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COMPARISON OF PREFERENCES FOR SALTY AND UMAMI FLAVOURS BETWEEN TWO ETHNIC GROUPS OF DIFFERENT DIETARY HABITS

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SUMMARY

Flavour potentiators such as MSG, IMP and GMP not only potentiate the flavours of foods at subthreshold levels and favourably alter preferences for foods, but also have their own distinctive palatable taste, umami, at higher than threshold levels. A sensory study was carried out to investigate whether any differences exist in preference for salty and umami flavours between two ethnic groups of different dietary habits. Preference for the flavour of natural and pure flavour potentiators was also investigated. Malaysian assessors significantly preferred the umami flavour of the flavour potentiators in comparison with the Scottish assessors who significantly preferred the salty flavour. The Malays showed significant preference for the flavour of pure flavour potentiators to natural ones whereas the Scots showed no significant difference in preference for them.

1. INTRODUCTION

A flavour potentiator is a compound which, when used in small quantities, produces no sensory response of its own, but modifies through intensification or some form of masking the sensory properties of the food system to which it has been incorporated (1). Flavour potentiators can favourably alter the preference for foods by enhancing desirable flavours and/or suppressing undesirable off-flavours (2,3). Flavour potentiators such as monosodium glutamate (MSG), inosine 5'monophosphate (IMP) and guanosine 5'monophosphate (GMP) not only potentiate the flavour of food at subthreshold levels but also have their own distinctive, delicious, savoury, palatable tastes at suprathreshold levels (4). These tastes extend the taste realm beyond the four basic tastes (5) and are referred to as umami by the Japanese, who have used natural sources of flavour potentiators in their cooking since ancient times (4). Umami is an integral part of cuisines world-wide, from the dashi and tan broths of Japan and China to the bouillon of Europe and fish sauces of South East Asia (6).

Flavour potentiators can be obtained from a wide range of natural sources, including yeast extract. It is the natural presence of nucleotides, a significant amount of glutamate and other amino acids which give yeast extracts their flavour potentiating properties (7).

Nutritional studies (8) showed that rats on a moderate or high protein diet preferred umami flavours, with consumption of the umami solution having a reducing effect on NaCl intake. No such preference for the umami solution was shown in rats fed a low protein diet. There has been speculation that a similar effect might be observed in man.

The aim of the work described here was to investigate whether any differences exist in preference for umami or salty flavours between two ethnic groups of different dietary habits, and to compare liking of the two groups for natural and pure flavour potentiators.

2. EXPERIMENTAL

2.1 Materials

Hedonic functions of taste substances cannot be predicted from the degree of liking in a model system or simple aqueous solution, but must be evaluated in foods or flavoured solutions compatible with added tastes (9). The umami and salty taste substances were therefore incorporated into a soup food system containing vegetables. Yeast extracts with varying NaCl and flavour potentiator levels were used to compare hedonic scores for soups containing high and low concentrations of NaCl and flavour potentiators, and pure flavour potentiators were used to compare these with the natural materials contained in the yeast extract.

Bouillon base (P.1970, instant), Paselli maltodextrin (MD20), vegetable (2M-44444), chicken (Y342.04.370) and beef (Y42.08.128) flavours, yeast extracts YEP LOC (38% NaCl, 5% MSG), YEP 990C (10% NaCl, 3% IMP, 3% GMP, 5% MSG) and YEP 770C (38% NaCl, 2% IMP, 5% MSG), MSG, IMP and GMP were supplied by Quest International Flavour Centre (Bromborough Port, Wirral, Merseyside, L62 4SC, England). Bouillon base (37.5 g), maltodextrin (7.5 g) and vegetable, chicken or beef flavour (7.5 g) were mixed with 2.5 litres of water and 0.533 kg each of carrots, onions and potatoes. Flavour potentiators were added, each soup mixture was simmered for 30 minutes and then blended for approximately 2 minutes using

a Kenwood Quisine Food Processor with large cutting blades at speed setting 2, as follows:

Soup 1 - Yeast extract YEP LOC (5 g) providing a low flavour potentiator concentration (0.059 g kg^{-1} MSG) and a high NaCl concentration (0.45 g kg^{-1}).

Soup 2 - Yeast extract YEP 990C (5 g) providing a high flavour potentiator concentration (0.059 g kg^{-1} MSG, 0.036 g kg^{-1} IMP and 0.036 g kg^{-1} GMP) and a low NaCl concentration (0.12 g kg^{-1}).

Soup 3 - Yeast extract YEP 770C (5 g) providing a high flavour potentiator concentration (0.059 g kg^{-1} MSG, 0.024 g kg^{-1} IMP) and a high NaCl concentration (0.45 g kg^{-1}).

Soup 4 - No additions to the basic ingredients.

Soup 5 - Flavour potentiators (0.25 g MSG, 0.15 g IMP, 0.15 g GMP) providing a high flavour potentiator concentration (0.059 g kg^{-1} MSG, 0.036 g kg^{-1} IMP and 0.036 g kg^{-1} GMP), and 0.5 g NaCl providing a low concentration (0.12 g kg^{-1}).

2.2 Assessors

Scottish and Malaysian ethnic groups were chosen for this study, because they have substantially different dietary habits and a sufficient number of each group where readily available and willing to take part. Scottish people on the whole have a generally higher protein diet than Malaysians, who tend to eat a high carbohydrate diet, having rice with all main meals including breakfast. Additionally many Malaysian residents of the UK follow a vegetarian diet, further limiting protein consumption. Twenty-nine Scottish students took part in the experiment, 17 female and 12 male, and twenty-five Malaysians, 7 female and 18 male. The ages of both groups ranged from 20 - 24 years.

2.3 Sensory Analysis

Individual assessors were given 30 ml of each coded soup formulation at 60 - 66 °C in white foamed polystyrene cups (Insulpak Ltd., Tower Close, Huntingdon, England) and were asked to taste each sample and indicate their degree of liking using a nine-point hedonic scale (10), ranging from 'Dislike extremely' (1) to 'Like extremely' (9). Individual tasting booths were used and data recorded using the PSA-System (Oliemans Punter & Partners, Utrecht, The Netherlands). The Malaysian students were only asked to taste soup samples made using vegetable flavour, whereas the Scottish students tasted all the soups. Data were analysed by one way analysis of variance using MINITAB.

3. RESULTS AND DISCUSSION

Analyses of variance of the hedonic scores are shown in Tables 1 and 2. Comparison of the results for soups 1 and 2 showed that the Malaysians preferred the umami flavour of the flavour potentiators to the salty flavour, when added to vegetable soup. In contrast, the Scottish assessors showed no significant preference, while the high salt-high flavour potentiator and low salt-low flavour potentiator soups were moderately liked and disliked, respectively, by both groups. The Malaysian assessors preferred the flavour of the pure flavour potentiators to the natural ones, whereas the Scottish assessors showed no significant difference in preference.

TABLE 1

Mean hedonic scores for Malaysian and Scottish assessors for vegetable flavour soup formulations.

Sample	Formulation	Malays[1]	Scots[2]	F^{1}_{52}
1	High NaCl Low FP	3.96	5.52[b]	9.95++
2	Low NaCl High FP	6.76[a]	5.21[b]	11.28++
3	High NaCl High FP	6.44[a]	6.69	0.49
4	Low NaCl Low FP	4.68	4.45	0.16
5	Low NaCl High Pure FP	7.48	5.31[b]	22.75+++
df		4,120	4,140	
F		17.81+++	6.34+++	

[a,b]means of samples which did not receive significantly different hedonic scores.
+, ++, +++ indicates significance at the 5%, 1% and 0.1% levels, respectively.
FP flavour potentiator.
[1]LSD = 0.64.
[2]LSD = 0.58.

The Scottish assessors showed no significant preference for salty and umami flavours in the vegetable and chicken flavoured soups, whereas they showed a significant preference for the salty flavour in the beef flavoured soup. For the vegetable and beef flavoured soups there was no difference in preference for the natural or pure flavour potentiators. Conversely there was a marked increase in preference for the chicken flavoured soup

containing the pure flavour potentiators. This suggested that the chicken flavour was best enhanced by the pure flavour potentiators.

TABLE 2

Mean hedonic scores for Scottish assessors for chicken and beef flavour soup formulations.

Sample	Formulation	Chicken[1]	Beef[2]
1	High NaCl Low FP	3.24[a]	6.24[bc]
2	Low NaCl High FP	3.03[a]	5.59[d]
3	High NaCl High FP	5.10	6.69[b]
4	Low NaCl Low FP	3.14[a]	4.38
5	Low NaCl High Pure FP	5.89	5.72[cd]
F^4_{140}		13.53+++	6.81+++

a,b,c,d means of samples which did not receive significantly different hedonic scores.
+, ++, +++ indicates significance at the 5%, 1% and 0.1% levels, respectively.
FP flavour potentiator.
[1]LSD = 0.62.
[2]LSD = 0.60.

The results of this study do not accord with previous reports (8) that rats on a moderate or high protein diet preferred umami flavours, whereas this study has shown that Scottish assessors, having a generally higher protein diet than Malaysians, have preferred salty flavours. This probably rather reflects a learned preference for the generally high salt content of the Scottish diet.

4. CONCLUSIONS

A group of Malaysian students significantly preferred soups containing high levels of flavour potentiators and low levels of salt, in contrast to a group of Scottish assessors who preferred a soup formulated with high levels of flavour potentiators and high levels of salt. The Malaysian assessors preferred pure flavour potentiators to natural ones in yeast extracts, whereas the Scottish assessors showed no significant preference.

REFERENCES

1 A. Kuninaka, in: M.S. Peterson and A.H. Johnston (Eds), Encyclopedia of Food Science, Avi Publishing, Westport, Connecticut, 1978, pp. 279-284.

2 K.N. Hall, in: M.S. Peterson and A.H. Johnston (Eds), Encyclopedia of Food Science, Avi Publishing, Westport, Connecticut, 1978, pp. 559-561.

3 S. Yamaguchi, in: J.C. Boudreau (Ed.), Food Taste Chemistry, American Chemical Society, Washington, D.C., 1979, pp. 33-51.

4 A. Kuninaka, in: R. Teranishi, R.A. Flath and H. Sugisawa (Eds), Flavour Research Recent Advances, Marcel Dekker, New York, 1981, pp. 305-353.

5 R.H. Cagan, K. Tori and M.R. Kare, in: L.J. Filer, S. Garrantini, M.R. Kare, W.A. Reynolds and R.J. Wurtzman (Eds), Glutamic Acid: Advances in Biochemistry and Physiology, Raven Press, New York, 1979, pp. 1-9.

6 Y. Kawamura, Food Technology International Europe, (1990) 151-155.

7 G. Schmidt, Food Flavourings Ingredients Packaging and Processing, 9(5) (1987) 25-30.

8 S. Kimura, Y. Yokomukai and M. Komai, in: Y. Kawamura and M. Kare (Eds), Umami: A Basic Taste: Physiology, Biochemistry, Nutrition, Food Science, Marcel Dekker, New York, 1987, pp. 611-634.

9 S. Yamaguchi, in: Y. Kawamura and M. Kare (Eds), Umami: A Basic Taste: Physiology, Biochemistry, Nutrition, Food Science, Marcel Dekker, New York, 1987, pp. 41-73.

10 E. Larmond, Laboratory Methods for Sensory Evaluation of Food, Publication 1637, Research Branch Canada Department of Agriculture, Ottawa, 1977.

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ENZYMATIC HYDRATION OF (4R)-(+)-LIMONENE TO (4R)-(+)-α-TERPINEOL

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SUMMARY

The enzyme-catalyzed hydration of the citrus by-product, limonene, to the important flavor and aroma chemical, α-terpineol, was investigated. Particulate-associated α-terpineol dehydratase was recovered from Pseudomonas gladioli, solubilized, and partially purified using detergent extraction and gel filtration.

Activity of α-terpineol dehydratase was low in non-aqueous solvents. α-Terpineol dehydratase was characterized in buffers containing 0.1% (w/v) Triton X-100. In 10 mM MES, 10 mM BIS-TRIS PROPANE buffer, the pH optimum was 5.5 and the stability optimum was pH 8.0. The temperature optimum at pH 7.0 was 25°C in 10 mM HEPES buffer. Using temperature-activity data for 10-25°C, E_a and Q_{10} of α-terpineol dehydratase were determined to be 21.6 ± 2.9 $kJ \cdot mol^{-1}$ and 1.37 ± 0.07, respectively. Activity was inhibited by Triton X-100. The effects were an increase in apparent K_m and decrease in apparent V_{max}. Average apparent K_m of α-terpineol dehydratase was 2.18 ± 0.19 mM in 10 mM HEPES buffer, pH 7.0 containing 0.1% (w/v) Triton X-100.

α-Terpineol dehydratase stereospecifically catalyzed the hydration of (4R)-(+)-limonene to (4R)-(+)-α-terpineol or (4S)-(-)-limonene to (4S)-(-)-α-terpineol. The enzyme was also stereoselective, since the rate of hydration of (4R)-(+)-limonene was approximately ten times faster than the rate of hydration of (4S)-(-)-limonene.

INTRODUCTION

In recent years, there has been increasing consumer preference for food products containing "natural" flavors over those containing artificial (synthetic) flavors. This preference has led to an increased demand for natural flavor and aroma chemicals (1, 2). Biotechnological processes (processes involving the use of microorganisms, plant cell cultures, or enzymes) offer many possibilities for the production of natural flavor and aroma chemicals.

In addition to their use for production of flavor and aroma chemicals, biotechnological processes provide simple systems for studying the biosynthetic pathways involved in the formation of

many important flavors and aromas. These processes have several advantages over alternate physical or chemical processes, the most important advantage being their ability to catalyze specific reactions which may not be possible with less selective processing methods. Another advantage is that biotechnological processes generally can be accomplished under mild conditions (i.e., ambient temperature, atmospheric pressure, and pH values near 7). This automatically results in lower energy consumption and decreased substrate and product damage.

The suitability of a process for the production of flavor and aroma chemicals depends on the market demand (total usage), commercial value (price) of the product and the technological state of the process. The use of a biotechnological process for the production of α-terpineol from limonene has economic potential. α-Terpineol is an important flavor and aroma chemical. Its annual consumption for flavor purposes has been estimated at over 13,000 kg, which places it among the top 30 most commonly used flavors (2).

Citrus essential oils are unusual because they contain pure (4R)-(+)-limonene at concentrations approaching 95% for orange and grapefruit oils (3). In addition to its high chemical and enantiomeric purity, limonene derived from citrus is in abundant supply, with 8.7 million kilograms being recovered during the 1988-89 Florida processing season (4). Limonene from citrus is also relatively inexpensive, the price being approximately 25% lower than the price of α-terpineol (5).

Presently, α-terpineol is commercially available only in racemic form, which is primarily recovered as a by-product from the pulp and paper industry. The properties of flavor and aroma compounds often depend on their enantiomeric purity. For example, (4R)-(-)-carvone has properties characteristic of spearmint oil; whereas, the properties of (4S)-(+)-carvone resemble caraway oil. Biotechnological processes are usually stereospecific; therefore it may be possible to produce pure (4R)-(+)-α-terpineol from (4R)-(+)-limonene by using this type of process.

Kraidman et al. (6) have shown that Cladosporium spp. (T_{12}) converts (4R)-(+)-limonene into pure (4R)-(+)-α-terpineol. Similarly, Penicillium digitatum (DSM 62840) was shown to produce (4R)-(+)-α-terpineol from either (4R)-(+)-limonene or racemic limonene (7). This could be explained by the exclusive hydration of (4R)-(+)-limonene. Cadwallader et al. (8) demonstrated that

Pseudomonas gladioli produces pure (4R)-(+)-α-terpineol from (4R)-(+)-limonene; however, the yield of α-terpineol was low due to the utilization of limonene by the bacterium for metabolic purposes. An enzyme, α-terpineol dehydratase (α-TD), has been isolated from P. gladioli, which catalyzes the hydration of limonene to α-terpineol (9). These researchers found that the enzyme was particulate-associated and could be extracted with 2.0% (w/v) Triton X-100 and 0.5 M sodium trichloroacetate. α-TD solubility was maintained during gel filtration by inclusion of 1.0% (w/v) Triton X-100. Suitability of an enzyme for the production of flavor and aroma chemicals depends on its physical and kinetic properties. It is necessary to isolate and characterize enzymes to relate these properties to important parameters affecting reactions.

Stability should be high under the process conditions which may include extremes of pH and temperature, as well as the presence of solvents or other protein denaturants. Enzymes should also be highly specific for the reaction of interest so that side products are minimized. When it is desirable to produce pure enantiomers from enantiomerically pure substrates, then the stereospecificity of the enzyme is important. Stereoselectivity is important when inexpensive racemic compounds are used as substrates instead of more expensive pure enantiomers.

The above discussion particularly relates to the importance of this study, which has the major objective of characterizing some of the physical and kinetic properties of α-TD.

ENZYME PROPERTIES

Determination of the kinetic properties of α-terpineol dehydratase was complicated because limonene was insoluble in the enzyme medium. The potential of using water-miscible organic solvents to dissolve limonene in the enzyme reaction medium was examined. Formamide, dimethylformamide, ethanol, 2-propanol, and acetonitrile inactivated α-TD at the concentrations required to solubilize limonene (25-50%). Since water is a co-substrate of α-TD, it was originally thought that the observed decrease in activity was due to decreased water activity and subsequent shift in reaction equilibrium; however, α-TD did not catalyze the reverse reaction when glycerol was used to lower the water activity.

The effects of various nonpolar solvents on α-TD activity were similar to the effects observed with water-miscible solvents. Enzyme activity was assayed in 1:1 mixtures consisting of nonpolar