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Abstract: Peptone from ram horn was compared with a casein and other peptones for bacterial growth. First, horns were ground and 35 g of horn flour was hydrolyzed chemically (acid hydrolysis). As a result of this process, 30 g of the 35 g horn flour (85.7%) could be hydrolyzed. Hydrolyzed material was completed to 400 ml with deionized water, and this resulting solution was termed ram horn hydrolysate (RHH). The contents of protein, nitrogen, ash, some minerals, total sugars, total lipids and amino acids of RHH were determined. It was found that it has both organic and inorganic materials sufficient for use as a peptone in the growth of bacteria. The effects of different concentrations (1 to 10% v/v) of RHH on the growth of bacteria were investigated, and 4% of the RHH was found to be optimal. RHH media were tested against fish, casein and bacto peptone, for their ability to support growth of bacteria in pure cultures (aerobic and anaerobic) and natural samples such as soil, water, milk and meat (under aerobic and anaerobic conditions). The obtained results from parallel studies with surface streaking, pour plate procedures (for comparing the colony counts) and shaking culture (for comparing the biomass yields) showed that RHH media yielded significantly ($p < 0.05$) higher bacterial counts and biomass yields than did fish and casein, but these values were somewhat lower (not statistically significant) than values obtained from bacto peptone. In conclusion, RHH was found to be suitable as a peptone for bacterial growth.

Key Words: Bacterial growth, fibrous proteins, horn, slaughterhouse waste, protein hydrolysate, peptone.

Bakteriyal Üretim İçin Pepton Olarak Koç Boynuzu Hidrolizatının Kullanımı

Özet: Koç boynuzundan elde edilen pepton, bakterilerilerin üretimi için kazein ve diğer peptonlar ile karşılaştırıldı. Önce boynuzlar öğütüldü ve 35 g boynuz unu kimyasal olarak hidrolize edildi (asit hidrolizi). Bu işlemin sonucunda 35 gram boynuz ununun 30 gram'ı (%85,7) hidroliz edilebildi. Hidrolize materyal deiyonize su ile 400 ml'ye tamamlandı ve bu son solüsyon Koç Boynuz Hidrolizatı (KBH) olarak isimlendirildi. KBH'nin protein, azot, kül, bazı mineral, toplam şeker, toplam lipit ve amino asit içerikleri belirlendi ve bakterilerin çoğaltılmasında bir pepton olarak kullanılmasına yetecek kadar organik ve inorganik maddelere sahip olduğu bulundu. Farklı konsantrasyonlardaki (%1,2,3,4,5,6,7,8,9 ve 10) KBH'nin bakterilerin üremesi üzerindeki etkileri incelendi ve %4'lük KBH'nin optimal olduğu bulundu. KBH besiyerleri, saf kültürler (aerobik ve anaerobik) ve toprak, su, süt ve et gibi doğal örneklerdeki bakterileri (aerob ve anaerob şartlar altında) üretme potansiyellerini belirlemek için, balık, kazein ve bakto peptona karşı test edildi. Yüzeye yayma, dökme plak (koloni sayılarını karşılaştırmak için) ve çalkalamalı kültür (biyomas verimlerini karşılaştırmak için) çalışmalarından elde edilen sonuçlar, KBH besiyerlerinin balık ve kazein besiyerlerine göre önemli derecede ($p < 0.05$) yüksek bakteri sayısı ve biyomas verimi oluşturduğunu gösterdi, fakat bu değerler bakto pepton dan elde edilen değerlerden biraz daha düşüktü (istatistiki bakımdan önemsiz). Sonuç olarak, KBH'nın bakterilerin üretimi için pepton olarak uygun olduğu bulundu.

Anahtar Sözcükler: Bakteriyal üretim, fibröz proteinler, boynuz, kombina atığı, protein hidrolizatı, pepton.

Introduction

Peptones are defined as protein hydrolysates that are soluble in water and not heat coagulable (1). These products may have significant value for the fisheries industries as their market prices are somewhat higher than usual by-products such as fish silage and fish meal. Growth substrate costs often make up the major part of the production cost of microbial cells and bioproducts from the fermentation industry (2). The nitrogen source is usually the most expensive component of bacterial growth substrates and at present it is obtained from plants (3), dairy proteins such as casein (4) or whey (5), and slaughterhouse waste. On the other hand, peptones and fish hydrolysates are made either by acid hydrolysis or enzymatic digestion of proteins. Acid hydrolysis allows high yields; however, this process results in a high ash content in the final products as the neutralization step cannot be avoided (6).

Ram horns are significant waste products of the meat industry in Turkey. For example, the slaughterhouses in Turkey directly discharge about 600 tons a year. Fibrous proteins such as horn, feather, nail and hair are also abundant waste products. These waste products can be converted to biomass, protein concentrate or amino acids using proteases derived from certain microorganisms (7). Horns consist of α-keratin, which has a very high content of cysteine (up to 22%) and they also contain most of the common amino acids. They have the components of bone and blood tissues and are rich in some growth factors required by microorganisms (7-10). Ram horn protein hydrolysate as a peptone has not been investigated, and its use in industrial processes is poor.

Most of the research was done with a simple approach to the microbiological data; for example, microbial growth may be described using -, +, ++ signs or by sizing colonies on agar plates (11,12). We used three techniques: -, + signs, biomass measurements and colony count (under aerobic and anaerobic conditions with both pure species and natural samples).

It was assumed that ram horn material could be used as a raw material for bacteriological media. For this reason, we investigated the suitability of ram horn hydrolysate as a peptone in bacteriological medium. The aim of the present work was to see whether there are strong differences between ram horn peptone and the other peptones commonly used in biotechnology.

Materials and Methods

Materials

Horns were obtained from Erzurum Slaughterhouse in Turkey. The chemicals used in this study were analytical grade and purchased from Oxoid and Difco.

Peptones: Ram horn peptone was compared with reference peptones (Table 1).

Bacterial cultures and cultivation media: *Bacillus cereus* NRRL-3711, *Bacillus subtilis* NRS-744, *Lactobacillus bulgaricus* B-548, and *Lactobacillus plantarum* NRRL B-4496 were obtained from Dr C.P. Kurtzman (1815 North University Street, Peoria, Illinois 61604). *Streptococcus thermophilus* 70885 MCG-50, *Clostridium sporogenes* 413, *Clostridium perfringens* and *Salmonella* sp. were obtained from Dr. M. Kaya (Food Engineering Dept. Agricultural Faculty, Atatürk University, Erzurum, Turkey). *Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus, Citrobacter* sp., *Corynebacterium* sp., *Proteus* sp., *Clostridium tetani* and *Veillonella* sp. were isolated from patients by Dr. A. Kadanalı (Yakutiye Hospital in Erzurum, Turkey). *Pseudomonas putida* 39/D was obtained from Dr. Cruden (Iowa University, USA). *Listeria monocytogenes* B_2 was our isolate from white cheese. All strains were maintained between transfers on nutrient agar slants at 5°C.

Medium consisted of (wt/wt) 1.5% glucose, 0.5% of peptones (except ram horn peptone in liquid form, 4% v/v), 0.2% KH_2PO_4, 0.013% $CaCl_2$- 2 H_2O, 0.001% $FeSO_4$ –7 H_2O, 0.3% $MgSO_4$- 7 H_2O, pH 7.2. Solid media were prepared by the addition of 1.5% agar.

Methods

Hydrolyzation procedures of horn: Horns were washed with deionized water and dried in an oven at 100°C. The dry horns were cut into smaller pieces and ground with a grinder (Wiley Mill, Arthur Thomas, USA). Obtained material was termed horn flour (HF). Thirty-five grams of the HF were impregnated with 50 ml of HCl 6 N. The mixture was incubated at 80°C for 24 h. At the end of this period, the mixture was incubated at 130°C for 1 h by adding 100 ml deionized water. The solution was then cooled and the pH adjusted to 7 with 10 N NaOH. It was filtered twice through Whatman no. 1 filter paper. The volume was completed to 400 ml with

Table 1. Short presentation of the peptones used in study

Commercial name and raw material	Manufacturer	Presentation (aspect)	Dry matter (g DM/ 100 g of product)	Nitrogen content (g N/100 of product)
Fish Peptone No 1 (Fish)	Difco (USA)	Powder	94.55	10.1
Bacto Tryptone (Casein)	Difco (USA)	Powder	95.1	10.0
Bacto Peptone (Beef)	Difco (USA)	Powder	96.10	13.8
---- (Ram horn)	This study	Liquid	12.80 (g/100ml)	0.881 (g N/100ml)

deionized water. The final clear filtrate was termed ram horn hydrolysate (RHH) and stored at 4°C. As a result of this procedure, 30 g of 35 g HF was hydrolyzed. The various concentrations (1-9 and 10%) of the RHH were tested for growth of bacteria. Four percent RHH was found to be optimal.

Analysis of RHH: Amino acid analysis of RHH was carried out after hydrolysis with 6N HCl at 110°C for 24 h in a Biotronic LC-5001 Amino Acid Analyzer (Germany). Total sugar content, dry matter and ash analysis were estimated by AOAC methods (13). Total nitrogen was estimated by the micro-Kjeldahl method. Total lipids were determined according to Folch et al. (14). The elemental composition was measured by atomic absorption spectrophotometer (UV HS-360).

Plating method and sample collection: Bacterial colony counts were determined by the pour plate technique (15).

Three water samples [a spring water, a river water (Aras River) and tap water] were used in this study. Water samples were collected in sterile 1-liter polypropylene wide-mouth bottles containing 1.0 ml of a 10% solution of sodium thiosulfate ($Na_2S_2O_3$) to neutralize residual chlorine in the sample (15).

Three soil samples (forest soil, greenhouse soil and garden soil) were used in this study. The collection, preparation and inoculation procedures of soils were carried out according to the AOAC (13).

Separate food samples (milk: untreated milk, pasteurized milk, fresh milk; meat: from markets, from a butcher, from a slaughterhouse) were purchased from local retail markets, and analyzed in triplicate. Food samples were collected and prepared as described in the compendium of methods for microbiological examination of foods (16). All samples were returned to the laboratory and processed within 2 h of collection. Inoculated plates were incubated at 35°C and colony counts were performed at 24, 48 and 72 h.

Colony counts for pure cultures: Pure cultures were maintained on slopes of nutrient agar (Oxoid) by storage at 5°C after overnight growth at 35°C. Broth cultures were obtained by growth at 35°C for 24 h in nutrient broth of 100 ml in 250 ml conical flasks shaken continuously in an orbital shaker (ROSI 1000 Thermolyne). Triplicate plates were prepared for each culture and inoculated plates were incubated at 35°C. Colony counts on fish, casein, bacto peptone and RHH were performed at 48 h (17,18). Several microorganisms including aerobic, anaerobic and facultative bacteria were activated in NB medium for approximately 24 h at suitable temperatures, and 0.1 ml of the cultures were spread onto the surface of media prepared from fish, casein, bacto peptone and RHH.

Microbiological examinations: Samples were serially diluted in 9 ml of physiological salt solution. For total viable counts, dilutions were plated in fish, casein, bacto peptone and RHH media, and colonies were counted after 24, 48 and 72 hours of incubation at 35°C for each culture. For anaerobic bacteria, the plates were incubated at 37°C for 72 h in an anaerobic jar with steel wool and flushed with CO_2.

The biomass analysis was performed after growing cultures in 100 ml of four media in 250 ml conical flasks for 72 h. After incubation, biomass was harvested by centrifugation at 5000 rpm for 10 min and washed twice with distilled water and then dried in an oven at 60°C for 48 h and weighed (19).

Statistical analysis

All media were compared against each other. The experiments were replicated three times in a randomized block design. All data were analyzed using the general linear models procedure of SAS. Differences among means were tested for significance ($p < 0.05$) by Duncan's multiple range test.

Results and Discussion

The main chemical composition of RHH is shown in Table 2. These data show RHH to be rich in both organic and inorganic materials. Notably, it contains the essential substances required in microbial media such as sources of carbon, nitrogen and minerals. In addition, RHH is rich in amino acid content. The essential amino acids are present and among them arginine (4.66 mg ml^{-1}) is the highest. However, of all the amino acids considered, glutamic acid (8.17 mg ml^{-1}) was the most abundant. The absence of tryptophan and proline was probably due to their degradation during the acid hydrolysis of proteins, because the hydrolysis did not allow the determination of some amino acids, such as proline (20).

The chemical composition of RHH is in accordance with the findings obtained from the investigations on the elemental and amino acid composition of various fibrous

Table 2. The main chemical composition of RHH

Components g ml^{-100}		Amino acids mg ml^{-1}	
Nitrogen	0.881	Aspartic acid	3.90
Protein	5.500	Threonine	2.00
Dry matter	12.80	Serine	2.87
Ash	4.98	Glutamic acid	8.17
Total sugar	0.500	Glycine	5.19
Total lipids	0.300	Alanine	3.19
Mg	0.160	Cysteine	0.21
Ca	0.164	Valine	2.56
Cu	0.017	Methionine	0.41
Mn	0.036	Isoleucine	1.63
Zn	0.064	Leucine	4.02
Fe	0.123	Tyrosine	1.61
K	0.113	Phenylalanine	1.67
		Histidine	0.72
		Lysine	2.21
		Arginine	4.66
		Proline	0.0

proteins, such as nail (21,22), fish epidermis (8) and bovine hoof (9).

First, we investigated the effects of RHH in various concentrations (1-9 and 10%) on the colony counts. We found that the most suitable concentration for growth was 4% and the colony yields (log cfu/ml^{-1}) for this application were 7.22 of soil, 4.38 of water, 7.44 of meat and 6.33 of milk (Figure 1). Similarly, the highest biomass yields for *B. cereus, L. bulgaricus, L. monocytogenes, E. coli* and *S. thermophilus* were obtained from 4% RHH (Figure 2). It was found that applications higher than 4% had an inhibitory effect. For example, the lowest colony yields (6 of soil, 0.0 of water, 6.72 of meat and 5.47 of milk) were obtained from the application of 9% RHH. Furthermore, no growth was observed from the application of 10% of RHH (Figs. 1 and 2). This inhibitory effect may be due to the high BOD load of RHH and presence of cell wall cations and some toxic materials. Similar effects have been observed from effluents with high loads of organic and inorganic materials (23). Therefore, we continued the research (comparison with other peptones) with 4% RHH.

The amount of HF used was 35 g, and the amount of HF hydrolyzed was 30 g. According to these results, 85.7% of horn can be used as peptones for bacterial growth.

The comparison of growth of test microorganisms and the bacteria in natural samples on fish, casein, bacto peptone and RHH is shown in Table 3. The performance of RHH is similar to that of bacto peptone. The biomass yields of bacteria incubated in the four media for 72 h are shown in Table 4. The yields obtained from bacto peptone and RHH are generally higher than the ones from fish and casein, supporting the results shown in Table 3. When bacto peptone and RHH are compared, it is seen that the biomass yields of bacteria grown in bacto peptone are generally higher than in RHH, but differences are not significant ($p < 0.05$).

Table 5 contains the results of aerobic viable counts from the samples of soil, water, milk and meat on fish, casein, bacto peptone and RHH. The casein plates yielded the poorest results of all media tested. The total bacterial counts (log CFU ml^{-1}) obtained from bacto peptone, RHH, fish and casein were 256.39, 251.13, 24.2 and 221.54

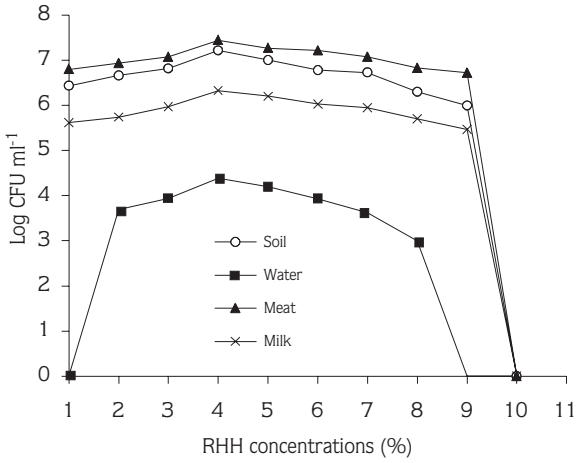

Figure 1. The effects of different RHH concentrations on the cell number of bacteria from natural samples

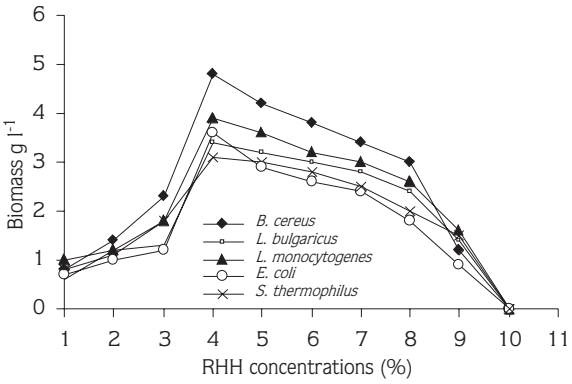

Figure 2. The effects of different RHH concentrations on the biomass yields of test microorganisms

Table 3. Comparison of growth of test microorganisms and aerobic and anaerobic bacteria in natural samples on Fish, Casein, Bacto Pepton and RHH

Bacteria and samples	Media			
	Fish	Casein	Bacto P.	RHH
B. cereus	+++	+++	+++	+++
B. subtilis	+++	++	+++	+++
L. bulgaricus	++	+	++	++
L. plantarum	+	+	+	+
E. coli	+++	+++	+++	+++
L. monocytogenes	++	++	+++	+++
S. thermophilus	++	++	+++	+++
E. aerogenes	+++	+++	+++	+++
P. putida	++	+++	+++	+++
S. aureus	++	++	+++	+++
Citrobacter sp.	++	++	+++	+++
Proteus sp.	++	+++	+++	+++
Corynebacterium sp.	++	+++	+++	+++
Salmonella sp.	+	++	+++	+++
Meat	+++	+++	+++	+++
Untreated milk	+++	+++	+++	+++
Pasteurized milk	+++	+++	+++	+++
Spring water	+++	+++	+++	+++
Tap water	+++	+++	+++	+++
ANAEROBIC				
Clostridium perfringens	+++	+	+++	+++
Clostridium tetani	+++	++	+++	+++
Clostridium sporogenes	+++	++	+++	+++
Veillonella sp.	+++	+	+++	+++
Meat	+++	++	+++	+++
Untreated milk	+++	++	+++	+++
Pasteurized milk	-	-	-	-
Spring water	+++	+++	++	++
Tap water	-	-	-	-

Samples of 0.1 ml of 24-h cultures were spread onto the surface of the agars.
+++ heavy growth; ++ little heavy growth; + very slight growth;
- no growth.

respectively. The differences between the results of fish and casein are not significant ($p < 0.05$). Similarly, the differences between the results of bacto peptone and RHH are not significant either. However, the differences between the results of bacto peptone-RHH and fish-casein are significant ($p < 0.05$). After 24 h of incubation for water (samples no. 1 and 3), no growth was observed on fish and casein. For the same samples, the mean colony counts (log CFU ml^{-1}) on bacto peptone and RHH were 2.44 and 1.90 (samples no. 1 and 3) and 2.06 and 1.21 respectively. There was also no significant difference in the performance of RHH when compared with bacto peptone for these samples. As seen in Table 5, the third water sample was chlorinated tap water and it may contain injured bacteria unable to growth in casein and fish. Rodrigues and Kroll (17) reported that many types of physical or chemical stresses were used in the food and other industries to eliminate or control the growth of microorganisms in products. This often results in the sublethal injury of cells unable to grow under certain environmental contidions and renders the cells susceptible to secondary stresses such as the selective agents commonly used in media for their enumeration. These organisms can pass undetected during routine microbiological examinations but, because they are capable of recovery and growth under suitable conditions, they can represent a considerable health hazard. In addition, Hurst (24) reports that a medium that is quite suitable for an uninjured organism may become inadequate for an injured one. It is thus essential to use

Table 4. Comparison of biomass yields of test microorganisms on fish, casein, bacto pep. and RHH

Bacteria	Media/ Biomass yields (g l^{-1})			
	Fish	Casein	Bacto P.	RHH
B. cereus	3.02 a	3.0 a	4.9 b	4.8 b
B. subtilis	2.30 a	2.6 a	3.1 b	2.8 a
L. bulgaricus	2.81 a	2.4 a	3.8 b	3.4 b
L. plantarum	00 a	00 a	1.2 b	0.9 b
E. coli	3.60 a	3.2 a	3.8 a	3.6 a
L. monocytogenes	3.12 a	2.6 a	4.2 b	3.9 b
S. thermophilus	2.80 a	2.4 a	3.6 b	3.1 b
E. aerogenes	3.10 a	3.4 a	3.2 a	3.3 a
P. putida	4.20 a	4.2 a	3.8 a	4.3 a
Citrobacter sp.	3.20 a	3.0 a	3.0 a	2.9 a
Proteus sp.	3.10 a	3.1 a	3.8 b	4.0 b
S. aureus	3.60 a	3.9 a	3.7 a	3.7 a
Corynebacterium sp.	3.10 a	3.6 a	4.0 b	4.0 b

Values with the same letter are not significant ($p < 0.05$)

Table 5. Comparison of growth of total aerobic bacteria in natural samples on fish, casein, bacto pep. and RHH

Samples	Incubation time (h)	Sample No.	Log CFU ml-1 on Fish	Casein	Bacto Pep.	RHH
		1	8.64 a	8.54 a	9.71 b	9.67 b
	24	2	6.70 a	6.51 a	7.83 b	7.63 b
		3	8.82 a	8.67 a	9.86 b	9.34 b
		1	8.96 a	8.94 a	10.04 b	9.98 b
Soil*	48	2	5.86 a	5.77 a	5.95 a	5.90 a
		3	10.01 a	10.95 a	11.01 b	10.98 b
		1	9.04 a	9.00 a	11.18 b	11.01 b
	72	2	6.00 a	5.95 a	6.48 b	6.34 b
		3	10.09 a	10.00 a	11.18 b	11.06 b
		1	NG a	NG a	2.44 b	2.06 b
	24	2	3.76 a	3.55 a	3.78 a	3.69 a
		3	NG a	NG a	1.90 b	1.21 b
		1	2.80 a	2.66 a	3.55 b	3.44 b
Water**	48	2	3.96 a	3.90 a	4.01 b	4.00 b
		3	NG a	NG a	2.14 b	1.98 b
		1	2.83 a	2.84 a	3.66 b	3.50 b
	72	2	4.03 a	3.96 a	4.96 b	4.85 b
		3	2.04 a	2.00 a	3.10 b	3.08 b
		1	5.91 a	5.81 a	6.66 b	6.48 b
	24	2	8.73 a	8.67 a	9.14 b	9.08 b
		3	8.70 a	8.76 a	9.58 b	9.38 b
		1	5.99 a	5.88 a	6.78 b	6.57 b
Meat***	48	2	9.66 a	9.14 a	10.84 b	10.68 b
		3	9.79 a	9.65 a	10.18 b	10.10 b
		1	6.12 a	6.08 a	6.94 b	6.86 b
	72	2	10.03 a	10.00 a	11.08 b	11.03 b
		3	10.11 a	10.10 a	11.04 b	10.96 b
		1	7.89 a	7.63 a	7.98 a	7.89 a
	24	2	4.67 a	4.18 a	5.12 b	5.04 b
		3	5.16 a	5.10 a	5.88 b	5.72 b
		1	7.93 a	7.88 a	8.25 b	8.09 b
Milk****	48	2	4.89 a	4.72 a	5.92 b	5.83 b
		3	5.78 a	5.56 a	6.34 b	6.14 b
		1	8.17 a	8.16 a	8.98 b	8.89 b
	72	2	5.00 a	4.98 a	6.01 b	5.89 b
		3	6.13 a	6.00 a	6.89 b	6.78 b
Total colony counts			224.2 a	221.54 a	256.39 b	251.13 b

[a,b]Means in row without a common superscript differ ($p < 0.05$). Means of three trials, and each trial were examined in duplicate. Values with the same letter are not significant ($p > 0.05$).

* Sample 1: Greenhouse soil; 2: Garden soil; 3: Forest soil.

** Sample 1: Spring water; 2: Water of Aras River; 3: Tap water.

*** Sample 1: From markets; 2: From butcher; 3: From slaughterhouse.

**** Sample 1: Untreated milk; 2: Pasteurized milk; 3: Fresh milk. NG: No Growth

enumeration media that allow the growth of both injured and noninjured bacteria.

We thought that the high numbers of colony counts obtained from RHH may have originated from only a few species. Therefore, we continued the tests on pure cultures belonging to different physiological groups, and the results are shown in Table 6. In accordance with the results of Table 5, RHH and bacto peptone yielded higher bacterial counts than did fish and casein. The test organisms were not uniform. They contained gram-positive, gram-negative, aerobic, facultative, rod and spherical bacteria. As seen in Table 6, the differences between the results obtained from six of the test organisms (*B. subtilis*, *E. coli*, *L. monocytogenes*, *S. aureus*, *Citrobacter* sp. and *Salmonella* sp.) are not significant ($p < 0.05$) for all of the media. While bacto peptone and RHH were statistically better than casein and fish for the other six test organisms. This shows that RHH does not demonstrate inhibitory effects on the organisms tested to any greater extent than standard peptones.

Table 7 shows the numbers of bacteria on four different media under anaerobic conditions. The total bacterial count (log CFU ml^{-1}) obtained from RHH

Table 6. Comparison of growth of test microorganisms on fish, casein, bacto pep. and RHH

	Log CFU ml^{-1} on			
Bacteria	Fish	Casein	Bacto Peptone	RHH
B. cereus	7.00 a	5.92 b	8.00 c	8.05 c
B. subtilis	9.17 a	9.15 a	9.16 a	9.17 a
L. bulgaricus	8.68 a	8.01 b	8.85 c	8.79 c
E. coli	9.09 a	9.04 a	9.03 a	9.10 a
L. monocytogenes	10.16 a	10.08 a	10.18 a	10.18 a
S. thermophilus	8.44 a	8.06 a	9.12 b	9.10 b
E. aerogenes	9.01 a	9.13 b	9.14 b	9.12 b
P. putida	9.41 a	9.31 a	10.11 b	10.03 b
S. aureus	9.21 a	9.18 a	9.25 a	9.24 a
Citrobacter sp.	9.32 a	9.34 a	9.37 a	9.35 a
Proteus sp.	HG	HG	HG	HG
Corynebacterium sp.	8.17 a	8.13 a	8.68 b	8.72 b
Salmonella sp.	9.07 a	9.00 a	9.04 a	9.03 a
Total colony counts	106.73 a	104.35 a	109.93 b	109.88 b

Values with the same letter are not significant ($p < 0.05$). HG: Heavy Growth (it could not be counted)

Table 7. Comparison of anaerobic bacterial count from food, soil and water samples on four media

Natural	Sample	Log CFU ml^{-1} on			
Samples	No.	Fish	Casein	Bacto P.	RHH
Soil	1	4.80 a	4.66 b	4.84 a	4.90 a
	2	3.68 a	3.50 a	3.74 a	3.77 a
	3	4.79 a	4.62 b	4.94 a	4.90 a
Water	1	1.30 a	0.90 a	1.25 a	1.20 a
	2	2.82 c	2.30 b	2.93 a	2.97 a
	3	NG a	NG a	NG a	NG a
Meat	1	3.80 a	3.38 b	3.90 a	3.94 a
	2	4.81 c	4.49 b	4.86 c	5.01 a
	3	4.89 c	4.62 b	4.96 ac	5.07 a
Milk	1	4.75 c	4.34 b	4.93 a	4.91 a
	2	NG a	NG a	NG a	NG a
	3	2.66 c	NG b	2.82 a	2.70 ac
Total colony counts		38.3 c	32.81 b	39.17 a	39.37 a

Values with the same letter are not significant ($p < 0.05$).
The samples are the same as in Table 4. ND: No Dilution, NG: No Growth

(39.37) was the highest. The differences between the results of RHH and bacto peptone were not significant ($p < 0.05$), but for fish and casein, the results of RHH were statistically significant ($p < 0.05$). Notably, the results indicate that RHH provided better conditions for growth of anaerobic bacteria than did fish and casein (Table 7).

There have been many papers comparing various media for their ability to support the growth of the largest number of bacteria from environmental samples (25-27). In addition to providing the highest bacterial counts, the medium should also be clear or only slightly colored, prevent growth of large spreading colonies, and be free of precipitates so that small colonies may be distingushed (28). RHH also has these properties.

Based on our results, we believe that RHH can be used as a peptone for bacteria growth or, at least for general microbiological purposes. The production of RHH could be one of the steps needed for the complete treatment of the horn. Furthermore, this process could be a very economical method, especially for countries that need to import a source for bacterial peptone. Other fibrous proteins such as feather, nail and hair should be researched for bacterial peptone as well.

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