CONTRIBUTION OF DIFFERENT MOLECULAR WEIGHT FRACTIONS ON BEEF BROTH FLAVOUR

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SUMMARY

Beef broth obtained by mixing one portion of minced meat with two volume of saline solution (0.75 % NaCl) and heated at 85°C for 60 min was ultrafiltered obtaining fractions of > 8 (fraction E), between 8 and 5 (D), 5 and 3 (C) 3 and 1 (B) and < 1 KDa (A). Total, 2 % trichloroacetic acid soluble, 5% phosfotungstic acid soluble nitrogens and free amino acids were determined in all ultrafilered fractions. Sensory evaluation of these fractions was performed by trained panelists, using quantitative descriptive analysis. Panelists detected brothy flavour only in the fraction A. In this fraction, a ~75 % of the nitrogen was peptidic and ~25 % amino acidic. Other fractions showed astringent, bitter and no brothy flavour, but the sensory analysis of several remixings of the above mentioned fractions allowed to deduce that peptides with molecular weight higher than 1 KDa do not contribute to beef broth taste but they enhance its palatability.

INTRODUCTION

It is well established that exists a relationship between molecular size and flavour ability. Fujimaki et al. (1973) observed that the brothy taste of a fish protein hydrolysate was mostly due to substances of molecular weight about 500 daltons. Likewise, Kazeniac (1961) reported that heating a raw chiken dialysate developed a flavour, which was considered chiken-like. This fact may signify that not dialysate substances contribute to the global chicken flavour.

Among substances involved in flavour, there is nitrogen compounds, being well known the role of amino acids (Kato et al., 1989), but peptide role remains uncertain. Since meat is a proteinaceous food, it may be an important source of aminoacidic and peptidic flavour. Nevertheless, only few hydrolitic products of proteins have been described as broth-like. Examples are the so-called "delicious peptide", which was isolated from beef treated with papain (Yamasaki and Maekawa, 1978), and some dipeptides, containing aspartic acid and/or glutamic acid (Arai et al., 1973; Noguchi et al., 1975; Ohyama et al., 1988). On the other hand, although chemical changes in protein due to heating are well documented (Davis and Anderson, 1984), only few studies (Spanier and Edwards, 1987; Spanier et al., 1988, 1990) deal with the release of peptides at cooking conditions and the role of peptides on flavour of cooked meat products. The objective of the present work was to study the role of different molecular weight fractions obtained by ultrafiltration on the beef broth flavour. In addition, it has been studied the nitrogen composition of these fractions in order to determine the possible importance of peptides and free amino acids on the flavour development of beef broth.

MATERIALS AND METHODOS

Preparation of broths

Beef (*M. sternomastoideus*) stored at 0°C for 3 days after slaughtering was used. Meat samples were minced in a grinder (Bizerba FW 70, West Germany) using a plate of 3 mm diameter holes. Conditions to obtain beef broth were studied. They were tested: NaCl concentration (from 0 to 1.5 %), meat / NaCl solution rate (1/1, 1/2, 2/5, 1/3) (w/v), cooking temperature (from 55 to 100°C), heating time (from 15 to 120 min) and the system to separate a clear broth from the solid meat residues after the cooking process (sieving through 0.2 mm diameter sieve, centrifuging at 3000 g for 15 min at 2°C and/or filtering through Whatman No 4 paper).

Tangential ultrafiltration

The beef broth obtained with the former conditions was filtered in a Filtron tangential ultrafiltration system, fitted with membranes with a nominal molecular weight cut-off of 8, 5, 3 and 1 KDa, obtaining fractions of molecular weitghs >8 KDa (fraction E), between 8 and 5 (D), 5 - 3 (C), 3 - 1 (B) and <1 (A). Each fraction was freeze-dried and reconstituted to the original broth volume.

Nitrogen fraction determinations

Aliquots (10 ml) of fractions were mixed with the same volume of 4 % trichloroacetic and 10 % phosphotungstic acid solutions to obtain nonprotein nitrogen (NPN) and 5 % phosphotungstic acid soluble nitrogen (PTN), respectively. Mixtures were left at 4°C for 60 min and the insoluble material was removed by filtration through Whatman No. 4 paper. Total nitrogen (TN), NPN and PTN were determined by the Kjeldhal method in a Büchi digester mod 425 and destiller mod 315.

The nitrogen content of free amino acids (NFAA) content was determined as described by Doi et al. (1981) by comparison of absorbances developed with those of different solutions of leucine. Therefore, NFAA concentration was expressed as leucine equivalents.

Protein nitrogen (PN) was estimated from the difference between the value of TN and NPN (i.e. TN - NPN) and peptidic nitrogen (PPN) was calculated from NPN - NFAA.

Amino acid analysis

Aliquots (10 ml) of fractions obtained by ultrafiltration were mixed with the same volume of 10% sulphosalicylic acid solutions. Mixtures were left at 4°C for 17 h. The insoluble material was removed by filtration through Whatman No 2 paper. An aliquot of 5 % sulphosalicylic acid soluble nitrogen fraction was used for free amino acids (FAA) determination, which was carried out by HPLC according to the method described by Diaz et al. (1993). FAA were identified by comparison of their retention times with those of standards (Sigma) and quantified by area peak measurements normalized against the peak area of L-norleucine (Sigma), which was used as internal standard.

The quantities of "potential" amino acids (PAA) were estimated from the differences between the values of FAA after fraction hydrolysis with 6N HCl for 24 horas at 120°C and FAA without this treatment. The PAA were considered to be amino acids from peptides and/or other amino acid derivatives.

Sensory evaluation

The sensory evaluation was performed by a trained jury composed by 6 females and 5 males.

The flavour of beef broths obtained by different methods was analyzed by a rank order-test to determine the conditions that yielded the best brothy flavour.

Panelists judged the flavour from fractions obtained by tangential ultrafiltration using a quantitative descriptive analysis (sweet, bitter, sour, brothy and astringent perceptions) and the taste intensity using a 4 point scale (+ weak, ++ medium, +++ strong and ++++ very strong). Fractions (reconstituted to the original broth volume and salt concentration) were presented together with a beef broth without fractionating at 40-45°C.

The relative strength of the brothy beef flavour of different remixtures was analyzed by a rank order-test using a 5-point scale (1, minimum broth flavour intensity and 5, maximum). Fraction A (the sole with beef broth flavour, see Reults and Discussion) was remixed with other fractions (i.e. A+B, A+B+C, A+B+C+D, and A+B+C+D+E) and at different ratios of fraction A and the complex B+C+D+E [i.e. A/(B+C+D+E): 1/0, 1/1, 1/2, 1/3, and 1/4, v/v]. In both cases, five samples were presented simultaneously.

RESULTS AND DISCUSSION

As the best beef broth flavour was attained by mixing one part of minced meat with two parts of 0.75 % NaCl solution heated at 85°C for 60 min and eventually filtered through Whatman No 4 paper, only the tangential ultrafiltration fractions corresponding to this broth were considered for nitrogen fractionation and sensory evaluation. At this respect, the impressions perceived by panelists were sour (fractions B, C and D), astringent (fractions B and D), bitter (fraction C), off-flavours (fraction E), and brothy flavour solely in fraction A, which was composed by substances of molecular weights lower than 1 KDa. These results agree the

findings of Kazeniac (1961), Fujimaki et al. (1973) and Warendorf et al. (1992) and suggest that low molecular weight compounds are important to meat flavour development.

The influence of no brothy flavour fractions on the global beef flavour was studied remixing them with fraction A. The best qualified was the remixing constituted by all fractions (A+B+C+D+E), indicating that all fractions played a role in the golbal flavour development. The complex A+B+C+D and fraction A alone were second in the rank. This seems to indicate that fractions B and C have not very important substances for the beef flavour. This hypothesis was confirmed mixing the fraction A with each fraction separately, because the most intense beef broth flavour was perceived in A+D and A+E and A alone, while A+B and A+C were much worse qualified. These results may be compared with those reported by Warendorf et al. (1992), who observed that the combination of low molecular weight substances (free glutamic acid and 5'-IMP) with some sour and salty components were determinative of the typical flavour of bouillon. These authors also showed that the contribution of high molecular components (i.e. gelatin) to the overall impression was marked. Hsieh et al. (1980) reported that gelatin enhanced mouthfeeling and bouillon-like notes, and suppressed undesirable sulphurous notes.

The importance of the no brothy flavour fractions was corroborated with other trial. Fraction A was mixed with different ratios of the complex B+C+D+E (1/0, 1/1, 1/2, 1/3, and 1/4, v/v) and samples were judged by panelists. The A/(B+C+D+E) rate with more intense beef flavour was 1/3 (v/v) and therefore, it may be concluded that substances with molecular weights higher than 1 KDa contribute to the brothy beef palatability, although beef flavour was not appreciated in such fractions. The fact that rate 1/4 had less broth flavour may be explained because of the tasty substances masked the charasteristic beef broth flavour of lower than 1 KDa molecules of fraction A.

Nitrogen fraction determinations

The highest nitrogen content was found in fraction A (almost 0.5 g/100ml of beef broth) and was contained in peptides (~75 %) and amino acids (~25 %). These results (Fig. 1) together with results of the sensory evaluation studies, suggest that FAA and small peptides (molecular weight < 1 KDa; most of all < 600 Da because were PTN) may play a remarkable role in the flavour of beef broth. At this respect, Nishimura et al. (1988) observed that the increase of FAA and oligopeptides contributed to the brothy taste improvement of meat during storage. Spanier et al. (1988, 1990) reported that both, peptides and FAA, may act as flavour principles and/or precursors. The brothy flavour must not be attributted only to these compounds, because there are other substances (e.g. ATP metabolites) in this fraction, which importance in the flavour development is well known (Macy et al., 1964; Koehler and Jacobson, 1967).

As expected, practically all nitrogen of fractions B, C and D (0.05, 0.010 and 0. 014 g/100 ml of beef broth, respectively) was included in peptides. The nitrogen content of these fractions was lower than that of fractions A (see above) and E (0.09 g of 100 ml of broth). In the latter, approximately the 35 % of nitrogen was protein and the 65 % peptidic.

According to the results of the sensory analysis, the role of peptides contained in fractions B and C in the brothy flavour was not worthly. However, these peptides might influence the flavour with astringent and/or sour perceptions. The fraction D also was qualified as astringent and sour, and the E as untaste, but they seem to acquire importance role in the palatability of broths because their remixings with fraction A enhance the brothy flavour.

Free amino acids (FAA) and those obtained after acid hydrolysis (PAA)

Amino acid analysis of each fraction was carried out with and without acidic hydrolysis to know the FAA and PAA content (Table 1) in fractions obtained by tangential ultrafiltration. The fraction A contained almost all of FAA (more than 98.5 %) of the broth. The dominant FAA were Asp, Glu, Gln, Gly, His, Tau, Ala, Cys and M-His. Other authors (Nishimura et al., 1988; Jarboe and Mabrouk, 1974., Cambero et al., 1992) have found practically the same amino acids as major ones in beef broths.

Cambero et al. (1992) suggested that the increase in FAA contributed to the improvement of brothy flavour. These authors found no significant correlation between any free amino acid concentration and brothy taste intensity, suggesting that the combination of all FAA determined the complex brothy taste sensation.

It is also important that the PAA of fraction A was 62 % of total PAA in broth. The most abundant amino acids in PAA of fraction A were Glu, b-Ala, His, Tau and M-His. If it is taken into account the taste of peptides reported by several authors (Arai et al., 1973; Noguchi et al., 1975; Yamasaki and Maekawa, 1978 and Ohyama et al., 1988), it is likely that precursor peptides of the PAA of fraction A play a role in the broth

flavour. Although chemical changes in protein during cooking are well documend (Davis and Anderson, 1984), few studies deal with the role of peptides in determining the flavour of cooked meat products. With respect to this, Cambero et al. (1992) reported that a wide mixture of FAA, peptides of low molecular weight (< 300 daltons) and 5'-IMP played an important role in the flavour intensity of beef broth.

It is obvious that the typical beef flavour is developed during cooking. However, some meat native substances, such as the peptides anserine and carnosine, both described as slighty sweet and bitter (Suyama and Shimizu, 1982), are suggested to contribute to the flavour of beef broth, possibly in combination with glutamic acid (Ziegler, 1982)

Remainder PAA were distributed in fraction B (4 % of total PAA in broth), C (1,5 %), D (2 %) and E (30 %). The most abuntant amino acids were M-His, (fractions B, C and D) and Asp, Gly, Thr, Ala, M-His and Lys (fraction E).

Conclusion

As a general conclusion, it may be said that peptides with molecular weight higher than 1 KDa do not contribute to beef broth taste but enhance its palatability and, therefore, influence the flavour.

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