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Effect of varying oxygen concentrations on the shelf-life of fresh pork sausages packaged in modified atmosphere

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Abstract

A total of 105 fresh pork sausages were packaged in atmospheres varying in oxygen concentration, using the following mixtures (%O_2/%CO_2/%N_2): 0/20/80, 0/20/80 + O_2 scavenger, 20/20/60, 40/20/40, 60/20/20, and 80/20/0. In addition, two batches were subjected to vacuum packaging or over-wrap with O_2-permeable film. They were stored for 20 days at 2 ± 1 °C in the dark. Values of pH, CIE L^*, a^* and b^* color parameters, surface metmyoglobin percentage, TBA-reactive substances, psychrotroph aerobe bacterial numbers and sensory discoloration and off-odor were assessed throughout storage. Packaging in the absence of O_2, either under vacuum or in atmosphere with O_2 scavenger, led to extension of shelf-life in terms of both color and odor stability due to low oxidation rates. Increase of O_2 caused a significant enhancement of oxidation, decrease of shelf-life due to discoloration and off-odor development. The highest O_2 concentration gave rise to a significant color improvement, though only for a limited period of 8 days.

Keywords: Sausages (fresh); Packaging; Modified atmosphere; Oxygen; Oxidation; Color; Pork

1. Introduction

Fresh pork sausages are commonly presented for retail sale in over-wrapped trays. They often contain colorants and additives (mainly sulfite) to preserve fresh color and odor, since sodium sulfite can retard both enzymatic and nonenzymatic reactions (Cornforth, 1994). It also has an antimicrobial effect (Farouk et al., 1997). Reports of serious sensitivity reactions to sulfites in recent years have raised concerns regarding the over-use or, in some cases, the unnecessary use of sulfites in commonly consumed foods.

Color of meat is an important quality attribute that influences consumer acceptance of meat and meat products (Glitsch, 2000). Consumers prefer bright-red fresh meats. Meat color depends on the relative amounts of three pigments: bright-red oxymyoglobin, purple deoxymyoglobin, and brown metmyoglobin. Fresh meat color is short-lived, so that meat discoloration is inevitable during storage in the presence of oxygen (Zhu & Brewer, 1998). Bradford, Huffman, Egbert, and Mikel (1993) reported that color acceptability of fresh pork sausages decreased with increasing refrigerated storage time, but no studies have so far been devoted to the effect of varying oxygen concentrations on the color and shelf-life of fresh sausages packaged in modified atmosphere.

Lipid oxidation also occurs throughout storage, though it is not usually the major determinant of shelf-life in over-wrapped packaging systems as air-permeable films allow odor volatiles to escape from the package. The volatile off-odor-causing products of lipid oxidation are retained within the package in modified atmosphere packaging (MAP), thus allowing consumers to perceive off-odors when packages containing oxidised meat are opened. A strong relationship between lipid

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and myoglobin oxidation has been well documented in fresh meat during aerobic storage (Faustman & Cassens, 1989).

The most common gas mixture for retail-ready fresh meats contains approximately 70% O_2 and 30% CO_2, which gives the product an extended shelf-life compared to air (Gill, 1996). High O_2 concentrations enhance bright-red color, but they cause myoglobin oxidation. Oxidation is increased in fresh sausages by mincing and further manufacturing of meat. However, it has also been demonstrated that low concentrations of O_2 in the package are responsible for color fading of fresh meat (Jakobsen & Bertelsen, 2000). One way of reducing the O_2 concentrations below the critical level for discoloration is through the use of O_2 scavengers (Buys, 2004).

The objective of this work was to determine the effect of varying gas mixtures (from absence to very high levels of O_2, in combination with 20% CO_2) on the storage life of fresh pork sausages. This was compared to vacuum and over-wrap packaging, in an attempt to select the best conditions for preservation of color and extension of shelf-life of sausages with no sulfite or colorant added.

2. Materials and methods

2.1. Preparation of samples and atmospheres

Four pork forelegs (initial pH 5.5–5.7) were obtained at 48 h post-slaughter from a local supplier (MARBE, Zaragoza, Spain) external fat was trimmed off, and the sample was ground using an industrial grinder machine. The ground meat was stuffed into collagen casings, Colfan F (Viscofan S.A., Caseda, Spain), of 17 mm diameter. A total of 105 fresh pork sausages were formed. The fresh sausages were placed on polystyrene foam trays ($18 \times 13.5 \times 2.5$ cm), and either over-wrapped in polyethylene film or placed in a pouch made of polyethylene and polyamide of water vapour permeability 5–7 g/m^2/24 h at 23 °C and oxygen permeability 40–50 ml/m^2/24 h at 23 °C (Sidlaw Packaging-Soplaril, Barcelona, Spain) and subjected to vacuum or filling with one of the following gas mixtures, supplied by Abelló Linde S.A., Barcelona, Spain (%O_2/%CO_2/%N_2): 0/20/80; 0/20/80 + O_2 absorber (Ageless® FX-40, Mitsubishi Gas Chemical Co. Inc., Tokyo, Japan); 20/20/60; 40/20/40; 60/20/20; 80/20/0. The packs were stored for 20 days at 2 ± 1 °C in the dark. Three packs were opened for subsequent analysis for each atmosphere every 4 days of storage. The entire experiment was duplicated.

2.2. Determination of gas mixtures

Gas composition of the different mixtures was assessed by gas chromatography, using a Hewlett–Packard 4890 instrument with a TCD detector; the column used was CP-Carboplot (Chrompack), 25 m length, 25 μm thickness film, 0.53 mm internal diameter and 0.75 mm external diameter. Three samples were taken off the headspace trays and a 50 μl gas volume was injected with a Hamilton gas-tight 1710 N syringe.

2.3. Determination of meat pH

The pH of meat samples was measured using a micro pH-meter model 2001 (Crison Instruments, Barcelona, Spain) after homogenising 3 g of sample in 27 ml distilled water for 10 s at 1300 rpm with an Ultra-Turrax T25 macevator (Janke & Kunkel, Staufen, Germany). Each value was the mean of three replicates.

2.4. Color instrumental measurement and metmyoglobin percentage

Meat color was measured at the surface of fresh sausages, using a reflectance spectrophotometer Minolta CM-2002 (Osaka, Japan), 30 min after package opening. CIE L^*, a^*, b^* (CIE, 1978) parameters were recorded. Chroma (C^*) and hue-angle (h^*) were calculated by the following formulae: $C^* = (a^{*2} + b^{*2})^{1/2}$, $h^* = \tan^{-1}(b^*/a^*)$. Each value was the mean of 30 determinations.

The surface metmyoglobin percentage was estimated spectrophotometrically, using the procedure of Stewart, Zipser, and Watts (1965) by measuring reflectance at 525 and 572 nm. The average value of the ratios of $(K/S)_{572}$ to $(K/S)_{525}$ at the beginning of the experiment was fixed as 0% metmyoglobin (MetMb). The value of 100% MetMb was obtained after oxidising a sample in a 1% (w/v) solution of potassium ferricyanide. The average value for each fresh sausage was the mean of 30 determinations.

2.5. Lipid oxidation analysis

Lipid oxidation was measured in triplicate by the 2-thiobarbituric acid (TBA) method of Pfalzgraf, Frigg, and Steinhart (1995). Meat samples of 10 g were taken and mixed with 10% trichloroacetic acid, using an Ultra-Turrax T25 macevator (Janke & Kunkel, Staufen, Germany). Samples were centrifuged at 2300g for 30 min at 5 °C; supernatants were filtered through quantitative paper (MN 640 W, Machinery-Nagel GmbH & Co. KG, Düren, Germany). Two ml of the filtrate were taken and mixed with 2 ml of thiobarbituric acid (20 mM); tube contents were homogenised and incubated at 97 °C for 20 min in boiling water. Absorbance was measured at 532 nm. The concentration of the samples was calculated using a calibration curve. TBARS values were expressed as mg malonaldehyde/kg sample.

2.6. Microbial sampling and analysis

Twentyfive gram of meat were taken from the sausages and diluted in 225 ml 0.1% peptone water. Each sample was homogenised in a Stomacher Lab Blender (model BA6021; Seward Laboratory, London) for 1 min. Serial tenfold dilutions were prepared by diluting 1 ml in 9 ml of 0.1% peptone water. Two plates were prepared from each dilution by pouring 1 ml in to fluid agar. Counts of aerobic psychrotrophic flora were determined from plates bearing 30–300 colonies in plate count agar (PCA; Merck; Darmstadt, Germany) incubated at 7 °C for 10 days (Elliot, Clark, & Lewis, 1983). Counts were expressed as the $\log_{10}$ of colony–forming units (CFU)/g.

2.7. Sensory analysis

Samples of fresh sausages were evaluated by a six-member trained panel. Though already skilled in this kind of evaluation, panellists received further training prior to analysis according to the method described by Cross, Moen, and Stanfield (1978). Three open-discussion sessions were held to familiarise the individual with the attributes and the scale to use. The attributes studied were evaluated using a 5-point scale according to Djenane, Sánchez-Escalante, Beltrán, and Roncalés (2001): odor scores referred to the intensities of odors associated with meat spoilage: 1 = none; 2 = slight; 3 = small; 4 = moderate; and 5 = extreme. Discoloration scores referred to percentage of discolored surface: 1 = none, 2 = 0–10%, 3 = 11–20%, 4 = 21–60%, and 5 = 61–100%. Results were expressed as the predominant score given by panellists.

2.8. Statistical analysis

The entire experiment was duplicated. The significance of differences among samples at each day of storage was determined by analysis of variance (ANOVA) using the least square difference method of the general linear model procedure of SPSS (SPSS, 1999). Differences were considered significant at the $p < 0.05$ level. All data were subjected to analysis of variance (ANOVA). The statistical significance of differences between mean values was analysed by repeated measures and the Tukey test in the general linear model of the SPSS statistical package.

3. Results and discussion

3.1. pH

Muscle pH values ranged from 5.5 to 6.1 (data not shown) and did not differ significantly ($p > 0.05$) among samples, except for over-wrapped sausages, which were significantly different ($p < 0.05$) from all others; the pH of samples subject to this treatment rose to above 6.0 at day 12 and reached 7.0 at day 16. A slight increase of pH has already been reported by other authors (Moore & Gill, 1987).

3.2. Color

As shown in Fig. 1, there was a significant effect of oxygen concentration on the redness a^* value of fresh sausage samples. Sausages stored in the highest oxygen concentration showed a high a^* value of about 10 at the 8th day of storage ($p < 0.05$), in agreement with the results reported on beef by Jakobsen and Bertelsen (2000). However, values fell below 4 on the 12th day. Sausages with O_2 concentrations within the range 20–60% maintained a^* values around 7 until day 8 of storage, but also fell to values below 4 ($p < 0.05$) at the 12th day. This fall was greater with a larger oxygen concentration. Similar results were obtained by Guerrero, Usborne, and Ashton (1988), who reported that high O_2 concentration was detrimental to color preservation in fresh sausages.

Samples with 0% O_2 and no oxygen scavenger had values of around 6 at the 4th day ($p < 0.05$), and reached values around 5 throughout storage. Similar results were obtained by Jeremiah, Gill, and Penny (1992). At low temperatures, pork color has been shown to be stable at several hundred ppm of O_2 (Sørheim, Grini, Nissen, Andersen, & Lea, 1995). The actual concentration of residual O_2 was 0.3–0.7. Rousset and Renerre (1991) reported that a 0.2% residual O_2 concentration in CO_2 atmospheres led to oxidation of myoglobin.

Samples stored under vacuum kept a steady acceptable a^* value above 7 throughout the entire storage period. Similar results were found by Renerre, Gatellier, Minard, Roussel, and Lapinte (1996). No measurement of O_2 concentration was made on samples packaged under vacuum because of the difficulty of taking gas sam-

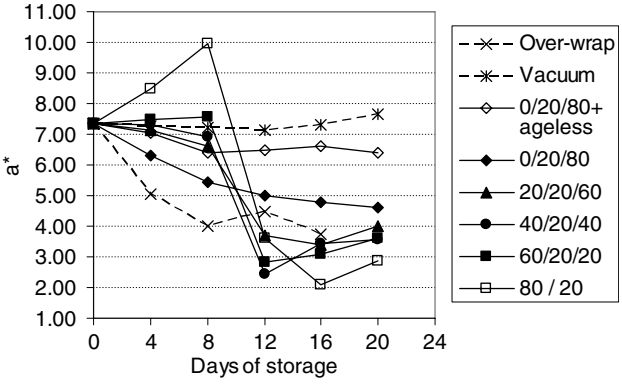

Fig. 1. Values of CIE a^* in fresh pork sausages packaged in different atmospheres and stored at 2 ± 1 °C.

ples devoid of weep. Samples packaged with 0% O_2 in the presence of scavenger showed an evolution similar to those under vacuum, owing to the low oxygen (values near 0–0.1% O_2 during all experiments), despite the presence of carbon dioxide. Similar results were found by Flodberg (1997) in sausages and Buys (2004) in pork chops. Over-wrapped sausages showed the lowest a^* values during the early early days of storage ($p < 0.05$).

According to these data, sausages packaged in a very rich O_2 atmosphere showed the most intense red color, but this lasted only a few days. On the other hand, samples packaged in the absence of O_2 (either under vacuum or with an O_2 scavenger) showed a moderate red color, which was stable for a long period of storage.

3.3. Metmyoglobin percentage

Fig. 2 shows the values of surface metmyoglobin percentage. The amount of metmyoglobin was lower ($p < 0.05$) in samples subjected to over-wrap, vacuum, and 0% O_2 in the presence of scavenger, which maintained a high level of acceptability (below 20%) after 20 days of storage. Djenane, Sánchez-Escalante, Beltrán, and Roncalés (2002) reported that about 30–40% metmyoglobin was needed to cause relevant meat discoloration. The absence of O_2 very effectively protected myoglobin from oxidation. Renerre et al. (1996) also found that metmyoglobin was very low in samples stored with O_2 scavengers.

At day 8 of storage, samples packaged with O_2 had metmyoglobin percentages that depended upon the concentration of O_2; they were higher at lower oxygen concentration. At day 12, metmyoglobin rose dramatically in all samples packaged with O_2, reaching values well above 80%. Samples packaged in 0% O_2 + 20% CO_2 + 80% N_2 in the absence of O_2 scavenger had a metmyoglobin percentage below 20 during the first 8 days, but they thereafter suffered a strong increase, reaching about 50% metmyoglobin at day 20 of storage.

It is noteworthy that over-wrapped sausages retained red color, even when already spoiled (see Figs. 3 and 4).

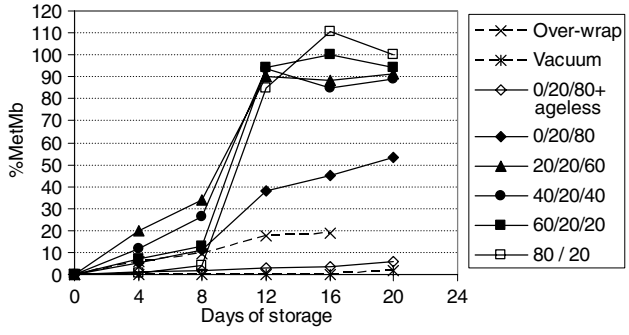

Fig. 2. Metmyoglobin percentage (MetMb %) in fresh pork sausages packaged in different atmospheres and stored at 2 ± 1 °C.

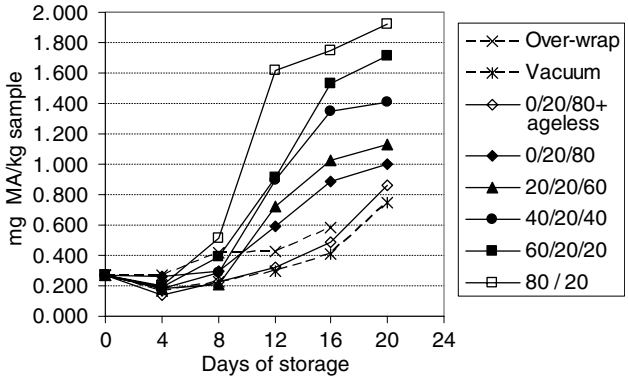

Fig. 3. TBARS (mg malonaldehyde/kg) in fresh pork sausages packaged in different atmospheres and stored at 2 ± 1 °C.

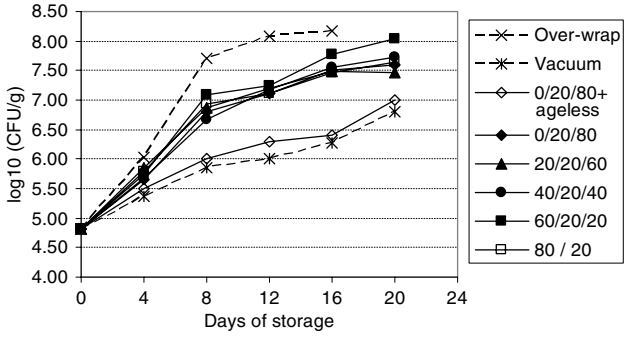

Fig. 4. Psychrotroph aerobe bacterial counts in fresh pork sausages packaged in different atmospheres and stored at 2 ± 1 °C.

This phenomenon was also observed by Rousset and Renerre (1991). According to Faustman and Cassens (1989), a reduction of metmyoglobin may be favoured by high populations (10^8 CFU/g) of psychrotrophic bacteria.

3.4. Lipid oxidation

Evolution of TBARS is shown in Fig. 3. Lipid oxidation rose rapidly with increasing time in all samples. However, large and significant differences ($p < 0.05$) were evident among samples, depending on the concentration of O_2, as already discussed concerning a^* values. Thus, the higher the oxygen concentration, the higher were TBARS values and their rate of increase, which was in agreement with results of Formanek et al. (2001) on beef patties. Fresh sausages packaged with 80% O_2 showed the highest values of TBARS ($p < 0.05$); they were well above 1 at day 12 of storage. On the other hand, samples under vacuum and with O_2 scavenger had the lowest values ($p < 0.05$); indeed, they did not reach 1 mg malonaldehyde/kg, even after 20 days of storage. Sausages packaged in the absence of O_2 and no oxygen scavenger had significantly higher TBARS values ($p < 0.05$) than these latter, due probably to residual O_2 within the package (Smiddy et al., 2002).

TBARS values of over-wrapped sausages were also high during the first 8 days of storage, but their rate of increase decreased strongly thereafter. This behaviour might be due to the reaction of malonaldehyde, a secondary product of lipid peroxidation, with protein degradation products. Similar results were found by Giménez, Roncalés, and Beltrán (2002).

Our results strongly agreed with those of Formanek et al. (2001) for beef, while Wang, Jiang, and Lin (1995) found lower oxidation in sausages packaged in a modified atmosphere of CO_2 and N_2 than in those vacuum-packaged. Lanari, Schaefer, and Scheller (1995) reported, too, that lipid oxidation increased in high-oxygen MAP pork chops compared with those air-stored. Fresh sausages were especially susceptible to oxidation when compared to meat steaks, because reduction in particle size, by grinding, disrupted membranes and led to incorporation of air and oxygen into the tissues. Both of these facts are known to increase tissue susceptibility to oxidation and to hasten development of oxidative rancidity.

3.5. Aerobic psychrotroph counts

Evolution of psychrotrophic aerobe counts is depicted in Fig. 4. High initial counts of about 4.8 in log values gave rise to strong microbial growth on all samples, showing no lag phase. Counts on over-wrapped sausages were significantly higher ($p < 0.05$) than on any other sample throughout the entire storage period. They reached values above 7.5 within 8 days, which were responsible for sausage spoilage. Similar results were obtained by Spahl, Reineccius, and Tatini (1981).

Samples stored without oxygen, either under vacuum or in the presence of an O_2 scavenger, showed the lowest values ($p < 0.05$), which did not reach 10^7 CFU/g, even after 20 days of storage. Sheridan et al. (1997) already noted that meat packaged under vacuum had slower aerobic bacterial growth than meat packaged in modified atmospheres with oxygen. It is known that packaging in anoxic environments retards microbial growth and delays spoilage due to slow proliferation of bacteria capable of tolerating anaerobic conditions.

Sausages packaged with varying concentrations of O_2 showed a similar evolution throughout the entire experiment, with no significant differences among samples at any time of storage ($p > 0.05$); but they were significantly different ($p < 0.05$) from over-wrapped and oxygen-free samples. The difference with sausages packaged in air (over-wrap) clearly evidenced the inhibitory effect of the presence of 20% CO_2 in modified atmospheres. According to Skandamis and Nychas (2002), storage of fresh meat at increasing CO_2 concentrations caused increasing inhibition of psychrotrophic aerobes and an extension of the shelf-life. On the other hand, the difference with sausages packaged without O_2 strongly supported the view that the presence of CO_2 is not sufficient to effectively prevent aerobic bacterial growth.

3.6. Sensory analysis

Sensory scores for discoloration and off-odor are summarised in Table 1. Discoloration of fresh sausages markedly increased throughout storage following all packaging treatments, except those including no oxygen (vacuum and 0% O_2 plus scavenger). Over-wrapped sausages were given a score of 3, corresponding to 11–20% discoloration, until the 12th day of storage. Oxygen-free samples were given the lowest scores, either 1 or 2 (below 10% discoloration), throughout the entire experiment. At day 16 of storage, only over-wrap, vacuum and 0% O_2 plus scavenger samples would be considered acceptable according to their color, while the rest of samples would be considered unacceptable. These results appeared to be in close agreement with those of metmyoglobin formation and redness a^* index (see Table 2).

Despite over-wrapped samples being considered acceptable with regard to their discoloration pattern, they ought to be considered unacceptable for consump-

Table 1
Effect of packaging with different atmospheres on sensory panel scores (predominant score) for discoloration of fresh pork sausages stored at 2 ± 1 °C

Atmospheres	Days of storage					
	0	4	8	12	16	20
Discoloration						
Over-wrap	1	1	2	3	3	nd
Vacuum	1	1	1	1	1	1
0/20/80 + scavenger	1	1	1	1	2	2
0/20/80	1	2	3	4	4	4
20/20/60	1	2	3	4	5	5
40/20/40	1	2	2	3	5	5
60/20/20	1	2	3	4	5	5
80/20/0	1	1	1	5	5	5

Table 2
Effect of packaging with different atmospheres on sensory panel scores (predominant score) for off-odor of fresh pork sausages stored at 2 ± 1 °C

Atmospheres	Days of storage					
	0	4	8	12	16	20
Off odor						
Over-wrap	1	2	4	5	5	nd
Vacuum	1	1	1	1	1	1
0/20/80 + scavenger	1	1	1	1	1	1
0/20/80	1	1	1	1	1	1
20/20/60	1	1	1	2	3	4
40/20/40	1	1	2	3	4	5
60/20/20	1	1	2	4	5	5
80/20/0	1	1	3	4	5	5

tion since microbial counts exceeded 10^7 CFU/cm^2 already at day 8 of storage. A reduction in metmyoglobin could be favoured by this high population of psychrotrophic aerobes, according to Faustman and Cassens (1989).

Regarding off-odor, scores increased throughout storage for all samples with oxygen concentrations above 20%, whereas they remained at their lowest value for vacuum and 0% O_2 samples, either with or without scavenger. Over-wrapped sausages reached a score of 4, corresponding to moderate off-odor, as early as 8 days after packaging. This odor score is due to microbial spoilage of these samples. Samples packaged in atmospheres varying in O_2 concentration showed different behaviour, depending on its percentage; the higher the O_2 percentage, the higher the rate of off-odor increase.

Sensory results on odor maintained a very close relationship with those of lipid oxidation; higher TBARS values corresponded to more intense off-odor. Only over-wrapped samples showed different behaviour, due to formation of odors related to microbial spoilage.

4. Conclusions

Packaging of fresh pork sausages in the absence of oxygen, either under vacuum or in an oxygen-free modified atmosphere with an oxygen scavenger, led to the extension of shelf-life in terms of both color and odor stability as a consequence of low oxidation rates. Increasing O_2 concentrations in packaging atmospheres caused a corresponding enhancement of oxidation and, therefore, a decrease of shelf-life, due to discoloration and off-odor development. Despite this, the atmosphere containing the highest oxygen concentration (80% O_2 + 20% CO_2) gave rise to a significant color improvement, though only for a limited period of 8 days of storage.

In conclusion, whereas oxygen-free packaging resulted in extended shelf-life, high-oxygen packaging improved the color appearance of sausages for a limited time. Further investigations appear then to be necessary for assuring oxidative stability of consumer-appealing fresh pork sausages packaged in a high-oxygen atmosphere.

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