PHYSICOCHEMICAL, SENSORY, FUNCTIONAL AND MICROBIAL CHARACTERISATION OF HORSE MEAT

CARACTERIZAÇÃO FISICOQUÍMICA, SENSORIAL E MICROBIOLOGICA DE CARNE DE CAVALO

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ABSTRACT

The objective of this work was to determine the technological feasibility of using horsemeat as a human food. Beef and horsemeat Longissimus dorsi was stored at 4°C for a total study time of 15 days. Collagen content was lower for horsemeat than for beef, although sensory analysis showed no significant difference in tenderness. Instrumental and sensory color analysis showed significant differences between species. Horsemeat proteins were significantly less functional than beef proteins. Coliform counts and final pH were similar in horsemeat and beef. Horsemeat can be used as a replacement of beef in meat products if other additives are used to improve functional properties. It can also be used to increase redness.

KEY WORDS: horsemeat, functionality, hydroxyproline, color, hardness

INTRODUCTION

Horses production is generally focused to sports and labor and are characterized for their long life span. Horse production as a meat source has been questioned for its edible quality although horsemeat has similar nutritive value as beef, in addition of being cheaper and with less fat content (DUFEY, 1996). Some cultural practices make horsemeat highly desirable item for human consumption. On the other hand, horsemeat is mainly used as an adulterant in some chopped meat products. A number of methods have been developed to detect species adulteration such as immunochemical and electrophoretical techniques (JANSSEN et al., 1990). However, when analyzed by sensory methods, horsemeat is difficult to detect due to its similarity with meat from other species when chopped and added with additives (GARCÍA et al., 1994). Microbial and chemical analysis must be carried out in horsemeat aimed for human consumption because of its high microbial counts due to inadequate handling practices, as well as to chemical contamination in the skeletal muscle and some internal organs, such as the liver. However, conversely as meat from other animal, there is scatter information on accumulation of chemical contaminants (ZIN, 1994). Protein functionality is determined by many factors such as its use as food ingredient, or by their performance during processing, storage, handling and consumption of foods. All these properties depend on physicochemical characteristics of the system (WILDING et al., 1984; WHITHING, 1988). Functionality of contractile proteins confers emulsion and gelation properties to meat products. Therefore, the evaluation of alternative protein sources is important in order to find cheaper proteinaceous materials with similar functionality as those traditionally used (SMITH, 1988). The objective of this work was to determine the technological feasibility of using horsemeat as a human food considering some physicochemical, sensory, functional and microbial characteristics.

MATERIAL AND METHODS

Horsemeat was obtained from *L. dorsi* muscle of 3 animals from a local equine abattoirs. Beef was obtained also from *L. dorsi* muscle of 3 carcasses. Samples were collected 24 hrs after animal slaughtering. No sex, breed, age or *pre-mortem* handling was recorded. The meat was freed from connective tissue and sectioned into 50-g pieces. Samples were stored at 4°C during a total study time of 15 days and analyzed for pH, water holding capacity (WHC) and microbial populations. Samples were also analyzed at 24 hours of storage for instrumental color, texture, hydroxyproline content, sensory attributes and functionality.

pH measurement was carried out according to the method described by OCKERMAN (1980a). Ten grams of samples were homogenized in a domestic blender with 90 mL-distilled water during 1 min and pH was determined with a Beckman potentiometer (Beckman Instruments).

Water holding capacity (WHC) was determined according to the method described by OCKERMAN (1980a). Ten grams of meat were ground in a mortar and 5 g placed in a centrifuge tube. Eight milliliters of a 0.6 M NaCl were added and the mixture was stirred with a glass rod during 1 min.

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The tubes were placed into a water bath during 30 min, stirred for 1 min and centrifuged at 8000 *g* for 15 min. The supernatant was decanted and measured. WHC was reported as mL of 0.6 M NaCl per 100 g of meat.

Instrumental color: It was measured using a Hunter-Lab colorimeter (model D25-PC2, Reston, VA). “a” and “b” coordinates were transformed into hue and chroma as described by LITTLE (1975). The standards used were: L=64.5, a=1.0, b=1.9. Samples were read in triplicate, rotating the sample port 90° before each reading.

Hardness: Meat hardness was measured by compression using an Instron Universal Testing Machine (Series 4502, Canton, MA). One cubic centimeter samples were compressed 50% of its total high applying a 10 N force perpendicular to the fiber direction.

Hydroxyproline content: Hydroxyproline was taken as an indicator of collagen content. It was analyzed following a modification of the technique described by WOESSNER (1961). One gram of sample was homogenized with 9 g of distilled water and centrifuged at 7800 x g for 30 min. The cell pack was placed into vials where 2 mL 6 M HCl were added. The vials were sealed and hydrolyzed for 24 h at 110°C. The samples were then filtered to remove any solid in suspension; this precipitate was again homogenized with HCl, washed with water and stored at 4°C. Before analysis, the samples were resuspended into 1-mL deionized water, and hydroxyproline was analyzed. The concentration of this aminoacid was obtained by mixing 100 mL of sample with 100 mL water + 100 mL of a T-chloramine solution (T-chloramine, deionized water, methylcellosolve and acetate buffer). The sample was stirred and left at room temperature for 24 h. It was then added with 100 mL 3 M perchloric acid, stirred and added with 100 mL 20% para-diaminobenzoic acid. Samples were heated at 60°C for 20 min, cooled down and read at 557 nm. The readings were interpolated to a standard curve.

Sensory analysis: Color was evaluated by triangular test. (PEDRERO & PANGBORN, 1996). Three-one cm^3 samples were presented to the panelists, one was the reference and corresponded to beef the other two were beef and horsemeat. The panelists were asked to compare the samples against the control in terms of raw meat color. An unstructured scale (PEDRERO & PANGBORN, 1996). Was used to evaluated flavor, juiciness and tenderness. The samples were cooked in a water bath until 80°C internal temperature was reached. The panelists were asked to describe on a 10-point scale the following attributes: flavor: 1=pleasent; 10=very unpleasant; juiciness: 1=very juicy; 10=very dry; tenderness: 1=very hard; 10=very soft.

Protein extraction: Muscle proteins from beef and horsemeat were extracted by a modification of the method described by NGAPO et al. (1992). Fifty grams of meat were homogenized with ice and water (1:1:1, w/w), stirred 10 minutes into an ice bath. Water was added in a 2:3 (v:v) proportion and stirred for another 15 min and filtered through a domestic metal mesh to remove connective tissue. The homogenate were centrifuged at 2,000 x g during 10 min at 4°C and the precipitate was resuspended with a 0.5% phosphate solution + 3.5% food grade NaCl + 0.1% sodium azide (Sigma Chemical Co., St. Louis, MO). Protein concentration was determined by the Biuret method (GORNALL et al., 1949) and adjusted with the same saline solution to 20 mg/mL of protein.

Emulsion capacity: It was determined by conductivity according to the method described by SWIFT et al. (1961). 0.5 mL of protein suspension were added with 2 mL of sunflower oil and homogenized during 1 min in 1.5 cm I.D. acrylic tubes fitted with electrodes connected to a multimeter. Conductivity of the suspension was recorded oil was continuously added at constant rate (2 mL/min) until phase inversion occurred and conductivity abruptly dropped. Emulsion capacity was reported as mL of emulsified oil per 100 g of protein.

Gel strength: It was determined by uniaxial compression of protein gels as described by MONTEJANO et al. (1984). Five milliliters of protein solution were placed into 13-mm I.D. test tubes and heated during 20 min at 70°C, cooled down in water and kept at 4°C until their analysis. Penetration tests were carried out using an Instron Universal Testing Machine model 4502 (Instron, Canton, OH) fitted with a 10 N load cell and 8-mm diameter probe penetrated 75% of the gel height (20 mm) was penetrated at 10-mm/min constant speed. Gel strength was reported as the maximum force registered during gel penetration.

Microbial analysis: Enterobacteria, total psychrothrophs, total mesophile counts, and yeasts and moulds counts were determined using brilliant green agar incubated at 37°C, nutrient agar at 4°C, nutrient agar at 37°C and potato dextrose agar at 30°C, respectively. Biochemical tests were carried out for strain identification: triple sugar-iron; urea broth, motility, sulphur-indol agar, lysine-descarboxilase agar (VANDERSANT & SPLITTSTOESER, 1992).

Statistical analysis: Data were the mean of at least 5 replications, and were subjected to student t-tests or F-tests, using a SAS package (SAS INSTITUTE, 1996).

RESULTS AND DISCUSSION

pH is a result of *postmortem* biochemical changes which continue during the storage period, and are directly related to storage temperature (DUSHYANTHAN et al., 1994). pH increased in horsemeat (Figure 1) as a result of amine-containing compounds production during spoilage. However, the increasing trend in pH was more marked in horsemeat than in beef. DUFEY (1996) reported similar pH values as those obtained in this study for meat from old horses. In these animals ultimate pH was high (above 6.0) and also presented dark appearance and reduced myoglobin oxygenation. As a result, bacterial attack was more likely to occur than in meat from other species (SILVA et al., 1998). Horsemeat pH was higher (5.8) (Figure 1) than beef (5.36) (PÉREZ CHABELA et al., 1999) at day 6 of storage.

Water holding capacity is defined as the ability of meat tissue to retain free water (not chemically bound) when an external force such as cutting, chopping or pressing is applied (OCKERMAN, 1980a). WHC is particularly important in ground or emulsified meat products in which the integrity of the muscle fiber was destroyed, therefore there is no physical water retention. The results show lower WHC in horsemeat as compared to beef (Figure 1), although a number of factors could affect these results such as fat content, pH and time elapsed after slaughtering. Another factor affecting the results

was animal age. In general, horses are slaughtered at older age than beef, this promoted in a significantly lower WHC in horse (75.7 mL/100 g tissue, Figure 2) than in beef (96.25 mL/100 g tissue) (PÉREZ CHABELA, 1998). However, when a sensory panel evaluated juiciness, there was no significant difference between animal species (P>0.0.5336) (Table 2).

Instrumental color was defined according to the CIE system, considered lightness hue and chroma. Lightness was significantly higher (P>0.0001) in beef (32.88) than in horsemeat (20.95) meaning paler meat (Table 1). This is the result of several factors such as higher myoglobin content, age of the animal as older animals have darker muscle color, and differences among species. Heme-pigment concentration increases rapidly after 24 to 30 months of age, it is further stabilized and decreased after 8 to 10 years. It was expected that, being older, horses will have higher concentrations of heme-pigments. PICALLO et al. (1998) found significant differences in color among bovines of different breeds. It is assumed therefore that differences among species such as equines and bovines could be more significant.

TABLE 1 - Physicochemical, sensory and functional characteristics of horsemeat as compared with beef

	Horsemeat	Beef	P>
Lightness	20.95	32.88	0.0001
Hue	24.93	40.68	0.0001
Chromaticity	12.75	13.82	0.3867
Hydroxiproline (mg/g tissue)	35.32	43.75	0.0001
Hardness (N)	0.1706	0.1493	0.0056
Emulsion capacity (oil mL/100 g protein)	71.35	113.54	0.0184
Gel strength (N)	4.22	5.25	0.0129

TABLE 2 - Sensory analysis of horse meat as compared with beef

Attribute	P>
Color (raw sample)	0.0020
Flavor	0.8499
Juiciness	0.5336
Tenderness	0.8542

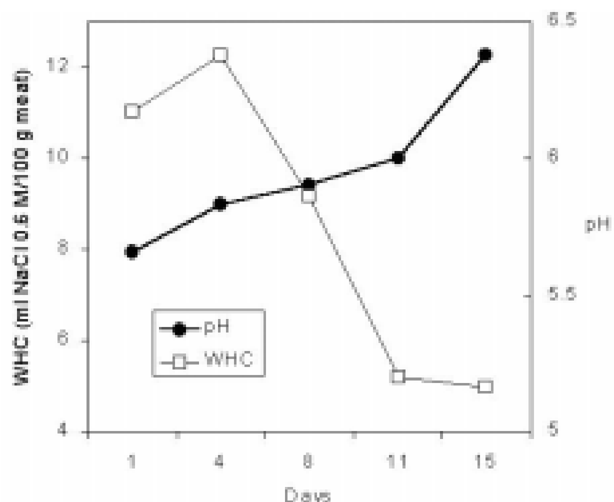

Figure 1 - pH and water holding capacity of horsemeat

Color was also detected by the panelists as being significantly different between animal species (P>0.0020) (Table 2). Hue was also significantly higher (P>0.0001) (Table 1) in beef (40.68) than in horse (24.93). In this component, 0º=red, 90º=yellow, the smaller the angle the closer the color to red color. Using redder meat in most sausages can be advantageous, as a more intense color can be desirable.

Sensory evaluation gave no significant differences in flavor (P>0.8499) and tenderness (P>0.8542) between horsemeat and beef (Table 2).

Collagen, the main constituent protein of connective tissue, has 12.8% hydroxyproline, higher than other proteins. Therefore this aminoacid is an indication of connective tissue in meat (GILLET, 1985). The amount of connective tissue results in tougher meat, and as the animal grows older this amount increases, although it also varies according to the anatomical section of the animal (BAILEY, 1984). The comparison of collagen content of horsemeat with meat from other animal species is difficult due that reports on horsemeat are scattered. In our study hardness was more evident in horsemeat than in beef, although, collagen content was lower. According to this fact secondary hardness, due to collagen content, was lower in horsemeat (35.32 mg/g tissue) than for beef (43.750 mg/g tissue). The force necessary to compress 50% of horsemeat samples was significantly higher (P>0.0056) in horsemeat (0.1706 N) than in beef (0.1493 N) (Table 1) probably due to larger fiber diameter. Conversely, sensory analysis showed no significant difference in tenderness (P>0.8542) in cooked meat. Although raw horsemeat showed more resistance to compression, heat treatment promoted protein denaturation and fiber disruption, therefore cooked horsemeat had similar tenderness as beef.

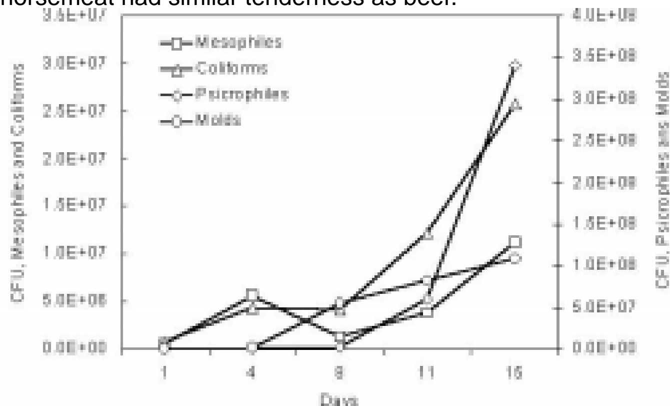

Figure 2 - Microbial population in horsemeat

Functional properties, in the context of this study, were referred to those attributes of macromolecules that impart physical properties to a food system. The meat source (animal species, specific muscle, age, etc.) determines this type of properties such as water and fat binding. The main functional properties in meat products are emulsion capacity and gel strength. These properties provide on a large extent the quality of a fabricated meat product. Good fat emulsification represents the possibility of incorporating fat and to keep it as a stable emulsion even after heat treatment. Gelling ability of meat proteins represents the formation of a stable matrix in which the system is formed by proteins, fat, water and additives. Emulsion capacity was significantly lower in horsemeat than in beef proteins (P>0.0184, Table 1) meaning that beef proteins have the ability to form larger interfaces in water-protein-oil systems than horsemeat proteins (SMITH, 1988). Gel strength was higher for horsemeat than for beef proteins (P>0.01297, Table 1). From this results, it can be concluded that horsemeat proteins were significantly less functional than beef proteins. This could have as a result the

need of other additives, such as gelling agent to achieve similar functionality in manufactured products where beef is replaced by horsemeat. As protein concentration for this analysis was standardized, variability was due to protein denaturation as a result of slaughtering and carcass handling, producing various degrees of proteolysis.

Coliform counts reached values around 10^6 cfu/g at day 11 of storage at 4°C. In previous studies (OCKERMAN, 1980b; PÉREZ-CHABELA et al., 1999) beef had a total microbial count of 10^5 cfu/g tissue at day 6 of storage at 6°C. Sanitation of horsemeat stored at 4°C became unsuitable at day 11 of storage, where coliform counts grew above 10^7 cfu/g.

CONCLUSIONS

Horsemeat can be used as a replacement of beef in meat products if other additives, such as gelling agents, are used to improve its functional properties because its gelling strength and emulsion capacity are lower than those of beef proteins. However, horsemeat can increase color in manufactured products when included in the formulation due to its more intense red color. From the sensory evaluation point of view, horsemeat and beef showed no significant differences, with the exception of color. As it has a lower concentration of collagen expressed as hydroxyproline, horsemeat secondary hardness is less than in beef, but hardness as expressed by compression is higher probably due to larger fiber diameter. From the sanitation point of view, when properly slaughtered, horsemeat has similar microbial populations as beef.

RESUMO

O objetivo deste trabalho foi determinar a viabilidade tecnológica da carne de cavalo na alimentação humana. O músculo Longissimus dorsi de bovino e cavalo foi armazenado a 4ºC por um tempo total de estudo de 15 dias. O teor de colágeno foi mais baixo na carne de cavalo do que na carne bovina embora a análise sensorial não mostrasse nenhuma diferença significativa na maciez. As análises de cor avaliados instrumental e sensorialmente mostraram diferenças significativas entre as espécies. As proteínas da carne de cavalo foram significativamente menos funcionais do que as proteínas da carne bovina. As contagens de coliformes totais e o pH final foram similares nas carnes de cavalo e bovina. A carne de cavalo pode ser usada como um substituto da carne bovina em produtos derivados se outros aditivos forem usados para melhorar sua propriedade funcional. Também pode ser usado para aumentar a pigmentação vermelha.

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