EVALUATION OF MICROBIOLOGICAL AND PHYSICAL-CHEMISTRY OF FROZEN HAMBURGER PATTIES EXPENDED IN MARACAIBO, ZULIA STATE, VENEZUELA

Evaluación Microbiológica y Físico-Químico de Hamburguesas Congeladas, Expendidas en Maracaibo, Estado Zulia, Venezuela

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ABSTRACT

In order to evaluate the microbiological quality (Aerobic plate count=APC; total coliforms=CT; fecal coliforms=CF; *Escherichia coli*=Ec; *Staphylococcus aureus*= Sa and *Salmonella spp*) and physical- chemistry quality (moisture, total solids, protein, fat and ashes) of hamburgers sold in Maracaibo, Zulia State, Venezuela, 27 samples of three hamburger brands were collected at random (brands A and B: beef, C: chicken) commercialized in three retail markets over three weeks. In APC, brand A obtained more than 2,8 $\log_{10}$ ufc/g with respect to brand C ($P<0.05$). For CT, brand B presented more NMP in relation to brands A and C in two of the retail markets sampled ($P<0.05$), while the results obtained for CT in all three brands were varied. *Salmonella* spp was present in beef hamburgers of brand B. The APC count and *E. coli* exceeded the limits allowed in all brands. In the proximal analysis brand B had a lower quantity of protein (14,66% vs A=18.25% and C= 16.36%) due to a higher proportion of fat (17.95% vs A=13.94% and C=14.56%) which was constant in the three sampled retail markets. The high count in the microbiological indicators reflects a poor microbiological quality, suggesting the lack of good sanitary practices in manufacturing. The variability in the content of protein and fat in the three brands evidenced not only the difference in the commercial formula, but also that these types of hamburgers do not comply with Venezuelan bromatological standards.

Keys words: Hamburger, *Salmonella*, proximal composition, microbiological quality, Venezuela.

RESUMEN

Para estudiar la calidad microbiológica (aerobios mesófilos=AM; coliformes totales=CT; Coliformes fecales=CF; *Escherichia coli*=Ec, *Staphylococcus aureus*=*Sa* y *Salmonella spp.)* y físico-química (humedad, sólidos totales, proteína, grasa y cenizas) de hamburguesas vendidas en la ciudad de Maracaibo, Estado Zulia, Venezuela, se recolectaron 27 muestras al azar de tres marcas de hamburguesas (marcas A y B de res; marca C de pollo), comercializadas en tres expendios de la ciudad durante 3 semanas. En AM, la marca A obtuvo más de 2,8 $\log_{10}$ ufc/g con respecto a la marca C ($P<0,05$). Para CT, la marca B presentó mayor NMP con respecto a las marcas A y C, en dos de los expendios muestreados ($P<0,05$), mientras que los resultados obtenidos para CT en las tres marcas evaluadas fueron variables. La *Salmonella* spp estuvo presente en las hamburguesas de carne de res marca B. Los recuentos de AM y *E. coli* sobrepasaron los límites permitidos en todas las marcas comerciales. En el análisis proximal, la marca B contenía menor cantidad de proteína (14.66% vs A=18.25% y C=16.36%) debido a la alta proporción grasa (17.95 vs. A=13.94% y C=14.56%) que se mantuvo constante en todos los expendios muestreados. Los altos contajes en los indicadores microbiológicos refleja una pobre calidad microbiológica y sugiere la falta de aplicación de buenas practicas de manufactura; y la variabilidad en el contenido de proteína y grasa en las tres marcas evidenció no solo la diferencia en formulas comerciales sino que las hamburguesas recolectadas no se ajustan a las Normas Bromatológicas Venezolanas.

Palabras clave: Hamburguesas, *Salmonella*, composición proximal, calidad microbiológica, Venezuela.

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INTRODUCTION

Changes in lifestyles in the last decade, have modified eating habits of Venezuelans. It has been predicted that these diet changes will result in some nutritional imbalances, since the current trend is towards the massive consumption of fast food in this case hamburgers, which are usually very rich in fat [4, 27]. Hamburger patty manufacturing commonly involves the varied combination of several manufacturing beef production processes, which may have wide different hygienic performances. During the hamburger patty processing, the muscular ingredient (lean meat) is mixed with animal fat which, in turn, increases saturated fat and cholesterol content, at the same time, the grinding process being is responsible of microbiologic spreading; therefore, the risk of food born illnesses (FBI) and excessive fat consumption exists. However, in Venezuela there is a lack of knowledge (so, ineffective control) about the genera and load of microorganisms contaminating ready-to-cook hamburger patties**.** In terms of bromatological standards, there is not an official compositional guide for domestic raw meat sources to be utilized during the processing of hamburgers nor any handbook detailing nutrient composition of the finished product. The objective of this study was to assess the microbiological and bromatological quality of the most popular domestically produced hamburgers at the retail level.

MATERIALS AND METHODS

Samples

Three types of frozen hamburgers samples nationally commercialized of each brand (brands **"A"** and **"B"** representing beef and brand **"C "**representing chicken) were taken from each three retail markets, differing in size (markets 1, 2 and 3 corresponding to large, medium and small retail markets, respectively) of Maracaibo city, Zulia State, Venezuela. The samples were collected on each day Wednesday for three weeks in a row (total 27 patties) from June 23 th to July 7 th, 1999. The storage temperature was recorded for the frozen display on point of sale during sampling. Samples were stored at 0º C and this storage temperature was controlled during and after transportation in an icebox with dry ice. Upon arrival to the laboratory, samples were prepared immediately for subsequent analyses.

Microbiological analysis

Microbiological tests were conducted according to the methodologies described by the standards of the Venezuelan Commission of Industrials Norms (COVENIN) as follows: aerobic plate counts (APC) [7], total coliforms (CT), fecal coliforms (FC) and *Escherichia coli* [8], *Staphylococcus aureus* (positive coagulase)[11], and *Salmonella spp* [10].

Bromatological analysis

Bromatological quality was assessed by proximate composition. Moisture and total solids were determined according to COVENIN methodology [8], protein content was determined according to AOAC [1], fat determination followed the Folch [13] procedure and ash was determined according to AOAC [2].

Statistical Analysis

A completely randomized design with factorial arrangement of 3^2 (n: factors, k: levels) and three repetitions were used. Data were subjected to analysis of variance using SAS [24] by the least squares method. The variable distribution was adjusted with $\log_{10}$ for interpretation.

The following Linear Additive (probabilistic) Model was used:

$$Y_{ijkl} = \mu + B_i + M_j + (B \times M)_{ij} + T(M)_{k(j)} + T(B \times M)_{k(ji)} + E_{ijkl}$$

Being:

I = 1,.....M: 3

j = 1,.....E: 3

K = -3,. T: 4

where:

Y_{ijkl} = Response variable corresponding to the k-sample from the i-brand, in the j- market.

μ = Overall mean under study.

B_i = Effect produced by the i-brand.

M_j = Effect produced by the j-market.

$(B \times M)_{ij}$ = Effect of the interaction of i-brand and j-market.

$T(M)_{k(j)}$ = Effect of the interaction of k-temperature on j-market.

$T(B \times M)_{k(ij)}$ = Effect of the interaction of k-temperature on i-brand and j-market

E_{ijkl} = Experimental Error.

In order to show the assumptions of normality of variance to the microbiological variables that do not comply with these assumptions, transformations to $\log_{10}$ were performed.

RESULTS AND DISCUSSION

ANOVA showed nested components such as Temperature (market) and Temperature (brand x market) only contribute to explain variation of total coliform in this study.

Microbiological quality

Brand effect

ANOVA detected the effect of brand (P<0.05) as an independent source of variation on APC and total coliforms, TABLE I.

APC numbers was greater in product from brand A (16.02 $\log_{10}$ ufc/g), respect to brand C (13.22 $\log_{10}$ ufc/g), brand B obtained medium values (15.35 $\log_{10}$ ufc/g), but showing no statistic difference from the other brands. Although these differences, all brands presented APC that exceeded allowed limits established by Venezuelan Commission of Industrials Norms (< 7 $\log_{10}$ ufc/g) [12], those of the Oregon Department of Agriculture (< 6 $\log_{10}$ ufc/g) [19] and under bacteriological standards ruling in other countries such as Israel (7.69 $\log_{10}$ ufc/g) [21]. The high APC level of hamburgers, as an indicator of spoilage microorganisms, reflects a poor microbiological quality and suggests poor adherence to Good Manufacturing Process [22]. Therefore, a shorter shelf life could be expected [26] for these products.

Since total coliforms was affected by the interaction Brand x Retail markets, the independent brand effect will not be explained.

TABLE I shows *E. coli* counts in the three brands (A= 4.02 $\log_{10}$ NMP/g; B= 4.27 $\log_{10}$ NMP/g and C= 4.30 $\log_{10}$ NMP/g) being higher respect to the limits established by COVENIN (< 2 $\log_{10}$ NMP/g); this indicates that fecal contamination may have been present. This number implies that the patties were manufactured from compromised product rather than being abused after their manufacturing. Furthermore, the use of meat recovered for their manufacture must be suspected.

Brand x Market Interaction

No interaction of brand x market was detected for APC and *E. coli.* Brand x market interaction (P<0.05) was detected for TC and FC (TABLE III).

Hamburgers of the B brand contained a greater number of CT respect to brands A and C in two of the sampled retail markets (P<0.05), while brand C in retail market 3 got the greatest counts (NMP 11.60 vs A= 8.48 and B= 8.44 $\log_{10}$). Results obtained for CF were varied, where the samples bought at market 1, brands A and B (NMP 9.11 and 8.78 $\log_{10}$, respectively) reached the major counts respect to brand C (NMP5.41 $\log_{10}$). Opposite to this, in market 2, brands B and C (NMP 10.38 and 10.23 $\log_{10}$ respectively) showed high counts in relation to brand A (NMP 7.23 $\log_{10}$). When the three brands of hamburgers in market 3 were compared, there was no significant difference among them.

The variability in total coliform and fecal coliform in the patty brands from retail markets lead to some deduction of the poor microbiological quality of the product from which hamburgers were manufactured; it also indicates a poor adherence to GMPs [22], as well as the conditions under which the patties are stored and display, due to possible prolonged or abusive storage or chilling.

TABLE I
EFFECT OF THE BRAND OVER THE MICROBIOLOGICAL VALUES IN HAMBURGERS

	BRAND		
	A	B	C
APC	16.02 ± 0.69 [a]	15.35 ± 0.69 [ab]	13.22 ± 0.69 [b]
CT	8.88 ± 0.49 [a]	11.07± 0.49 [b]	9.69± 0.49 [ab]
CF	8.12 ± 0.47	8.76 ± 0.47	7.90 ± 0.47
EC	4.02 ± 0.68	4.27 ± 0.68	4.30 ± 0.68

[a.b.]: Different letters indicate significant differences (P<0.05)

TABLE II
BRAND EFFECT OVER THE PHYSICO – CHEMISTRY VALUE IN HAMBURGERS

Componente g/100g	Marca		
	A	B	C
Protein	18.25 ± 0.5 [a]	14.66 ± 0.52 [b]	16.36 ± 0.5 [c]
Fat	13.94 ± 0.40 [a]	17.95 ± 0.40 [b]	14.56 ± 0.40 [a]
Total Solids	34.53 ± 0.87	37.19 ± 0.87	37.58 ± 0.87
Moisture	65.49 ± 0.87	62.50 ± 0.87	62.41 ± 0.87
Ashes	1.88 ± 0.17	2.14 ± 0.17	1.92 ± 0.17

[a.b.]: Different letters indicate significant differences P<0,05.

However, according to Venezuelan standards, coliform count and fecal coliform; this is, high numbers of TC and FC do not determine whether the product is potentially hazardous due to contamination of fecal origin [14].

Levels of *S. aureus* (positive coagulase) did not vary among the three brands and were similar to those reported by American researchers [28, 25]. However, it is well known that *S. aureus* cannot compete in highly contaminated products, such as those represented by all brands, secondary contamination represents potential hazards of food poisoning because many strains of this organism are capable of producing heat-resistant enterotoxine [25]. The presence of *Salmonella spp*, without any exception, in all B brand samples collected at the different markets, violated the mandatory standards established by the COVENIN ("zero tolerance") and USDA/FSIS [12,29] norms.

Venezuelan research [3], who have developed microbiological analyses of hamburgers of raw meat and chicken meat, collected in different markets in the metropolitan area of Caracas, agree with these findings. *Salmonella* isolation by other investigators indicated that the isolation frequency has varied from zero to 4.3% in beef hamburgers [14,15]. The persistence of *Salmonella* in hamburger patties has suggested the implementation of control measures not only within the processing plant and distribution channels but also at the farm level [26].

TABLE III

BRAND x MARKET INTERACTION OF MICROBIOLOGICAL AND PHYSICO – CHEMISTRY INDICATORS IN HAMBURGER PATTIES

	Brand A Beef			Brand B Beef			Brand C Chiken		
	Market 1 n=3	Market 2 n=3	Market 3 n=3	Market 1 n=3	Market 2 n=3	Market 3 n=3	Market 1 n=3	Market 2 n=3	Market 3 n=3
Total Coliform log10 NMP/g	9.77 ± 0.85 a/c	8.38 ± 0.85 a/c	8.49 ± 0.85 a/c	12.38 ± 0.85 a/cd	12.38 ± 0.85 a/d	8.44 ± 0.85 b/c	7.43 ± 0.85 a/d	10.05 ± 0.85 ab/c	11.63 ± 0.85 b/d
Fecal Coliform log10 NMP/g	9.11 ± 0.81 a/c	7.28 ± 0.81ª/c	8.01 ± 0.81a/c	8.78 ± 0.81 ab/c	10.38 ± 0.81ª/d	7.12 ± 0.81 b/c	5.41 ± 0.81ª/d	10.23 ± 0.81 b/d	8.06 ± 0.81 b/c
Salmonella presence				presence	presence	presence			
S.aureus ufc/g	<20	<20	<20	<20	<20	<20	<20	<20	<20
Fat g/100 g	11.88 ± 0.70 a/c	14.83 ± 0.70 b/c	15.11 ± 0.70b/c	18.06 ± 0.70 a/d	18.28 ± 0.70 a/d	17.51 ± 0.70 a/d	15.71 ± 0.70 a/e	13.88 ± 0.70 a/d	14.18 ± 0.70 a/d

ab/: Means in the same row not having a common superscript; different between markets, within the same brand (P< 0.05).
/cd: Means in the same row not having a common superscript; different between brands, within the same market (P< 0.05)

Bromatological quality

Brand effect

ANOVA detected the independent effect of brand on protein (P= 0.003) and fat content (P= 0.0001). The lowest protein content corresponds to B brand (14.66%), followed by C brand (16.36%) and the highest protein content was in A brand (18.25%). These results were due to the variation of fat content present in the three brands, where B presented the highest percentage of fat (17.95%), followed by C (14.56%) and A (13.94%) (non-significant difference between C and A brand), TABLE II.

Variation in protein content among finished products can been related to the leanness of the meat used in the product manufacturing and to the amount of fat added during mixing operations [4, 2]; therefore, each meat plant had different practices for meat patty processing. These observations are pointed out by other researchers [15, 5]. When comparing these protein values to those established by COVENIN standards [12] and USDA compositional guides [20], it was observed that only brand A complied with both reports.

Brand A presented moisture values exceeding top allowed limit demanded by COVENIN [12]; this is in line with the lowest fat content constantly found for this brand. The inverse proportion between water and fat content reported for fresh and processed meat products is widely known [5, 23].

Total solids, in spite of not being officially listed as a bromatological criterion for describing the hamburger quality in Venezuela, reflects the total content of macro and micro nutrients in the samples.

Ash levels found for inl the samples are relatively high as compared to other reports [4, 5,18, 30] meaning an abundant use of salt, spices and condiments added during processing.

Brand x Market Interaction

No interaction of brand x market was detected for moisture, total solids, proteins and ashes. Significant interaction was only detected for fat (P>0.05) (TABLE II). The B brand was more constant in fat content in each market (18%). On the other hand, A and C brand varied in more than one percent among market (11 to 15%). When different brands of patties were compared within the same market, B brand always presented higher proportions of fat.

Fat content in all three brands was lower than what it is demanded by COVENIN standards [11] and USDA compositional reports [30]. Contrary to what it was expected, hamburger samples in this study are graded “Extra lean” by the American standards [16,18]. However, this important nutritional fact was not labeled in any package. Furthermore, this finding indicates that prediction of nutritional imbalances based on the assumption of high “fat-rich hamburgers” consumption in Venezuela must be revised. These products are classified as extra lean, this may be due to amount of water added to increase the profitability during processing; also, temperature and pressure undergone by the product in the market may produce loss of humidity and variation in the fat content [6]. Another reason that support this may be due to the fact Venezuelan meat has less than 5% of fat, what may explain the grading of beef hamburger sampled for this study as “extra lean” [17].

CONCLUSIONS

This survey has demonstrated that there is a lack of uniformity concerning microbiological standards of commercially produced patties. Risk levels for *S. aureus* is quite low according to the national standard consulted. The presence of *Salmonella spp* in hamburger patties “B” may represent a health hazard, therefore the fact that they are cooked does not guarantee its ubiquity. The variability in total and fecal coliform indicates that the patties were the result of poor microbiological conditions during manufacturing, and prolonged or abusive storage. However, the high number of *E. coli* indicate poor microbiological condition of raw material.

Variations in protein and fat values among hamburger brands explains the variability in commercial recipes and storage practices. Venezuelan hamburger manufacturers should consider the use of Extra lean labeling for promoting their products within the segment of health-conscious consumers.

It is necessary for industries to adopt adequate standards and controls to improve their production processes, and for markets to identify and monitor appropriate practices for the storage and display of chilled patties.

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