa.

H2: The apples were heated in a microwave oven at 0.8 kW for 3 min and then heated in an electric oven with grill (2.0 kW) for 10 min. The fruits were cooled and packed as described in the H1 process above.

H3: The apples were heated in an electric convection oven using the bottom heating element (2.5 kW heated to 200 °C) and an air convection setting where the air speed was 1.5 m  $s^{-1}$ . The apples were heated under these conditions for 30 min. The fruits were cooled and packed as described in the H1 process above.

H4: The apples were heated using an electric oven with grill (2.0 kW, heated to 172 °C) for 7 min. The fruits were cooled and were packed as described in the H1 process above. Once the baked apples were packed, the apples were then pasteurized using a horizontal cylindrical autoclave (Ilpra Systems, corp. Barcelona, Spain). A heat treatment was then applied to the apples for 13 min at a temperature of



85 °C (pasteurization value PV70 in core 411.09 min). These parameters exceed the time and temperature parameters that are needed for microbiological stability. Finally the pasteurized products were cooled in a refrigerator (Ilpra Systems, corp. Barcelona, Spain), so that the temperature of the apples dropped to a temperature of 10 °C in 30 min (Universidad de León, 2009).

**3.3. Conservation of roasted apples:** The apples were stored at two different temperatures (3 °C and 20 °C). Each of the two groups of roasted apples were split into three subgroups, one for the microbiological analyses, one for the difference test, and one for the quantitative descriptive analysis (QDA) test. The maximum storage period was 140 days. A total of 146 apple samples were selected for each type of processing of the roasted apples, storage temperature and storage time.

**3.4. Microbiological tests:** The apples were extracted from the two different storage areas each seven days (up until the 140<sup>th</sup> day). When the apples were extracted microbiological and sensory analyses were conducted on them (sensory analyses were conducted only until the 70<sup>th</sup> day of storage). Since the pH of the 'Reinette du Canada' apple is very low, with usual values being close to 3.3 (Marcelo *et al.*, 2010), there is the risk of invasion by *Clostridium botulinum*, thus the microbiological tests were limited only to the counting of mesophilic bacteria, enterobacteriae, moulds and yeasts, according to a very strict Spanish regulation (Boletín Oficial del Estado, 2001). This regulation was repealed by community regulation (The Commission of the European Communities, 2005) which is less strict. In addition it was analysed that there was an absence of *Escherichia coli* and *Staphylococcus aureus* (both microorganisms are indicators of lack of hygiene) and absence of *Salmonella* spp and *Listeria* spp (microorganisms pathogen).

To obtain the 10<sup>-1</sup> dilution needed to perform the analysis, a 10 g sample was obtained from one apple piece using sterile techniques and the sample was blended with 90 ml of sterile buffered peptone water (0.1% w/v) for one minute in a sterile stomacher bag using a stomacher (model 400 circulator Seward, I.C.T., S.L., La Rioja, Spain). Two replicate counts were carried out for each bag each time the microbiological analyses were performed.

Mesophilic bacteria, enterobacteriae, yeast and mould counts were carried out weekly over the 140 day period. The mesophilic bacteria count was performed using a plate count agar (PCA) (ISO

4833, 2003). Yeast and mould counts were performed using a chloramphenicol glucose agar (CGA) (ISO 21527, 2008). All the plates were incubated at room temperature for 3-5 d. The enterobacteriae were isolated using a crystal violet red bile glucose agar (VRBG) with a temperature of 37 °C for 24 h (Oxoid, Basingstoke, The United Kingdom) (ISO 21528, 2004). The presence or absence of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp. and *Listeria* spp. were determined by using techniques outlined by ISO techniques.

**3.5. Sensory evaluation:** Once the results of the microbiological tests were obtained, and only when the results were within the limits allowed by the normative, the sensory analyses were performed. A triangle test was performed every seven days. Each panellist received three coded apples (two of them identical and the other one different) randomly distributed in order to compare the roasted apple that was packed and stored with a newly roasted apple. Forty-four panellists were asked to identify the apple which was different (ISO 4120, 2004). The number for each apple per sample was 132 (44 panellists × 3 apples) for each type of processing and storage time. The panellists were trained for two months in eight different sessions (ISO 5496, 2006). They were selected according to the criteria of being a frequent consumer of the investigated product (Moskowitz *et al.*, 2006). The end of sensory shelf life period for roasted apple was calculated as the storage time when significant differences between the newly processing apples and the stored apples (7 to 70 days) were found ( $p < 0.05$ ).

Quantitative descriptive analyses were carried out by a panel of twelve panellists from the Universidad de León trained to assess roasted apple attributes (Marcelo *et al.*, 2010). For each roasted apple, the perceived intensity of each attribute was indicated by placing a vertical line along the line scale (0 = low, 10 = high) (Cliffe-Byrnes and O'Beirne, 2007).

**3.6. Data analysis:** Combined analysis of variance was performed using SPSS version 17.0.1, (SPSS Chicago, IL, USA) to test the differences between the effects of the processing, the storage temperature and the storage time were considered to be fixed effects. Means comparison was based on the Tukey test. A PCA without rotation was performed on the mean sensory scores to determine the relationship between the attributes and between the samples.

## 4 RESULTS

**4.1. Microbiological tests:** The apples that were stored at 20 °C and that were processed using the H1, H2, and H3 types were contaminated (CFU g<sup>-1</sup>: Colony forming units per gram. Mean plate counts in duplicate samples) from aerobic mesophilic bacteria (H1= 1.8 × 10<sup>3</sup> CFU g<sup>-1</sup>; H2=2.0 × 10<sup>4</sup> CFU g<sup>-1</sup>; H3=1.1 × 10<sup>3</sup> CFU g<sup>-1</sup>), moulds and yeasts (H1= 2.1 × 10<sup>2</sup> CFU g<sup>-1</sup>; H2=1.8 × 10<sup>4</sup> CFU g<sup>-1</sup>; H3=3.2 × 10<sup>2</sup> CFU g<sup>-1</sup>) during the first week. Only the apples that were processed using the H4 procedure and that were stored at 20 °C were contaminated by aerobic mesophilic bacteria during the thirteen week (H4= 2.3×10<sup>3</sup> CFU g<sup>-1</sup>), and the existence of moulds and yeasts (H4= 3.6 × 10<sup>2</sup> CFU g<sup>-1</sup>) were detected during the analysis conducted on the 84<sup>th</sup> day. Contamination by enterobacteriae was detected during the second week of storage for the samples that were elaborated using the H1, H2, and H3 types and stored at 20 °C (H1= 2.0 × 10<sup>4</sup> CFU g<sup>-1</sup>; H2=3.4 × 10<sup>5</sup> CFU g<sup>-1</sup>; H3=2.7 × 10<sup>3</sup> CFU g<sup>-1</sup>). The apples that were elaborated using the procedure H4 and

which were stored at 20 °C were contaminated by enterobacteriae (H4= 1.4 × 10<sup>3</sup> CFU g<sup>-1</sup>), during the thirteen week of storage. The samples that were processed using the types H1, H2 and H3 were considered unacceptable for consumption according to the legislation (Boletín Oficial del Estado, 2001).

After 20 weeks, the longest storage time for the present study, no contaminations were detected (<10 CFU g<sup>-1</sup>) for aerobic mesophilic bacteria, or for moulds and yeasts, or for enterobacteriae in the samples that were stored in the refrigerator (3 °C). It is important to remark that the presence of *E. coli*, *S. aureus*, *Salmonella* spp. and *Listeria monocytogenes* were not detected at any moment during the present study.

### 4.2. Sensory evaluation. Triangle tests

No differences were found during storage time for sensory quality, according to the triangle test, until the 42<sup>th</sup> day of storage for the apples that were stored at 3 °C (Table 1).

**Table 1. Storage time effect on the sensory characteristics for the roasted apple cv. 'Reineta' according to the triangle test**

Time (days)	H1		H2		H3		H4	
	3 °C	20 °C	3 °C	20 °C	3 °C	20 °C	3 °C	20 °C
7	ns†	-	ns	-	ns	-	ns	ns
14	ns	-	ns	-	ns	-	ns	ns
21	ns	-	ns	-	ns	-	ns	*
28	ns	-	ns	-	ns	-	ns	*
35	ns	-	ns	-	ns	-	ns	**
42	ns	-	*	-	ns	-	ns	***
49	*	-	*	-	*	-	ns	***
56	*	-	**	-	**	-	ns	***
63	***	-	***	-	***	-	*	***
70	***	-	***	-	***	-	***	***

† ns = non significant, \* p<0.05, \*\* p<0.01, \*\*\* p<0.001

This means that a storage period of 35 days will be the limit for sensory shelf life of the product, independent of the processing type. From the 42<sup>th</sup> day on, differences were found depending on the type of processing performed. The H2 processing was the type of processing which was conducted that produced the worst results, because differences between the stored apples and the newly processing apples appeared earlier than for the other three types of processing, whereas samples processing

using the H4 procedure did not to show differences between the newly elaborated apples and stored apples until the 63<sup>th</sup> day of storage. For the apples that were stored at 20 °C it was only possible to conduct the triangle test on the apples that were elaborated using the H4 procedure.

### 4.3. Sensory evaluation. Descriptive tests:

External appearance and internal colour were the attributes that showed differences between storage times (Table 2). There was a gradual loss of external



appearance, so after 63 days of storage, the external appearance of the roasted apples was lower than the external appearance of the newly processing apples. A gradual loss of internal colour happened as well, so after 35 days of storage the internal colour of the roasted apples that were stored was lower than the

internal colour of the newly processing apples. The interaction between the types of processing × storage times was not significant for any of the visual attributes.

**Table 2.** Mean sensory scores of attributes for baked apple cv. ‘Reineta’ according to the type of processing and the storage time (n= 12; scale: 0= low, 10 =high).

Type of processing	External appearance	Internal appearance	Internal colour	Odour intensity	Sweet taste
H1	6.7 a†	7.0 a	5.9 a	7.1 b	6.9 b
H2	7.0 a	7.0 a	5.9 a	7.1 b	6.9 b
H3	7.0 a	7.0 a	5.8 a	7.6 a	7.4 a
H4	6.9 a	7.0 a	6.0 a	7.8 a	7.0 a, b
Storage (days) 7	7.3 a	7.1 a	7.2 a	8.0 a	7.7 a
14	7.3 a	7.0 a	7.0 a	7.9 a	7.6 a
21	7.2 a, b	7.0 a	6.9 a	7.9 a	7.5 a, b
28	7.1 a, b, c	7.0 a	6.7 a, b	7.8 a, b	7.4 a, b, c
35	7.0 a, b, c	6.9 a	6.4 b, c	7.7 a, b	7.2 a, b, c, d
42	6.9 a, b, c	7.0 a	6.0 c	7.4 a, b, c	7.0 b, c, d, e
49	6.8 a, b, c	7.0 a	5.4 d, e	7.2 b, c	6.8 c, d, e, f
56	6.7 a, b, c	6.9 a	4.9 e, f	7.0 c, d	6.7 d, e, f
63	6.5 b, c	6.9 a	4.5 f, g	6.6 d, e	6.6 e, f
70	6.5 c	6.9 a	4.0 g	6.3 e	6.4 f
Effect of processing	ns <sup>¶</sup>	ns	ns	***	***
Effect of storage time	***	ns	***	***	***
Effect proc. × storage	ns	ns	ns	ns	ns

† Means with the same letter in the same column are not different (Tukey, p<0.001)

¶ ns = non significant, \* p<0.05, \*\* p<0.01, \*\*\* p<0.001

In regards to olfactory-gustatory attributes, odour intensity and sweet taste were influenced by the type of processing conducted. The apples that were processed using the H1 and the H2 techniques showed lower values of odour intensity than the apples processing using the H3 and the H4 techniques. It seems that the use of the microwave oven influenced the value recorded for odour intensity and for sweet taste. No differences were found between the four different processing techniques for acid taste and alcoholic flavour. All the olfactory-gustatory attributes (odour intensity, sweet taste, acid taste and alcoholic flavour) changed during storage. Alcoholic flavour increased after the 49<sup>th</sup> day of storage and was higher until the end of the storage period, whereas the rest of attributes showed decreases during the storage period. Even though type of processing × storage time interactions were not significant, for any of the olfactory-gustatory attributes.

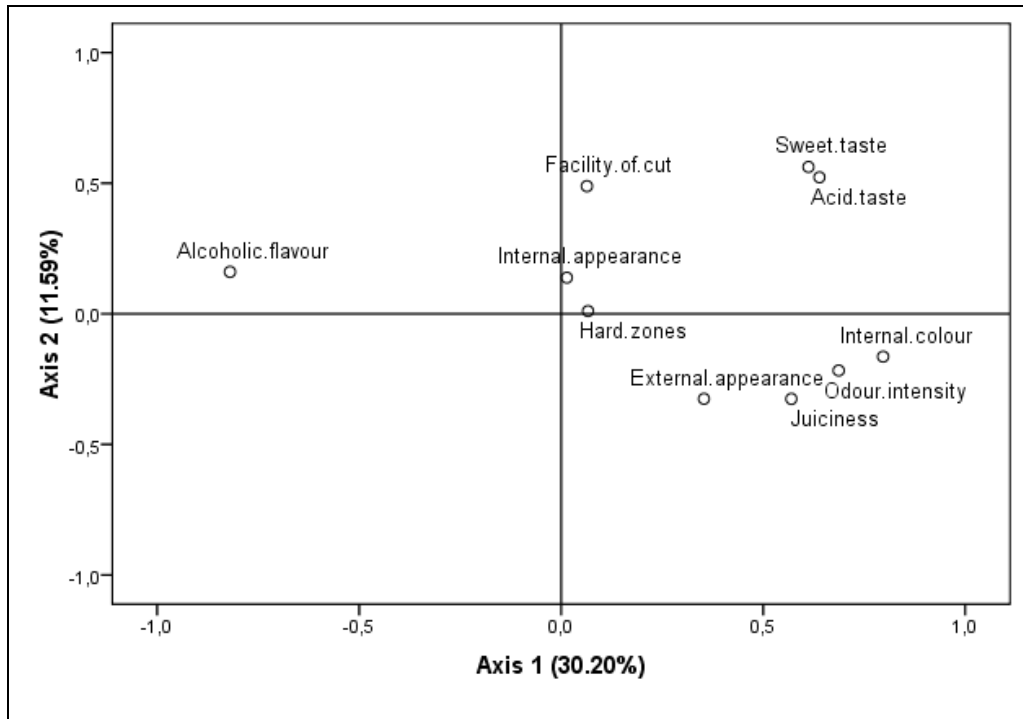
Juiciness was the only textural attribute that changed over the storage period (Table 2). Neither the facility of cut nor the presences of hard zones were different between the types of elaboration techniques or the length of storage time. The juiciness of the samples decreased as the storage time increased.

**4.4. Principal component analysis:** A PCA was conducted on the correlation matrix which was produced from the sensory data from the different type of processing during the storage period. The PCA plots gave a visual overview of how the sensory quality was influenced by the different types of processing and the different lengths of storage periods. The principal components of 1, 2, 3 and 4 all had eigenvalues that were greater than 1.0. The first three principal components (PCs) explained 62.52% of the total variance (PC1: 30.20%; PC2: 11.59%; PC3: 10.56%) (Figure 1), and an additional 10.17% was explained by PC4 (not shown). The



loading plot for the first two PCs indicated that some of the attributes described the same variation among the samples. The underlying dimension for factor I were the colour, olfactory gustatory attributes and juiciness, with attributes such as internal colour (0.80), odour intensity (0.69), acid taste (0.64), sweet taste (0.61) and juiciness (0.57)

loading positively; whereas alcoholic flavour (-0.82) was negatively loaded and place on the left side of the plot. The second PC explained 11.59% of the variance and was loaded positively with presence of sweet taste (0.56) and acid taste (0.52). The third PC was negatively loaded with facility of cut (-0.52).



**Figure 1.** PCA loadings of the quality attributes of roasted apple derived from the different types of elaborations at different storage times.

The PCA scores of the type of elaboration conducted on the roasted apples gives a visual representation of how the type of elaboration contributed to maintaining the initial characteristics of the roasted apple (Figure 2). Roasted apples that were elaborated using the H3 technique were lightly

positively loaded on axis 2, whereas roasted apples elaborated using the H1, H2 and H4 techniques were lightly negatively loaded on axis 2. There was a minimum distance between the four different types of elaborations. This means that the difference between the four types of elaborations is minimal.

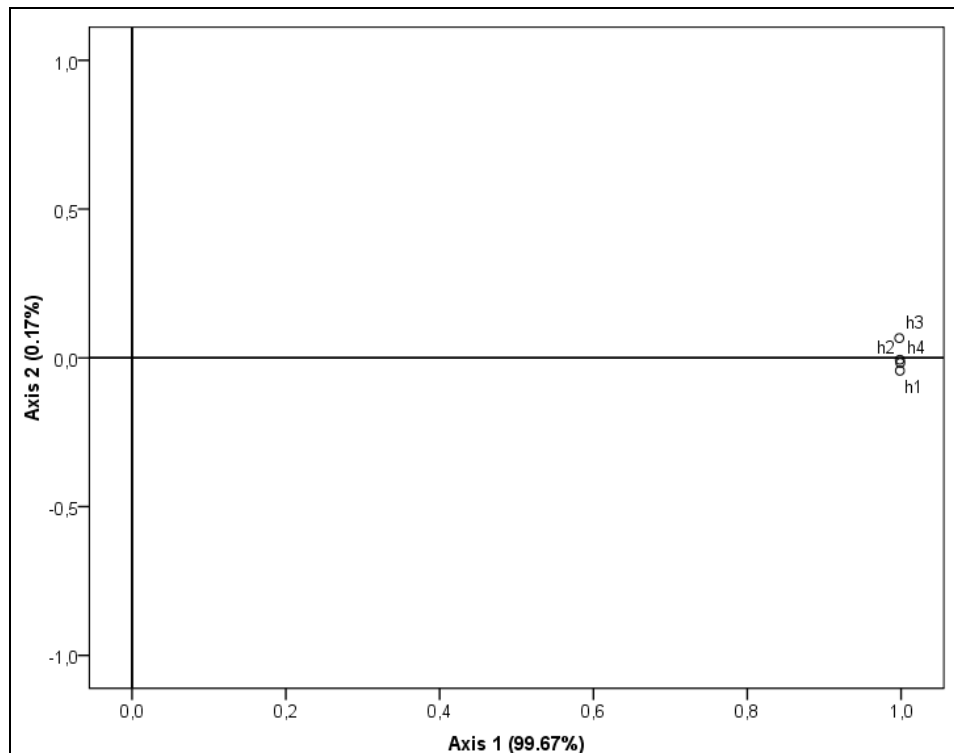


Figure 2: PCA scores of four different types of elaborations for the roasted apple sample.

## 5 DISCUSSION

**5.1. Microbiological tests:** Several authors reported that some of the major microbiological hazards that are associated with chilled storage products were the growth of pathogens at low temperatures, e.g. *Listeria monocytogenes*, *enterotoxigenic*, *Escherichia coli* and spore-formers such as *Bacillus cereus* which may survive when the heating process is inadequate (Nyati, 2000). According to strict Spanish regulations (Boletín Oficial del Estado, 2001), the processing type H4 obtained a microbiological life of 77 days for roasted apple cv. Reineta which was packed and stored at 20 °C. This is because the pasteurization process prevents a later contamination. Some authors reported that the concentration of aerobic bacteria in soybean sprouts decreased after pasteurization but increased during storage (Koo *et al.*, 2008). The microbiological life of the rest of the processing types (H1, H2, and H3) and stored at 20 °C is very short, and therefore it will be necessary to use refrigeration. González-Fandos *et al* (2005) found that the final mesophiles and anaerobes counts were lower ( $p < 0.05$ ) for samples stored at 2 °C than in those stored at 10 °C for all heat treatments in salmon slices processed using the sous vide method.

**5.2. Sensory evaluation:** Marcelo *et al* (2010) reported a shelf life of 42 days for roasted apples cv. Reineta that were packed and vacuum sealed before being pasteurized. For this work the storage period time limit for an acceptable product was decreased to 35 days. This decrease in the time for which the product is acceptable could be due to the fact that during the processing H1, H2 and H3 the apples were packed after being processed. This process can potentially cause contamination and therefore the apples can lose their original sensory qualities faster. For the apples that were stored at 20 °C it was only possible to conduct the triangle test on the apples that were processed using the H4 procedure. The H4 procedure is interesting since it demonstrates that this method of processing has a sensory shelf life of 15 days when stored at 20 °C. It is obvious that lower storage temperatures contribute to longer retention times for the original sensory qualities of the apples. Nyati (2000) did an evaluation of the effect of storage and processing temperatures on microbiological status and found that lower storage temperatures extended the shelf-life of sous vide products. She found that during the fifth week of



storage at 3 °C, 18 out of 19 products were still acceptable organoleptically and microbiologically. Chiralt (2002) reported that the loss of colour during storage is due to the alteration of the product's pigments or to the reaction of colourless compounds induced by the treatment. In agreement with this study results, Wang and Kays (2001) point out that the cooking method can influence how the heat penetrates, how the temperature is attained, and the environment surrounding the sample, each of which can influence the synthesis of new compounds as well as the rate of release or the degradation of existing components in sweet potato. The baked samples processed all of the odour-active compounds identified and in higher concentrations than either the boiled or microwaved samples. Yaylayan *et al* (1994) reported that the major differences between microwaved and baked foods were the lower concentrations of Maillard reaction aroma and the lack of nonenzymatic browning reaction aroma products. The apples roasted using the H3 processing technique had a sweeter taste than the apples roasted using the other processing techniques. This can be explained by the microwave heating process, water vapour migration to the surface of the food and rapid heating throughout the sample which all effect flavour (Schiffmann, 1994). Due to low surface temperature, short cooking time, and high surface water activity, Maillard reactions proceed at a much slower rate. In addition, caramelization

reactions are essentially not possible under microwave conditions (Van Eijk, 1994).

Marcelo *et al* (2010) obtained similarly results except for alcoholic flavour which increased after 84<sup>th</sup> day of storage when stored under MAP conditions. This can be explained by the fact that MAP conditions prevent the appearance of off-flavours. Yesudhasan *et al* (2009) reported that based on sensory score and microbiological data, the shelf life of seer fish steaks increased 9 d when samples were packed in MAP. They also reported that there was a loss of sweetness and acid taste during the storage period. Perkins-Veazie (1995) found that the sugar to acid ratio is possibly more important for the perception of sweetness for the sensory panel than the amount soluble solids alone. Guerra *et al* (2009) also observed that the total soluble solids to titratable acidity ratio were correlated positively with sweetness during storage. The juiciness decreased as the storage time increased. It is well-known that cooking fruit causes an initial loss of firmness due to membrane disruption and this is associated with the loss of turgor (Thiel and Donald, 2000).

As final conclusions, this study shows that storing roasted baked apple cv. Reineta at 3 °C is useful to extend the sensory shelf life up to 35 days. The type of processing that the apples were heated using an electric oven with grill for 7 min, then the fruit were cooled and were packed and the end the apples were then pasteurized was the only type that kept the original properties of roasted apple cv. Reineta when stored at 20 °C for a 14 day period.

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