Bacteriology and Meat Quality of Moisture Enhanced Pork from Retail Markets in Canada

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Abstract

Packages of moisture-enhanced and conventional pork chops were collected from six Canadian retail stores on five sampling days. The composition of injection brines differed between retailers, but all contained polyphosphates and salt as main ingredients. Meat quality characteristics and bacteriology were analyzed from collected meat samples. Moisture enhanced chops had a higher pH and a higher water holding capacity than conventional. Juiciness and overall tenderness were improved in moisture enhanced chops. The surfaces of moisture enhanced chops were discoloured; the chops were darker and displayed less colour saturation. Total numbers of aerobes, psychrotrophs and lactic acid bacteria were not affected by moisture enhancement but numbers of *Enterobacteriaceae*, pseudomonads and *Brochothrix thermosphacta*, bacteria frequently associated with microbial spoilage, were approximately 1 log CFU g⁻¹ higher in moisture enhanced samples. This work shows moisture enhancement with injection brines containing salt and phosphates can result in a more palatable product.

Keywords: pork, moisture enhancement, retail survey, meat quality

1. Introduction

Moisture enhancement involves the injection of brine solutions containing polyphosphates and salt into meat; these procedures have been widely used in the meat industry to improve water holding capacity (Bendall, 1954; Detienne & Wicker, 1999) and eating quality of meat products (Cannon, McKeith, Martin, Novakofski, & Carr, 1993; Jones, Carr, & McKeith, 1987; Prestat et al., 2002b; Vote et al., 2000). A major portion of pork produced in the United States and some in Canada undergoes moisture enhancement procedures (Mandell & McEwen, 2011). Moisture enhancement of fresh pork is thought to improve eating quality by increasing juiciness and tenderness (Brewer, Gusse, & McKeith, 1999; Sheard, Nute, Richardson, Perry & Taylor, 1999; Smith, Simmons, McKeith, Bechtel, & Brady, 1984; Wynveen et al., 2001). Additionally, injection of brine may improve tolerance to some cooking abuse (Baublits, Meullenet, Sawyer, Mehaffey & Saha, 2006).

Although the needle injection applied during the moisture enhancement process appears to improve meat palatability, this process disrupts the integrity of the meat surface. Such disruptions carry a risk of introducing bacteria from the meat surfaces into the interior of the muscle. Similar contamination has been shown for the blade tenderization of beef inoculated with *E. coli* O157:H7 (Phebus, Mardsen, Thippareddi, Sporing, & Ortega, 2002), for needle-injected pork inoculated with *Salmonella* spp. (Kastner et al., 2001), and for needle-injected chicken breast inoculated with *Clostridium perfringens* (Mead & Adams, 1979). However, there is only limited information on the bacteriology of moisture-enhanced meat cuts at retail, and how bacterial counts compare to conventional meat (Banks, Wang, & Brewer, 1998; Bohaychuk & Greer, 2003; Wen, Li, & Dickson, 2014) and further research regarding the food safety and bacterial spoilage of moisture-enhanced products is necessary.

The aim of the present study, therefore, was to determine meat quality of conventional and moisture-enhanced pork products of different suppliers available at Canadian retail outlets and to enumerate bacteria in raw meat.

2. Method

2.1 Sampling of Pork Products

Fifty packages of moisture-enhanced and 38 packages conventional pork chops were purchased from six Canadian stores (3 retailers) on five sampling days. Stores 1-4 were from Western Canada and stores 5-6 were from Central Canada. The pork chops were boneless thick, grill-style chops and all products were in-store packaged. One retailer, however, did not offer boneless conventional chops; therefore only moisture enhanced chops from that store were included in analyses. Moisture-enhanced chops differed in ingredients and volume of injection brine, according to the specifications of the manufacturers, but all contained polyphosphates and salt as main ingredients. Purchased meat was transported to the Agriculture and Agri-Food Research and Development Centre, Lacombe, AB, Canada and stored overnight at 2° C. Samples were subsequently analysed for physico-chemical, sensory, and bacteriological meat quality characteristics.

2.2 Physico-chemical Meat Quality

Meat colour was measured using a Minolta Chroma Meter CR 300 (Minolta Canada Inc., Mississauga, ON, Canada), recording CIE L* (brightness), a* (red-green axis), and b* (yellow-blue axis) values (CIE, 1976). Results were transformed to hue angle ($H_{ab} = \arctan [b^*/a^*]$) and chroma ($C_{ab} = [a^{*2} + b^{*2}]^{0.5}$). Chops were subjectively evaluated for muscle colour (5-point scale: 1 = extremely pale, 5 = extremely dark), surface discolouration (7-point scale: 1 = no surface discolouration, 7 = complete surface discolouration), and retail appearance (7-point scale: 1 = extremely undesirable, 7 = extremely desirable) by three experienced raters; these evaluation scales have been previously used by Nattress and Baker (2003). Temperature adjusted pH was measured using an Accumet ATC temperature probe (Fisher Scientific, Edmonton, AB, Canada) and an Accumet 1002 pH meter equipped with an Orion Ingold electrode (Urdorf, Switzerland). Meat was homogenized using a Robot Coupe Blixer BX3 (Robot Coupe USA Inc., Jackson, MS, USA) and homogenates were analysed for expressible juice (a measure of water holding capacity) by centrifugation at 16,000 RPM for 15 min at 2 °C. Moisture content of homogenized meat was determined as the weight loss after heating samples at 102 °C for 24 h (Juárez et al., 2009). Crude protein content was first measured from dried samples using a Leco Nitrogen/Protein Determinator CN2000 (Leco Corp. St. Joseph, MI, USA), protein content from fresh meat was then calculated (Method 992.15) (Association of Official Analytical Chemists [AOAC], 1995b). Crude fat was extracted from dried samples at 105 ℃ using a Soxtec Extraction Unit/Service Unit HT-1043 (FOSS Tecator, Högan äs, Sweden) and petroleum ether as the solvent (Method 960.39) (AOAC, 1995a).

Two chops out of each package were grilled to an internal temperature of 71 $^{\circ}$ C on an electric grill (surface temperature 210 $^{\circ}$ C) for determination of maximum shear force and sensory evaluation following procedures described by Ju árez et al. (2009). Briefly, cylindrical cores (1.9 cm in diameter) were removed from one cooked chop parallel to the longitudinal axis of the muscle fibre and cores were sheared perpendicular to the muscle fibre with a Warner-Bratzler Shear head attached to an Instron Material Testing System (Model 4301, Instron; Burlington, ON, Canada).

The second chop was trimmed of all fat and cut into 1.9 cm cubes. Trained panellists rated cooked samples for initial and overall tenderness, initial and sustained juiciness, pork and other flavour intensities using a nine-point descriptive scale (9 = extremely tender, extremely juicy, extremely intense for pork flavour, extremely intense for other flavours; 1 = extremely tough, extremely dry, extremely bland for pork flavour, extremely bland for other flavour). Flavour desirability and overall palatability were rated using nine-point hedonic scale (9 = extremely desirable; 1 = extremely undesirable); these factors have been previously used to characterize pork sensory characteristics by Prieto et al. (2015).

2.3 Bacteriological Analysis

Samples (10 g) of surface and subsurface muscle from chops were homogenized using a Stomacher Lab-Blender Model "400" (Seward Laboratory, London, England) following Nattress and Jeremiah (2000). Dilutions used for plating, selective media, and incubation procedures described by Nattress and Jeremiah (2000) were used to enumerate: pseudomonads, *Brochothrix thermosphacta*, lactic acid bacteria (LAB), and *Enterobacteriaceae*. Total aerobic bacteria and psychotrophs were enumerated on Plate Count Agar and plates were incubated for 2 d at 25 °C and 10 at 4 °C respectively (Difco Laboratories, Becton Dickinson Microbiology Systems, Sparks, MD, USA). The lower limit for enumeration of *Enterobacteriaceae* was log 1 cfu g⁻¹ and log 2 cfu g⁻¹ for other bacteria.

2.4 Statistical Analysis

Statistical analyses were carried out with SAS (SAS, 2009) applying a mixed model procedure with meat type

(conventional or moisture enhanced) as a fixed factor; sampling day and store were included as a random factors. LS-Means were tested for significant differences by Scheffe's multiple comparison. Where appropriate, Pearson's correlation values were calculated amongst factors attempting to establish relationships of quality measures. Additionally, summary statistics (minimum, maximum, and mean) were calculated for bacteria counts from each store.

3. Results

3.1 Physico-chemical Meat Quality

Overall, the majority of the physico-chemical meat quality traits were significantly different (P < 0.05) between moisture-enhanced and conventional samples of retail pork (Table 1). Moisture-enhanced pork had a higher pH (P<0.001), a lower expressible juice (P = 0.002), and lower shear force (P = 0.001) (0.5 kg lower) than conventional samples.

Objective measures of colour showed enhanced chops were darker (L*: P < 0.001), meat colour was less saturated (Chroma: P < 0.001), and less red (Hue: P = 0.049) than in conventional chops. Moisture-enhanced pork was also rated higher in subjective scoring for surface discoloration during retail display vs. conventional pork (P=0.005). Additionally, subjective retail appearance of moisture enhanced pork was less desirable (P=0.016).

Moisture-enhanced pork contained more water (P < 0.001) and less protein (P < 0.001) than conventional pork. Fat content was not significantly different between moisture enhanced and conventional chops, however, tended to be numerically lower in moisture-enhanced chops (P = 0.116). From the chops cooked for the taste panel, cooking losses were significantly lower for moisture enhanced pork vs. conventional pork (P = 0.035).

There was significant variation in many of the physico-chemical traits amongst stores reflecting a range in pork quality which included variations in pH, colour attributes and moisture content of the muscle.

	Cont	Control Mo		Moisture Enhanced	
Physico-chemical characteristic	mean	sem ^c	mean	sem	Р
Shear (kg)	4.85 ^a	0.12	4.31 ^b	0.11	0.001
pH	5.84 ^b	0.04	6.07^{a}	0.03	< 0.001
Expressible Juice mg g ⁻¹	93.2 ^a	6.1	67.3 ^b	5.3	0.002
Cook loss mg g^{-1}	270.4^{a}	7.3	249.7 ^b	6.3	0.035
Objective Colour					
L*	53.70 ^a	0.62	49.90^{b}	0.54	< 0.001
Chroma	11.23 ^a	0.31	9.11 ^b	0.27	< 0.001
Hue	40.13 ^a	1.00	37.47 ^b	0.88	0.049
Subjective Retail Scores					
Surface colour	2.94 ^b	0.06	3.35 ^a	0.06	<.0001
Surface discoloration	1.63 ^b	0.14	2.16^{a}	0.12	0.005
Retail appearance	5.20 ^a	0.18	4.60^{b}	0.16	0.016
Crude Nutrient Composition					
Moisture, mg.g ⁻¹	724.5 ^b	1.5	744.3 ^a	1.3	< 0.001
Crude fat, mg.g ⁻¹	21.7	1.7	18.1	1.5	0.116
Crude protein, mg.g ⁻¹	251.0^{a}	1.4	228.2 ^b	1.2	< 0.001

Table 1. Physico-chemical meat quality characteristics

^{a,b}Least squares means (LSMean) within a row without a common superscript were significantly different (P < 0.05)

°Standard error of means

3.2 Sensory Meat Quality

Overall palatability of the pork chops was improved by moisture-enhancement (P<0.001) (Table 2). Moisture-enhanced pork was also rated as more tender and juicier than conventional pork. Pork flavour intensity however, was significantly lower (P<0.001) and other flavour intensity was significantly higher in enhanced vs. conventional chops (P<0.001) (Table 2).

	Control		Moisture Enhanced		
Sensory characteristic	mean	sem ^c	mean	sem	Р
Initial tenderness	4.82 ^b	0.17	6.48 ^a	0.15	< 0.001
Overall tenderness	5.02 ^b	0.15	6.44 ^a	0.13	< 0.001
Initial juiciness	4.48^{b}	0.15	5.72 ^a	0.13	< 0.001
Sustained juiciness	4.28 ^b	0.14	5.61 ^a	0.12	< 0.001
Pork flavour intensity	5.49 ^a	0.17	3.54 ^b	0.15	< 0.001
Other flavour intensity	1.66 ^b	0.26	4.69 ^a	0.23	< 0.001
Overall palatability	4.86 ^b	0.11	5.58 ^a	0.10	< 0.001

Table 2. Panel ratings for moisture enhanced and conventional pork

^{a,b}Least squares means (LSMean) within a row without a common superscript were significantly different (P < 0.05)

°Standard error of means

3.3 Bacteriology

The numbers of aerobes, psychrotrophs and lactic acid bacteria were not significantly different between conventional and moisture enhanced pork (Table 3), with values ranging from 3.0 log CFU g⁻¹ to 7.8 log CFU g⁻¹. *Enterobacteriaceae* and pseudomonads, were significantly higher in moisture enhanced vs. conventional pork (*Enterobacteriaceae* P<0.001, pseudomonads P=0.002), however the differences were less than 1 log CFU g⁻¹ (Table 3). The numbers of *B. thermosphacta* were 1 log CFU g⁻¹ higher in moisture enhanced than in conventional chops (*B. thermosphacta* P = 0.002). In addition to differences between moisture enhanced and conventional chops, mean bacterial counts were different between stores (Table 3). Store 5 had lower numbers of all groups of bacteria, while stores 2 and 4 tended to have higher numbers of bacteria.

Table 3. Bacteria counts $\log (cfu g^{-1})$ for moisture enhanced and conventional pork

Set type		Ν	aerobes	Pseudomonads	B. thermosphacta	lactic acid bacteria	psychrotrophs	Enterobacteriaceae
Pork	C ^c	38	5.18	2.96 ^b	3.18 ^b	5.09	5.18	2.10 ^b
	\mathbf{M}^{d}	50	5.31	3.82 ^a	4.18 ^a	5.20	5.22	2.94 ^a
Store	1	14	5.47	3.56	3.93	5.44	5.42	3.05
	2	16	5.83	4.29	4.78	5.79	5.78	3.40
	3	16	4.89	3.11	3.54	4.72	5.01	2.43
	4	14	5.96	3.26	3.69	5.90	5.85	3.19
	5	18	4.48	3.07	2.82	4.36	4.38	1.35
	6	10	5.00	3.44	3.92	4.80	4.84	2.17

^{a,b}Least squares means (LSMean) between C and M without a common superscript were significantly different (P < 0.05)

^cconventional pork

^dmoisture enhanced

4. Discussion

4.1 Physico-chemical Characteristics and Eating Quality

Meat pH was significantly higher in enhanced chops than in conventional; this is similar to effects reported in pork from previous studies involving polyphosphate injections (Detienne & Wicker, 1999; Smith et al., 1984). Higher pH, further from the isoelectric point of the myofibril proteins, results in a greater charge allowing higher levels of water binding to the protein matrix (Huff-Lonergan & Lonergan, 2005).

However, there was some variability in pH of enhanced products among the different retailers (ranging from 5.99-6.37); this may have been caused by differences in final concentration of polyphosphates in meat. These differences could result from either lower concentration of polyphosphate in injection brines or a lower injection volume. Injection volume can vary according to the recipes of manufacturers, but usually, meat is injected between 7% to 10 % of fresh weight (Miller, 1998). At higher injection volumes, retail appearance can be impaired due to higher package purge; this has been shown for loins pumped to 12% or 18% of fresh weight (Brashear, Brewer, Meisinger, & McKeith, 2002).

Moisture enhanced pork also had higher moisture and lower protein content in the present study. It has been

shown the composition of brine injections is important when considering changes in water and protein content; for example, injection of pure water, does not increase water content in meat as a large proportion of injected water is lost as package purge (Sheard et al., 1999). Higher moisture content in enhanced chops, therefore, is likely the result of less package purge and higher water holding capacity of myofibril proteins due to the increase in meat pH and ionic strength through the addition of polyphosphates and salt. As well, Offer and Trinick (1983) reported a swelling of myofibril proteins following exposure to polyphosphates due to phosphate binding to myosin, preventing cross-bridge formation.

Moisture enhanced pork in the present study had a lower expressible juice. Similarly, Cannon et al. (1993) reported significantly less free water and lower purge loss after marinating pork with sodium tripolyphosphate. In addition to the effects of a higher pH and phosphate binding to myosin, salt also leads to swelling of muscle fibres by increasing ionic strength (Offer & Trinick, 1983). An interaction of salt and polyphosphate in the injection brines for chicken meat has been reported to improve water holding capacity (Shults & Wierbicki, 1973). Additionally, packaging purge was lower for pork injected with combinations of sodium tripolyphosphates and salt, rather than a single ingredient alone, confirming the additive effect on water holding capacity (Detienne & Wicker, 1999). Thus, salt included in injection brines likely would have contributed to the low proportion of expressible juice in moisture-enhanced pork of the present study.

Moisture enhanced pork had significantly lower cooking losses than conventional pork. In agreement with the present results, pork loins injected with combinations of salt and tripolyphosphate (Detienne & Wicker, 1999; Apple, Dikeman, Simms, & Kuhl, 1991) or marinated with tripolyphosphate (Cannon et al., 1993) have been shown to have lower cooking losses than conventional meat. The reported effects of polyphosphates on cook loss in pork, however, are not entirely consistent; cooking loss has also been shown to slightly decrease (Sheard et al., 1999) or display no change after polyphosphate injection (Sutton, Brewer, & McKeith, 1997).

Moisture enhancement also had an effect on sensory quality traits of pork chops. The improved moisture content of the enhanced chops was reflected in higher taste panel scores for juiciness. In accordance, palatability was closely correlated with initial juiciness (r = 0.76), sustained juiciness (r = 0.79), initial tenderness (r = 0.73), and overall tenderness (r = 0.77). Improved perception of juiciness for moisture-enhanced pork is in agreement with Cannon et al. (1993), where pork was marinated with polyphosphate. Additionally, cook loss was moderately correlated with panel scores for initial (r = -0.64), and sustained juiciness (r = 0.63), while the correlations between moisture content in chops and subjective juiciness attributes were weaker (initial r = 0.42, sustained juiciness r = 0.51). Thus, higher moisture content in meat does not necessarily improve juiciness if water does not remain in the meat during the cooking process.

Improved tenderness in pork treated with dilutions of polyphosphates, with or without salt, has also been reported in previous studies (Cannon et al., 1993; Sheard et al., 1999; Smith et al., 1984). The higher taste-panel scores for tenderness in moisture enhanced pork for the present study corresponded with the lower shear force values of enhanced chops. Further, subjective scores for overall tenderness, were moderately correlated with shear force (r = -0.51). The hypothesized mechanisms for improvement in tenderness by polyphosphates are similar to those affecting moisture content (i.e. changes to pH and ionic strength). The swelling of the muscle fibres caused by increased pH and ionic strength (Bendall, 1954) lead to disruption of myofibril protein structure and thus increased tenderness (Offer & Trinick, 1983; Voyle, Jolley, & Offer, 1986).

Tripolyphosphate use in injection brines has been observed to decrease pork flavour intensity and increase abnormal flavour intensity in pork (Sheard et al., 1999). A similar effect was noted in the present study, where enhanced chops were rated lower for pork flavour but higher for other flavour intensity. Other authors, however, found no effect of polyphosphate injection on off-flavour and pork flavour intensity (Brewer et al., 1999); Smith et al., 1984). Thus, it cannot be excluded that differences in flavour obtained in the present study were caused by ingredients of the injection brines other than polyphosphates.

Objective meat colour characteristics were affected by moisture enhancement. Enhanced pork chops were lower in L*-value and chroma, additionally, surface colour was subjectively rated as darker. These results are in agreement with some previous studies (Banks et al., 1998; Glaeser, Nattress, Greer, Gibson, & Aalhus, 2005; Prestat, Jensen, McKeith, & Brewer, 2002a). In contrast, Sutton et al. (1997) found polyphosphate injection had no effect on meat colour of pork. The higher water holding capacity of enhanced meat is thought to result in less light scattering on free water molecules and thus darker meat (Fern ández-L ópez, Sayas-Barber á P érez-Alvarez, & Aranda-Catal á 2004). Additionally, the lower chroma of enhanced chops has been associated with changes to the oxidation state of myoglobin in higher salt concentrations (Fern ández-L ópez et al., 2004).

Corresponding to the objective measures of lower saturation and darker surface colour, enhanced pork was given

higher subjective scores for surface discolouration than conventional pork. Surface discolouration in injected chops was mainly the result of grey or dark surface areas along the path of the injection needles (Figure 1). So-called two-toned meat is one of the major quality defects identified in needle-injected fresh meat products (Miller, 1998) and is thought to be the result of disruption of myofibril structure by high salt concentration around the injection sites (Voyle et al., 1986). Meat colour has been identified as highly important for consumer choice (Dransfield et al., 2005). As such, higher discoloration in enhanced chops may negatively affect the purchase intent of consumers and will likely contribute to their perception of quality. Corresponding to this, enhanced chops in the present study were rated lower for retail appearance. It has been observed certain additives containing antioxidants (e.g.: hydrolyzed plasma protein or oregano oil) when included in injections brines, improve measures of colour stability relative to loins only injected with salt and polyphosphate (Scramlin et al., 2010; H. Seo, J. Seo, & Yang, 2016). However a detailed study on methods to alleviate striping, including changes to the brine, enhancement level, or tumbling following injection, was unsuccessful (Gooding et al., 2009).

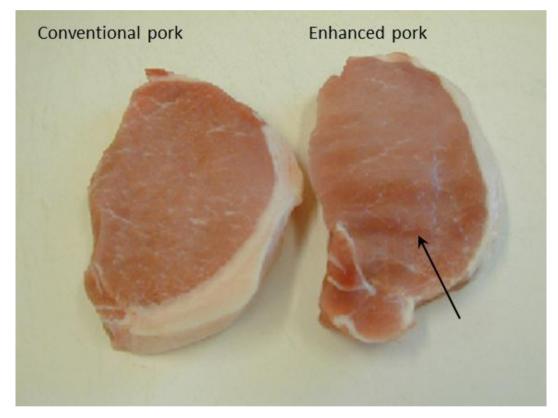


Figure 1. Surface discoloration in moisture-enhanced pork chops. Arrow pointed at region of discolouration

4.2 Bacteriology

Bacterial load in injection brines have been shown to increase with processing time by up to 1.5 log CFU ml⁻¹ through the duration of the moisture-enhancement process (Greer, Nattress, Dilts, & Baker, 2004). Additionally, Bohaychuk and Greer (2003) in their analysis of commercial pork loins observed 2 log CFU g⁻¹ higher numbers of psychrotrophic bacteria in moisture-enhanced pork when compared to non-injected product; numbers of lactic acid bacteria, *Enterobacteriaceae* and *B. thermosphacta* were not different. However, in cooked chops the moisture enhancement process did not result in increased risk for survival of the pathogenic bacteria *Salmonella typhimurium* or *Campylobacter jejuni* (Wen et al., 2014). In the current study, there were no significant differences in numbers of aerobes, psychrotrophs and lactic acid bacteria but increases in numbers (0.84-1 log CFU g⁻¹) of *Enterobacteriaceae*, *B. thermosphacta* and pseudomonads were observed. Increases in these bacteria resulting from moisture enhancement could be associated with bacterial spoilage (Borch, Kant-Muermans, & Blixt, 1996). However, it is currently unknown whether the bacterial increases observed here would result in increases to spoilage of commercial importance. Storage trials would be required to determine whether these differences in bacterial levels would impact storage life of the meat. However, Bohaychuk and Greer (2003)

concluded there was no evidence of a compromised storage life for moisture-enhanced pork loins when compared to non-injected pork.

There was some variability in bacterial counts between stores (Table 3), with the mean counts of *Enterobacteriaceae* and *B. thermosphacta* being most variable. The range in mean counts of all bacteria measured here between stores were greater than 1 log cfu g^{-1} . This may indicate the hazard analysis critical control point (HACCP) procedures, among the retailers and suppliers could contribute to overall differences in meat bacterial levels.

5. Conclusions

Moisture enhanced pork had higher levels of some bacteria associated with spoilage. It is currently unknown how these increases to bacteria affect shelf-life of moisture enhanced pork. Future research should explore how spoilage rates are affected by moisture enhancement under different retail conditions.

Many factors associated with eating quality of pork were improved by moisture enhancement in the present study. The improvements to eating quality of moisture enhanced chops included subjective panel scores for juiciness, palatability, and tenderness. Additionally, moisture enhanced chops had a lower shear force. Despite the improvements to the textural factors associated with eating quality and higher overall palatability, enhanced chops were rated lower for pork flavour intensity and had higher ratings of other flavour intensity.

Moisture enhanced pork displayed a higher surface discolouration and lower colour saturation. Since colour is an important consideration when the consumer chooses a product, further means to reduce striping should be considered in producing moisture enhanced pork for retail markets.

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