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ASSOCIATION OF FOOD SCIENTISTS & TECHNOLOGISTS, INDIA

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(INDIA)

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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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Research Notes

REVIEW

Physiology of Perception of Odour and Taste

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General principles of the morphology and physiology of smell : nd taste perception are presented and discussed for readers not particularly specialized in biology; selected details about curren research are included.

Gustation and olfaction are considered together as "chemical senses". This means, from the usual point of view, that the primary process of excitation of the respective sensory cells is a chemical interaction between certain membrane sites of these cells and the stimulating odorous or tasting molecules.

The difference between taste organs and smell organs, which is fairly evident in man and in all mammals, is not as well manifested in insects and fish. One could say, however, that for all animals except for those living in the water, taste stimuli are characterized as solid, liquid or gaseous substances soluble in water and to a certain degree in lipids. Odorous stimuli are represented by molecules in the gas phase, and they should be water— and/or lipid soluble and adsorbable, and also volatile. More general and more practical is the difference in threshold intensities: olfactorous stimulation usually requires lower average concentrations than taste awareness — 10^2 to 10^{14} molecules per ml air (Table 1) and 10^{13} to 10^{20} molecules per ml solution, respectively (Table 2)[1,2,5,6,10]. Dogs are aware of the smell of 10^3 molecules of diacetyl per ml air, and the silkmoth (*Bombyx mori*) of 10^2 molecules of "bombycol" per ml air, which will be discussed later. Man is able to differentiate many thousands of different odours. The human sense of smell is, however, far less sensitive in comparison to other living beings.

Chemoreception of Olfaction

Our knowledge of the anatomy and physiology as well as the psychology of olfaction could well be increased by research efforts on this topic. As is well known, the human nose is divided by a septum into a left and right cavity into which the nasal conchae (or turbinate bones) project from the lateral wall, forming bony hills covered with mucus. As shown in Fig 1 the inspiratory air stream goes through the nasal ducts formed by the conchae (or turbinates) and is in part reversed by turbulences and directed to the highest dome of the nose. Although the entire surface of this nasal cavity is covered with respiratory mucosa cells forming the "respiratory region", there is just one little area of about 2.5 cm^2 in the top just above the upper concha which is called the "olfactory region", and it alone bears olfactory sensory cells (Fig 1). There are about 20 to 50 million olfactory receptor cells in this region within an area, including both nasal cavities[1,51], of about 5 cm^2. In the schematic diagram in Fig. 2 three olfactory receptor cells of a macrosmatic mammal, e.g., a dog, are shown. In the last years new evidence has been gathered, which shows that olfactory cells definitely regenerate, whereas a few years ago they were considered to be permanent and stable nonregenerating elements of the nervous system[16,17,34]. In the center of Fig. 2 one basal cell is just changing into a mature receptor cell; its apical dendritic process has not yet completely reached the surface and the cilia have not yet emerged, which appear to be important for the first contact with the olfactory stimuli. The yellowish colour of the olfactory region in the middle of the red respiratory region is caused by the many lipids included in the sustentacular cells and the surface is covered with a layer of mucus which must be penetrated by the stimulating molecules. This mucous however is produced only in part by the sustentacular cells in the olfactory region; the greatest part is excreted by mucous cells in the respiratory region.

Not all the olfactory information, however, is processed by olfactory cells; there is also a strong tactile component of odours via the trigeminal nerve—the V^{th} cranial nerve. Its free nerve endings, which are not shown in Fig. 2, are stimulated very easily by certain molecules, such as ammonia, and also by higher concentrations of odorous molecules[19]. This is the explanation for people with torn olfactory nerves (as the result

Paper presented at the Central Food Technological Research Institue, Mysore, India on 6th Nov. 1974.

TABLE 1. OLFACTORY THRESHOLDS IN VERTEBRATES AND INSECTS[24]

Number of molecules per cm^3 in log 10 units	Man	Dog	Eel	Bee	Bee single cell	Locusta single cell	Bombyx single cell ♂	Bombyx single cell ♀	Bombyx ♂	Bombyx ♀	Necrophorus
Trinitrobutyltoluene	*7_{67}										
Coproic acid	11_{67}	4_{50}		11_{66}	12	9					
Butyric acid	10_{67}	*4_{50}		11_{66}							
∝ I-onon	8_{54}	$4_{52\cdot53}$	6_{73}	10_{66}							
Diacetyl		*$3_{52\cdot53}$									
Phenyl ethyl alcohol			*3_{73}								
Phenyl propyl alcohol	10_{66}			*9_{66}							
Queen substance	∞				*8						
Hexenal						*8					
Bombykol	∞						*7 ∞		*2 ∞		
Skaiote	9_{67}										14
CO_2					16						

Maximum concentration at 1 atm. = 10^{19} particles per cm^3
*The lowest threshold value of each organism is indicated by asterisk mark..
The superscripts and subscripts refer to the number under References

of fractures at the base of the skull) who may still have a residual ability to smell. The majority of nerve fibers in this region, three of which are shown on the bottom of Fig. 2, are branches of the first cranial nerve—the olfactory nerve called "Fila olfactoria"—which extends to the olfactory bulb situated on the top of the cribriform plate of the ethmoid bone. The olfactory bulb is a part of the brain involved in processing olfactory information, called the rhinencephalon, which belongs to the limbic system[32,35,37]. During the last few years neurophysiologists as well as psychologists have found the limbic system to be highly involved in the production of emotions and affections[78].

TABLE 2. GUSTATORY THRESHOLDS IN MAN AS INDICATED BY THE ABSOLUTE NUMBER OF MOLECULES PER ML TASTE SOLUTION FOR STIMULI REPRESENTATIVE FOR THE FOUR TASTE QUALITIES.[10]

Sweet		**Bitter**	
Sacharose	6.2×10^{18}	Caffeine	9.4×10^{16}
Lactose	3.9×10^{19}	Quinine sulfate	6.5×10^{13}
Sacharine	1.6×10^{15}	Strychnine Hydrochloride	4.9×10^{14}
Sour		***Salty**	
Acetic acid	6.0×10^{18}	NaCl	7.2×10^{18}
HCl	4.3×10^{17}	KCl	4.0×10^{19}
Tartaric acid	9.3×10^{16}		

*The only substance tasting purely salty is NaCl; KCl in this concentration has a sweetish taste. More concentrated KCl will have a bitter component in addition to salty. This holds for most of the other salts.

One of the main difficulties in studying olfaction by physiological means is the delivery of an accurately controlled stimulus. A large number of devices have been designed over the years to deliver a jet of air with a constant velocity and containing a known quantity of the odorous substance under study. Fig. 3 shows the principle of an olfactometer used by Allison and Goff[13] similar to one constructed by Finkenzeller[31] to study changes in EEG in man after odorous stimulation. Of particular interest are what we call

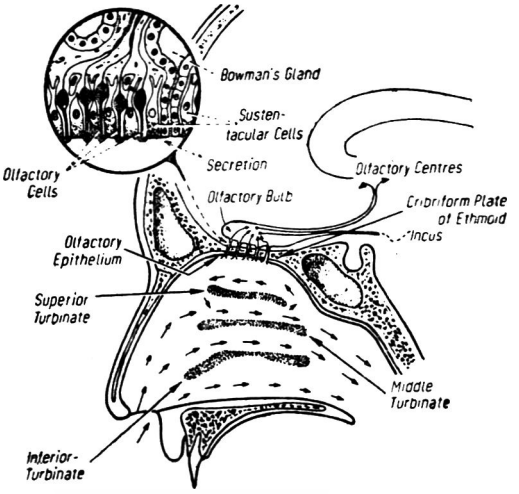

Fig. 1. Sagittal section of human nose showing the nasal fossae after removal of the nasal septum. Small arrows indicate the direction of air flow during normal respiration. Turbulences are generated at the end of the superior turbinate to direct the air flow upwards the olfactory region (see enlarged view top left; compare Fig. 2). The turbulences can be increased by "sniffing". Adapted from C. Eyzaguirre, *Physiology of the Nervous System*, Year Book Medical Publ Chicago: 1964.

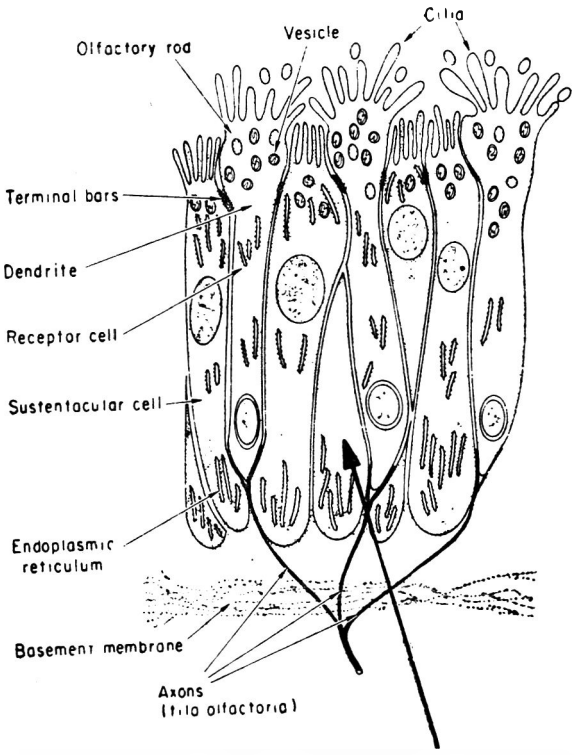

Fig. 2. Schematic drawing of the olfactory mucosa based on electron-microscopic studies of A.J.D. de Lorenzo. The various cell types and their relationships are shown; the large arrow indicates a basal cell just maturing into an olfactory (or sustentacular) cell. (Ref. 11).

"olfactory evoked cortical potentials". Some of these olfactory potentials are shown in Fig. 4, which demonstrates the great difficulty in attaining quantitative differences in potentials related to specific stimuli, during cortical processing of olfaction. Certainly this problem might be more easily solved in animals with easy access to the brain structures. In classical experiments of Fox, *et al*[32] the olfactory bulb was electrically stimulated at certain places and the evoked electrical activity recorded by electrodes placed directly on the surface of adjacent nervous structures of the lateral olfactory tract, the olfactory tubercle, the prepyriform cortex and the pyriform lobe, which are situated behind the two adjacent olfactory bulbs and lateral to the optic chiasm[35,74]. This is certainly easier than recording the EEG from the intact skull of man, in which case a sophisticated evaluation for averaging or cross-correlating of the EEG is necessary to improve the signal-noise ratio (Fig. 14).

This is an objective way to ascertain whether or not an animal or a man is able to smell (compare EAG and EOG later). There is, however, no information yet from these potentials concerning the quality of odours (Fig. 4). We have to confess that in contrast to most other sensory modalities we have not really been able to precisely identify different olfactory qualities. Some early investigators have proposed as many as 18 or 24

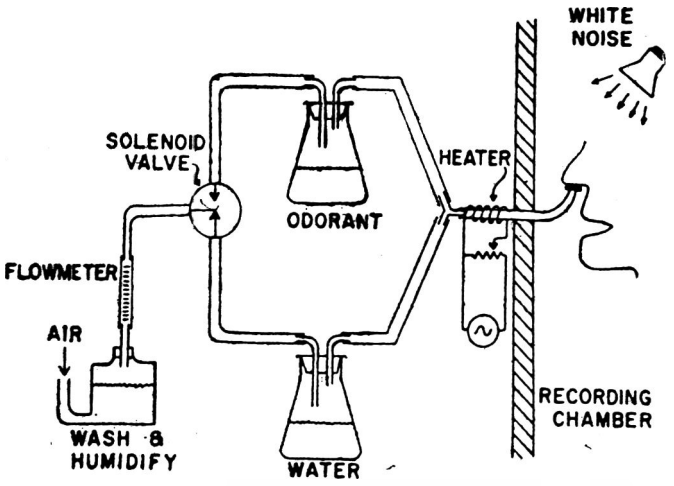

Fig. 3. Apparatus for presenting odorant pulses to a subject. The EEG is recorded and computed similar to the diagram shown in Fig. 13 for taste. (Ref. 13).

primary olfactory qualities, while others have reduced the number to a very few[1,5,6,10,23,29,38,39,48,61,62,80]. Based on theoretical considerations, namely the stereochemical hypothesis of odour by Amoore, many research workers assume seven primary odour qualities[14,15,75,77]. Fig. 5 shows not only the names of these primary odours but also the theoretical background: Amoore assumed that specific and complex receptor sites situated at the dendritic membranes of the olfactory cells were more

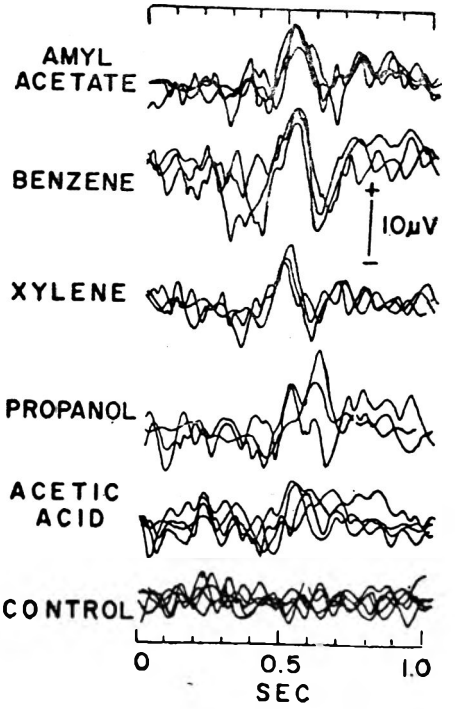

Fig. 4. Olfactory evoked cortical potentials elicited after presenting puffs of the substances listed on the left to a subject. Experimental device shown in Fig. 3. There were no differences found, in the potentials, which could be correlated to the differences of the stimuli. (Ref. 13).

complicated for five of the seven primary odours, as shown in the diagram. The sixth odour, pungent, is characterized by a simple negative site attracting protons, such as those of formic acid, which is a very good example of a pungent or stinging smell. Putrid odours, such as hydrogen sulfide, are formed at a positive membrane site. The rest of the stereochemical relations can be well understood by Fig. 5, which shows that characteristic odor substances are able to elicit a specific odourous sensation if they fit into the form of the receptor sites. Amoore is quite convinced that a large number of the predictions from this stereochemical hypothesis of odour are in good agreement with the experimental results of different laboratories, which would appear to verify his assumptions.

We must mention, however, that despite the fact that the seven qualities of odour characterized by Amoore are widely accepted as the correct number of subdivisions, not all physiologists concerned with smell and chemoreception agree with Amoore's stereochemical hypothesis. R. H. Wright in Vancouver, Canada, strongly advocates the "vibrational theory of odour", which basically assumes that the molecular quality which we perceive as odour is due to certain vibrational movements of odourous molecules[75,76,77,78]. To quote Wright: "Such things as volatility, water–or lipid-solubility, adsorbability, and so on may affect the strength of an odour, but its essential quality will depend on what vibrational frequencies are present and in what relative proportions in any given assemblage of molecules. The shape or profile of a particular molecule may however, help to determine which of the molecule's vibrational modes is most effective in triggering a sensation". Wright supports his theoretical considerations by comparing Raman and far-infrared spectra of complex organic compounds with their odourous properties. It might also be mentioned in this context, that practically none of the inorganic molecules has really odourous properties.

To touch upon another point, it is well known that great difficulties arise when we try to control the threshold of an odoruous substance if the olfactometer, the machine delivering the odourous stimuli, is not perfectly designed. So threshold values gained by different devices can hardly be compared with each other (Table 1). The clearest devices providing means for doing basic research in olfaction are the "electroantennogramms (EAGs)", which study the antennae of bugs or butterflies; these are comparable to the "Electro-olfactogramm (EOG)" developed for vertebrates by Ottoson[55]. By means of the electroantennogramm Kaissling and Priesner in Germany proved that receptor cells of the silk spinning *Bombyx mori* are stimulated by just one molecule of an odourous substance[41] (Fig. 6*a* and 6*b*). This is particularly true for those substances which play an important role in the reproductive cycle of these butterflies. Certain sexual attractants, "pheromones" such as the famous "bombycol" have been extensively investigated[23,24,40,41,63]. Schneider and his colleagues[24,41,63] found that there are about 32,000 olfactory receptor cells on one antenna of a male *Bombyx mori*, of which 25,000 (about 80 per cent) give specific responses to bombycol secreted by the females[41]. Thus when a male butterfly is fixed in place and subjected to female

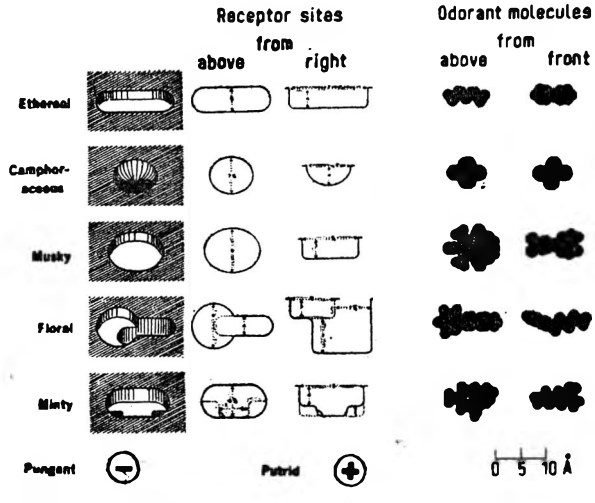

Fig. 5. Amoore's scale models of olfactory receptor sites at the membranes of olfactory cells and some respective odorant molecules. The compounds shown as top and front silhouettes on the right from the top down are diethyl ether, hexachloroethane, 2, 4, 6-trinitro-3, 5-dimethyl-(tert.) butylbenzene, alpha-amylpyridine and l-menthol; they elicit the respective odorous qualities isted on the left. Pungent is thought to be produced by protons le.g. from formic acid) being bound to negative sites, and putrid by (electro-negative stimuli, such as hydrogen sulfide. (Ref. 14).

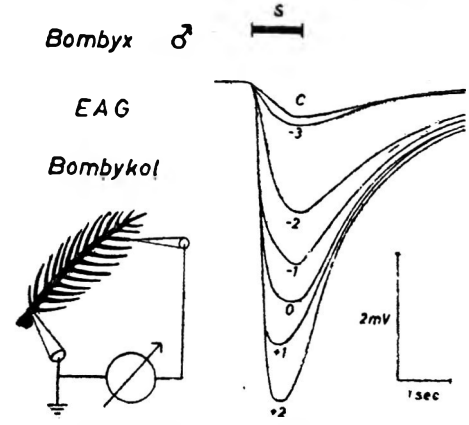

Fig. 6*a*. Electroantennogram (EAG) of a male silkmoth *Bombyx mori*. EAG-response to a control paper (C) gives the tactile (trigeminus) component of the air pulse by which bombycol of increasing concentration is applied (numbers indicate exponents to the base 10 for the quantity of bombycol in μg on the stimulus paper); S = duration of stimulus. The picture in the bottom left corner shows the recording procedure: one recording micropipet is inserted near the tip and another one at the base of an antenna, (Ref. 24).

bombycol, a significant whirring of his wings—which normally would propel him to her—indicates the specific response to this bombycol[68,69].

The remaining 20 per cent of the sensory cells on the antenna can also be excited by other odourous substances, as revealed by single cell recordings. However, the intensity of this excitation, caused by the same number of molecules, is very low compared to the bombycol excitation of the specific cells; in addition, these other substances produce a limited excitation in the specific cells, which is however much less than the one elicited by a much smaller number of bombycol molecules. This is the reason that Gesteland[33] — based on the results of Boeckh *et al.*[24] and Schneider[62] — divided the olfactory sensory cells into "specialized cells" and "generally responding cells". The generally responding cells are predominant in vertebrates.

After prolonged exposure we adapt to a specific odour. Everybody knows the experience of going into a room and noticing an unpleasant odour. After a while however we do not any longer register a particularly offensive odour. This is known as adaptation of the olfactory receptors or habituation of synapses in the central olfactory pathways to an odour. More complicated is, when the offensive odour is provoked by several different substances. In this case one may have adaptation or habituation to a particular odour without altering the threshold to other single odour.

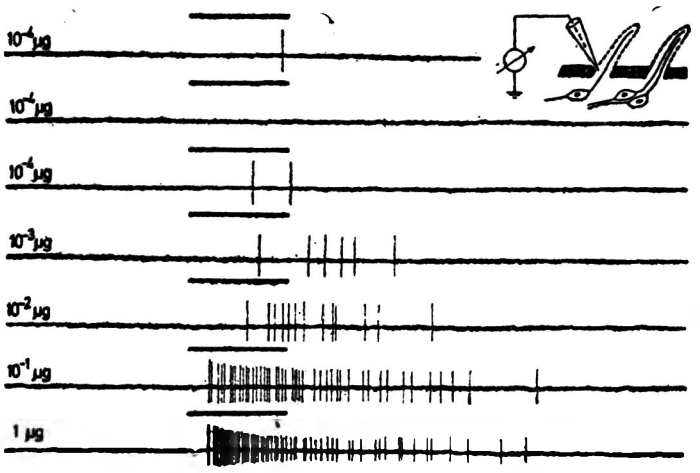

Fig. 6*b*. Spike responses of a receptor cell highly sensitive to bombycol applied in increasing concentration. Stimulus duration S = 1 sec marked by the horizontal lines above each recording trace. A recording glass micro-electrode is inserted at the base of one olfacory sensilla and recorded versus a reference electrode in the haemolymph of the antenna. (Note the difference between this recording and the EAG, which is gained by intracellular penetration of the recording electrode into the dendritic process of just one olfactory cell in one sensillum close to the tip of the antenna). It is estimated that $10^{-3}\mu g$ bombycol equal in the mean about 7 molecules/sec per cell, $10^{-2}\mu g$ equal 70 molecules/sec per cell etc. (Ref. 41).

Similar to adaptation is the interesting phenomenon of masking of odours: offensive odours are in effect eliminated by an ambient deodourant, which merely covers up the offensive odour with a relatively pleasant odour. This general principale of sensory physiology is used in a great number of comn ercially available deodourants which for the most part mask or decompose the odour evoked by bacterial decomposition of human perspiration, but this principle may be of importance for food technology too.

On the other side of the fence are the perfumers, who are interested in pleasant smells. It is well-known that in perfuming, concentration plays a very important role. There are quite a number of substances of which small concentrations produce pleasant smells but very high concentrations unpleasant sensations. Sensations provoked by odourous stimuli are in many cases assigned terminologies of either pleasant or unpleasant, but are seldom registered as neutral, in contrast to sensations received in other sensory channels like vision and audition. This is in part caused by the close anatomical connections between the olfactory system and the limbic system in the brain, which is responsible for emotions, as mentioned earlier.

Chemoreception of Taste

General internal chemoreceptors automatically control oxygen or hydrogen or CO_2 concentrations in the arterial blood. Other ones regulate the intake of food and—by additional osmoreceptors—of water by the general sensations of hunger and thirst. These chemoreceptors are neither part of gustation nor olfaction but taste and smell are also closely connected to the input of food, and both their receptors located around the mouth, are very important for checking meals for pleasant or annoying or even poisonous contents.

When I started out in taste research almost 10 years ago I memorized the fact that the taste receptors in mammals were independently discovered in 1867 and 1868 by Schwalbe[64,65] and by Lovén[46]. So by the time of the centennial in 1967, I expected that everything about the functions of taste would be well-known, with nothing left to be explored. Taste appeared to be a very simple sensory modality, since its sensory cells are so nicely accessible on the surface of the tongue which can be conveniently protruded out of the mouth. It is well-known of course that this hope has not been fulfilled and that although we now know many facts on taste physiology, many problems and questions in gustation are still open for discussion. The sensation of taste can be studied by a psychophysical method in which different substances or even electric current are applied to the tongue of an individual who is then questioned as to what he tastes and how intense it is. This can be done in

normal man and also in those who have suffered ablations of some part of the cerebral cortex[25,26]. It is difficult, however, to use this method in animals. Behavioural responses like the whirring reaction of the butterflies to bombycol discussed earlier are one aid. The study of conditioned responses to taste is another. This time-consuming conditioning method is very widely used to study taste preferences and food habits. A more objective way of studying taste in animals is to record the electric activity of sensory nerves from the tongue or of the receptor cells themselves.

Most of the receptors responsible for the sensation of taste, are located on the tongue and are called the taste cells within the taste buds. The adult human is supposed to have between 4,000 and 5.000 taste buds[17a,57], of which 80 to 94 per cent are actually situated on the tongue; the rest are scattered on the epiglottis, glosso-palatine arch, the soft plate and even the esophagus. One taste bud consists of between 30 and 60 taste cells (Fig 7), which are arranged in a pattern resembling the

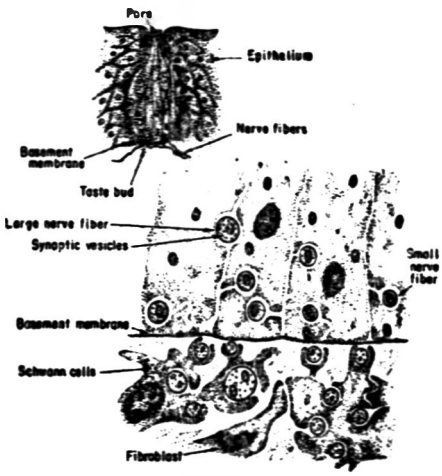

Fig. 7. Organization and ultrastructure of taste buds in papillae of the rabbit tongue. Slightly modified from A.J.D. de Lorenzo. (Ref. 11). Four resp. five cell types can be distinguished in the rabbit foliate bud according to Murray 49: 1) Dark cells (Type I) 2) light cells (type II), 3) Type–III cells, 4) basal cells which are able to develop to the cells of type I to III and 5) perigemmal celis which might be precursors of the basal cells or belong to the connective tissue outside the bud.

staves of a barrel. These elongated and cylindrical cells form an opening on the surface of the tongue which is the taste pore. The taste buds are grouped in papillae distributed on different parts of the tongue, for instance, the circumvallate papillae along the V-shaped line as seen in Fig 8, the foliate papillae on both edges of the tongue and the fungiform papillae which are scattered all over the tongue tip and lateral edges. The central parts of the tongue are covered with filiform papillae which have no sense of taste at all; one can assume that these keratinized papillae are just a mechanical aid in

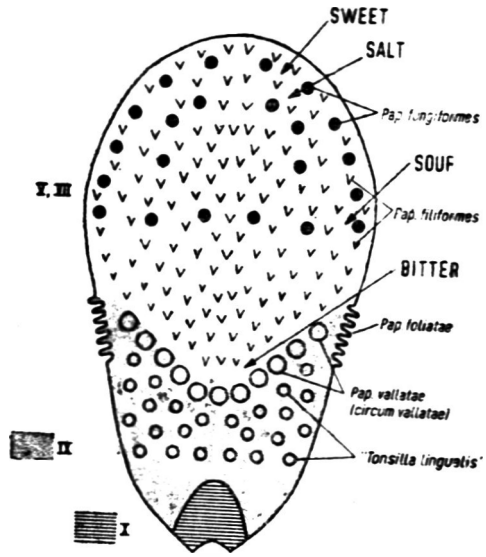

Fig. 8. Schematic representation of the tongue, the location of the different tongue papillae and the areas innervated by the cranial nerves (V = Trigeminus, VII; Facilis, IX; Glossopharyngeus and X = Vagus). The different taste qualities are located predominantly, but not exclusively in the areas indicated. Compare Fig. 9/10 and Ref. 56 to 59.

shifting the contents of the mouth about in conjunction with the tongue (Fig. 8). In man the tongue papillae infrequently carry several such taste buds and on the average only about 50 per cent of the taste papillae are equipped with even one bud; the rest is completely bare[17a,59]. Fortunately for neurophysiologists doing research in taste there are also animals, in particular the Norwegian rat, that bear precisely one taste bud on the top of each papilla.

The neural afferent fibers coming from the different taste papillae appear to be more complicated than the olfactory afferent nerves. As seen in the gross picture of the tongue, (Fig. 8), three cranial nerves—Number V, the trigeminus, Number VII, a certain part of the facial nerve and Number IX, the glossopharyngeus act together in processing taste sensations. To be more exact, the anterior two-thirds of the tongue is innervated from the trigeminus by the lingual nerve, which provides the general somesthetic sensitivity of the tongue to touch and temperature but which also carries gustatory fibers. These reach the 7^{th} cranial nerve via the tympanic chord, which runs through the middle aar. The posterial one-third of the tongue beyond the V-shaped-line mentioned before is innervated mainly by the glossophyngeus nerve. This nerve provides fibers for general sensitivity not only of the base of the tongue but also, together with the vagus, of the throat and even the esophagus. The scheme is well known, thought not completely correct, that the taste qualities are located on different parts of the tongue surface; sweet at the tip of the tongue is followed

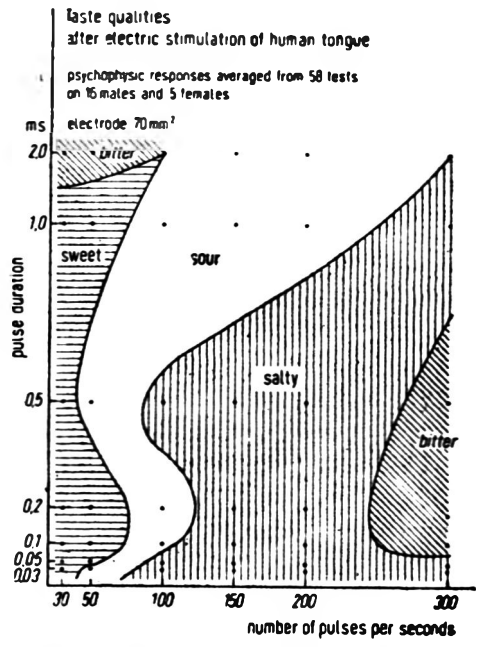

Fig. 9. Taste qualities after electric stimulation of the human tongue. The qualities were elicited from the upper tips of 21 students' tongues. The kind of taste quality depends only on the combination of pulse frequency and pulse duration (Ref. 58).

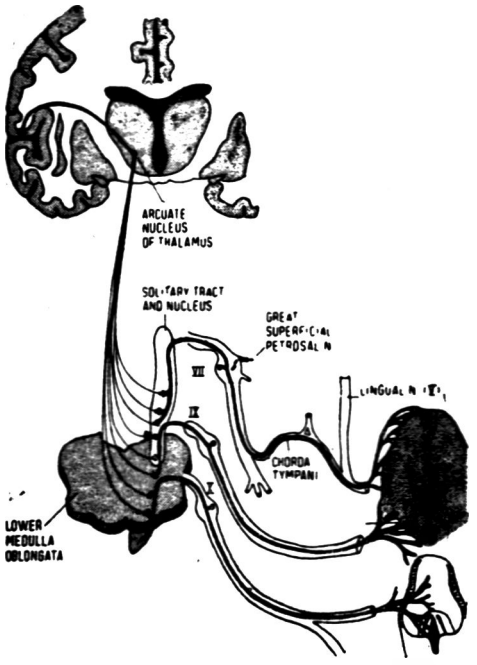

Fig. 10. Neural interconnections between taste cells and gustatory cortical representation on the Gyrus postcentralis (Brodmanrea 43). The nerve cells of the first neurons beyond the taste buds are located in the geniculate ganglion for the facial nerve (right of number VII in the picture), in the petrosal ganglion for the glossopharyngeal nerve (below number IX) and in the nodose ganglion for the few vagal taste fibers (below number X). There are no synaptic relays for taste in these ganglia; however, the gustatory information is switched after passing these "pseudounipolar" neurons to the second neuron in the solitary nucleus and to the third one in the arcuate nucleus of the thalamus. (Adapted from Netter F. H. *The Ciba Collection of Medical Illustrations. Vol.* 1, *Nervous System, Ciba, Basel:* 1972.)

by salty, while sour is thought to be located at both edges of the tongue and bitter at its base. In regard to this I might mention a few results from our own experiments in electrical stimulation of taste. With the background of von Békésy's taste research[21,22] we could prove, that electric square-wave pulses elicit *all* the four known taste qualities of sweet, sour, bitter and salty from the tip and the upper anterolateral edges of the human tongue. Single electrical impulses were applied via gross electrodes of about 70 mm² and varied in frequency and duration (Fig. 9). The classical tongue areas of taste quality just provide regions of higher probability for eliciting a certain desired taste quality[58].

Some of the details on the interconnections between taste buds and the central nervous system will now be summarized. The nerve cells of the taste fibers mentioned above are located in the geniculate ganglion, which is associated with the tympanic chord. The glossopharyngeal taste nerve cells are located in the petrosal ganglion and the cells of the vagal taste fibers in the nodose ganglion (Fig. 10).

The fibers from these ganglia proceed toward the nucleus solitarius which is located in the pontobulbar area of the brain stem[36]. Here the first synaptic relay of the taste fibers is situated (after the one between the cell and the first order neurons) and the second order neurons terminate in the nucleus ventralis posteriomedialis of the thalamus[30]. Experimental or accidental destruction of these nuclei produces loss of taste sensations in animals and in a few known cases also in man. The final cortical representation of taste qualities was doubtful until a few years ago. It now seems to be well established that it is located in the gyrus postcentralis of the parietal lobe very close to cortical areas receiving projections from the other somesthetic modalities of the tongue and from nearby zones in the mouth and the face[25,26,57]. There is nothing known however about the topological order of this cortical area in regard to the taste qualities: the local distribution of factory qualities on the olfactory bulb are comparably better described[80]. The psycho-physical phenomenology of the four taste qualities sweet, sour, bitter and salty is well known. It should be mentioned however, that salty substances like sodium or potassium chloride, which produce a very definite salty taste if applied in sufficient concentration, will produce sweet or more bitter sensations if the concentrations are very low[6,10] (Table 2). Sourness seems to correlate to the pH of the solution, which must usually be less than 4. On the other hand we know that different acids with the same pH produce different intensities of sour sensation.

Our knowledge of the initial gustatory sensory mechanism on the membrane of the taste cells is about as poor as our knowledge of the primary olfactory processes. However the phenomenon of taste modification might give a few further clues towards better understanding. This holds especially for gymnemic acid from a shrub native originally to India and Africa called *Gymnema silvestre*. The Indian name means, as I was told, "making honey tasteless". When Indian and Africans chewed the leaves of this shrub they found that for several hours afterwards sugar would taste like sand. Gymnemic acid also eliminated the sweet taste of artificial sweeteners like cyclamate, saccharine or berrylium chloride. The active principle in the shrub leaves was found to be the potassium salt of gymnemic acid; the structure of this acid was clarified between 1967 and 1969 as a glycoside of glycuronic acid and gymnemagenin, which is derived from hexahydro-triterpen[2,72].

Other taste modifiers are miraculin, monellin and thaumatin. An interesting hypothesis by Kurihara and Beidler[44,45] concerning miraculin attempted to explain the mechanism by which this protein, contained in the "miracle fruit" (*Synsepalum dulcificum*), turns sour tastes into sweet tastes–e.g. a lemon may taste like an orange. This taste modifying protein is a large molecule of 44,000 molecular weight, 6.7 per cent of which consists of the sweet tasting sugars arabinose and xylose. Because of its size this molecule is certainly unable to penetrate rapidly into the taste cell, so it must be assumed that it reacts on the outside of the cell membrane. Kurihara and Beidler's explanation for the sweet-inducing mechanism of this taste modifying protein is, briefly, that the sweet parts of the molecule are unable to fit into postulated "sweet receptor sites" of the receptor membrane, unless this receptor membrane changes its conformation under the influence of hydrogen ions and makes the sweet receptor site swell out of the level of the membrane. This also substantiates Beidler's theory of taste receptor stimulation,[18] pioneered in 1954, in which he assumed that all gustatory substances must be adsorbed on the surface of taste cells primarily in order to ultimately elicit the neural activity along the taste nerve. Beidler supported his hypothesis with strong evidence from theoretical considerations on the basis of the mass-action law.

To understand the problems of taste coding better we should have another look at the ultramicroscopic view of a taste bud. Fig. 7 gives a schematic diagram of the principle features of the cells in a rabbit foliate bud as revealed by Murray[49]. There are 5 different cell types shown, of which 4 are taste bud cells, most likely each with receptive properties for all four taste qualities. The fifth cell is a "perigemmal" cell with prominent fibril bundles and ribosomes enclosing a perigemmal nerve process. These perigemmal cells are quite exciting for explaining an effect which had first been found for taste and then also for olfactory sensory cells but which had been thought to be almost unimaginable for sensory cells up til now. This is the phenomenon of regeneration mentioned earlier in regard to the olfactory sensory cells[16,17,79].

It was Beidler and Smallman[20], who after many previous experiments on the reappearance of taste buds and taste bud regeneration after nerve regeneration, proved by injection of radioactive thymidine that there are indeed mitoses of these perigemmal cells. The cells Number 1, 2, 3 and possibly the base cells Number 4 (Fig 7) are then substituted for at the periphery of the taste bud. Radioactive tritiated thymidine when injected was first located in the vicinity of the taste bud, but about 100 hr later the dark points have moved directly into the taste bud[20]. This migration of thymidine can be inhibited by colchicine, which stops the normal mitoses of the cells and also significantly reduces neural taste responses. By this technique it could be estimated, that taste cells undergo physiological proliferation of growth and of age *via* division of the perigemmal cells; the life span of an average taste cell is about 250 ± 50 hr[20].

These changes in morphology of a taste cell might indicate that physiological properties change in the same manner. From the coding results of taste qualities from neurophysiological findings of many laboratories, it now seems to be quite clear that one taste quality is encoded not in one single afferent gustatory fibre but in the excitatory pattern of quite a number of gustatory afferencies[60]. This would mean that taste stimuli produce excitation in different nerve fibres at different levels, so that there could be one peak of excitation in one of those many excited fibres with the other ones being graduated lower in excitation level. This across-fibre pattern hypothesis was projected as early as 1941 by Pfaffmann[56], and it was seriously attacked by findings of Georg von Békésy[21,22], who in 1965 in electric or chemical stimulations of single human tongue papillae, claimed an absolute specificity of the taste papillae, which would produce only one kind of taste quality regardless of the kind of stimulation.

In continuation of our work on electrical taste[27,28], we rechecked human sensations elicited by single papilla stimulation[59]. Fig. 11 shows the set-up for these experiments which were done after chemical screening to exclude unsuitable subjects: one single fungiform papilla could be stimulated by a silver wire of 0.4 mm tip diameter. Of course it was extremely difficult at first to clearly identify certain tongue areas in order to locate after several hours, or in a few cases after several days, the one exact papilla which had been tested earlier. The

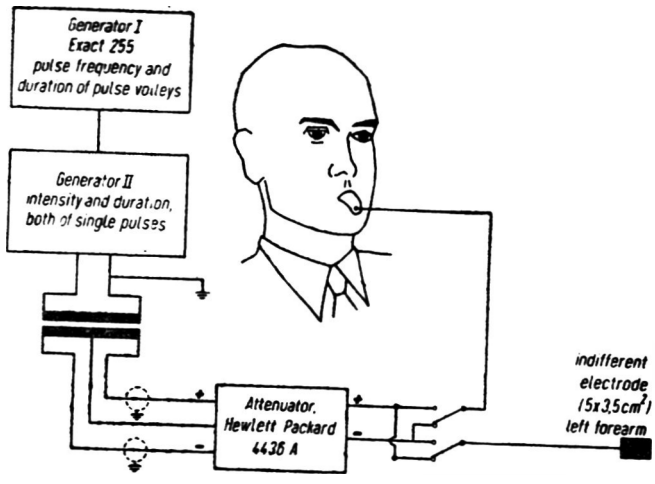

Fig. 11. Experimental arrangement for testing single human tongue papillae by electric stimulation. Block diagram, with a function generator (exact 255) controlling the frequency of stimulating pulses, which trigger another generator producing square-wave pulses of variable intensity and duration. For stimulus isolation a transformer was used, the secondary coil of which had been grounded halfway between its two exists, which were connected over an attenuator (Hewlett Packard 4436A) to a pole changing switch. With this reversal switch either the positive (anodic stimulation) or the negative voltage maximum (cathodic stimulation) or tactile stimulation could be applied to the tongue via a silver wire-electrode of 0,4 mm tip diameter versus a 5×3.5 cm² reference electrode on the subject's left wrist. For further details see Ref. 58. (Ref. 59).

TABLE 3. SURVEY OF TASTE QUALITIES ELICITED BY 84-FOLD ELECTRICAL AND MECHANICAL STIMULATION OF 144 SINGLE HUMAN FUNGIFORM TONGUE PAPILLAE IN 3 MALES AND 2 FEMALES, 18 TO 22 YEARS OF AGE. TASTE PREFERENCES AFTER STIMULATION ACCORDING TO THE PSYCHOPHYSICAL RESPONSES WERE EVALUATED BY x^2-TEST ($P \leq 0.05$)*

	Anodic stimulation		Cathodic stimulation		Touched only	
		%		%		%
Papillae						
Total tested	54	100.0	45	100.0	45	100.0
Giving taste response	27	50.0	19	42.2	5	11.0
Sweet	0	0.0	0	0.0	1	2.2
Sour	12	22.2	10	22.2	2	4.4
Bitter	2	3.8	0	0.0	0	0.0
Salty	1	1.8	1	2.2	0	0.0
Sour and sweet	0	0.0	4	8.9	1	2.2
Sour and salty	5	9.3	1	2.2	1	2.2
Sour and bitter	7	12.9	2	4.4	0	0.0
Bitter and salty	0	0.0	1	2.2	0	0.0

*Ref. 59

subjects also had to concentrate extremely hard to keep the tongue stationery. After a while our subjects could sit fairly comfortably for about fifteen minutes in the experimental position, in which the chin rested on a table with the forehead leaning on a bar which has been removed for this picture. The subject's tongue was gently pressed against the lower side of a plexiglass holder with a hole of 5.3 mm diameter, which had been mounted on the table at an angle of about 45 degrees. Stimulation, usually of three tongue papillae visible through the hole, was done *via* a micromanipulator and observed under a stereomicroscope.

The results are given in Table 3, which show that three modes of stimulation repeated 84 times for each papilla revealed that for anodic stimulation 50 per cent of 54 papillae tested gave taste responses and for cathodic stimulation 42.2 per cent of 45 papillae gave taste responses. On mere touching of the papilla with an electrode without current 11 per cent of the 45 tested papillae were still reported as producing taste sensations. The sensations elicited, however, where not constant for one papilla. The response patterns changed, as can be seen in Fig. 12. The same papilla (C_1) is classified as "sour-bitter" with a relative specificity, since there is a significantly higher occurrence of sour and bitter on electrical stimulation than on mechanical touch alone. (The

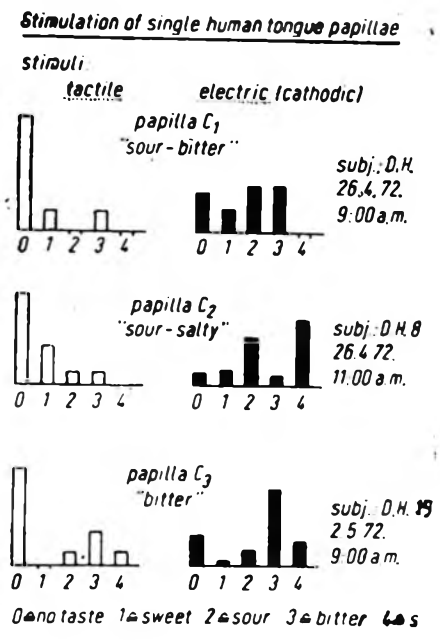

Fig. 12. Gustatory sensations reported on tactile and electric stimulation of single human tongue papillae. Attempt was made to find exactly the same papilla at different times, the results of C2 (second row from top) being gained 2 hr and the results of C3 (bottom row) being gained 6 days after C1. Between April 26th and May 2nd the same papilla was followed up four times per day after its vicinity had been marked by a mucosa-pencil. It is not completely proved however that C3 is precisely the same papilla as C1 and C2, since the tongue surface changed considerably in appearance from day to day. The similarity of C1 and C2 however is completely proved, and it is quite apparent that the gustatory pattern of this single papilla changed very clearly within 2 hr. The electric stimulus was switched to the anodic pole; for further details *see* Fig. 11. (Ref. 59).

classification could be a "no taste" papillae if there were no significant differences between the reported patterns on tactile and on electrical stimulation). The patterns found for these short moments changed however within a relatively short period of time. The results of C_2 (second row from top) were received after two hours and the results of C_3 six days after C_1, each from the same papilla. Similar changes of response patterns within a period of four hours were found on several single papillae but it was extremely difficult to follow up for a longer time, so that in fact we cannot really be quite sure despite all our endeavors, that the papilla C_3 is really identical with C_2. The fact however, that the across-fibre-pattern hypothesis is valid also as an "across-taste-buds-pattern" (or better "across-taste-cells-pattern"), appears to us to have been established also for man[2,56,59].

Another part of our research attempted to uncover information on taste intensity and quality coding from the EEG of an awake man. We used the technique of averaging cortically evoked brain potentials after electrical stimulation of the human tongue, since the electrical stimulus gives an almost rectangular initial flank at stimulus onset, while the onset of chemical stimulus rises at a flat slope. The experimental set-up is shown in Fig. 13 which in regard to stimulating is practically equal to the one used for stimulating papillae. The EEG is recorded via preamplifiers and amplifiers on a frequency modulated tape recorder and then off-line fed into a computer for averaging or correlation computation. As a result a series of typical brain potentials are shown in Fig. 14, with relatively slow deflections in the positive (down) or negative (up) direction after certain latencies; these amplitudes correspond to stimulus intensity, of which these potentials are a power function[42,43,57,58,70,71]. Unfortunately, however, we have not yet been able to find any dependence of these brain potentials on taste qualities.

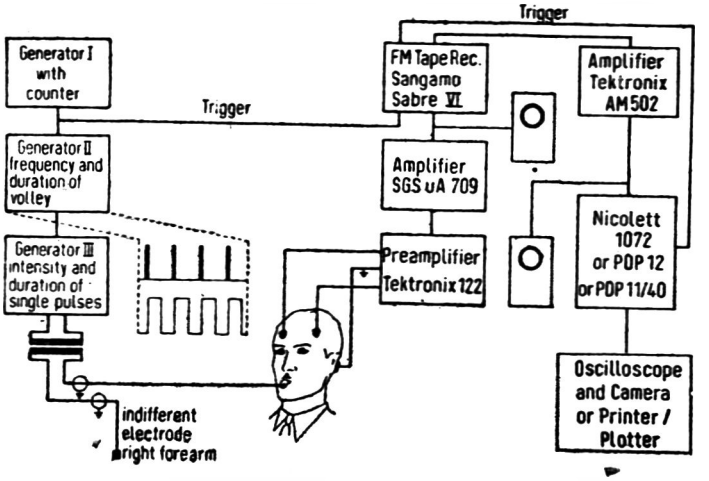

Fig. 13. Experimental setup for gaining evoked brain potentials in man after electric stimulation of tongue, which is followed by the sensations of electric taste (Ref. 59).

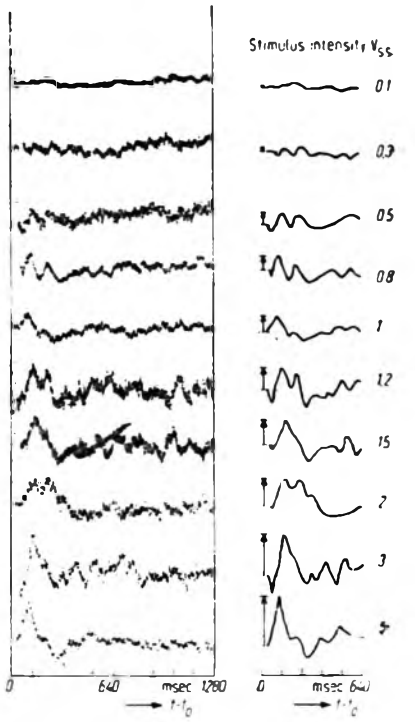

Fig. 14. Slow evoked cortical potentials recorded from the parietal area of man, contralateral to the position of electric tongue stimulation. Forty post-stimulus EEG-sweeps are averaged to obtain one potential, the amplitudes of which follow a power-function of stimulus intensity. The increase is marked schematically on the right together with the eliciting stimulus intensities in V_{pp} (Ref. 57).

I might finally summarize our present knowledge of the different modalities of taste by reminding you of the effects of applying a solution of a local anesthetic to the surface of the tongue. The psychophysical responses of the subject reveal that the first sensation that disappears under the effect of the anesthetic, for instance cocain, is pain. This is followed by the disappearance of sensations of first bitter, then sweet, then salty, sour and finally touch[37]. Our gustatory brain potentials also disappear under the influence of tetracain. They cannot normally be elicited when the stimulating electrode touches the lip or a part of the tongue surface without taste buds even if no a local anesthesia is applied. So we may state that the different qualities of taste are independent sensations for which certain specific receptor sites on the taste cell membrane must exist. In the future it must be proved, for one taste quality whether or not these sites are situated at the membrane of just one taste cell. It appears to be feasible but will have to be proved, that the developmental course of the taste cells by the regeneration procedure described above might switch the preference of sites for one or the other receptor cells.

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Long Term Toxicologic Assessment of Nickel in Rats and Dogs*

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Nickel sulfate hexahydrate was added to the diet of rats and dogs for 2 years in amounts yielding dietary concentrations of 0, 100, 1000, and 2500 ppm as nickel and 0, 250, 500, and 1000 ppm for 3-generation reproduction studies in rats. In the two year study in rats and dogs, growth was significantly depressed in rats on 1000 and 2500 ppm diets, and in dogs on the 2500 ppm diet. Hemograms in rats were within normal range, and in dogs on 2500 ppm diet slightly lower hematocrit and hemoglobin values were observed. Urinary findings were normal except for marked polyuria in 2 dogs on 2500 ppm diet. Tissue storage of nickel in various organs indicated no important storage sites. Organ-to-body weight data for rats indicated increased heart and decreased liver ratios for females on 1000 and 2500 ppm diets; in dogs on 2500 ppm diet increased kidney and liver ratios. In multigeneration study in rats, a higher incidence of stillborn was observed only in the first generation at all dietary levels of nickel and decreased body weights of weanings on 1000 ppm diet in all generations. Histopathologic studies on rats in the two year study and of F/3b weanlings revealed no lesions. Dogs on 2500 ppm diet showed lung lesions, and in two, granulocytic hyperplasia of the bone marrow was observed.

Nickel (Ni) has been referred to as a relatively non-toxic ubiquitous trace metal, it is found in the tissues of man, in many organs and tissues of animals, in a variety of plants, soil, and in sea foods. Of the 29 trace elements found in the tissues of man, nickel ranks third in the universe and solar atmosphere, eleventh in the earth's crust, fifteenth in sea water, and fifteenth in the body of man, in this respect ranking with the essential elements iron, cobalt, copper and zinc. Its physiologic role, if any, has not been established. As far as is known, nickel does not play any role in human, animal and plant nutrition, in disease, or is required in known enzyme systems.[1-3]

Acute and subacute toxicity of various nickel compounds in various species of experimental animals by different routes of administration have been documented in the literature. However, there is conflicting evidence on their systemic toxicity by mouth.[4,5] In humans, systemic poisoning from orally ingested nickel salts is almost unknown from occupational and non-occupational exposure.[4-6] In studies on the effects of feeding nickel carbonate, nickel soaps, and nickel catalyst to rats and monkeys, Phatak and Patwardhan[7] found no toxic effects after continuous feeding of nickel for 4 to 6 months at diet levels of 250, 500, and 1000 ppm nickel. In rats, reproductive performance was not significantly affected after 3 to 4 months of continuous feeding of nickel-containing diets. Further studies by Phatak and Patwardhan[8] on the retention and excretion of nickel at 4 month intervals for 16 months in rats on nickel catalyst diet containing 250 ppm nickel revealed no progressive accumulation of nickel in tissues assayed. Bone showed the highest concentration, about 8 mg per cent, kidney, heart, and spleen about 2 mg per cent, liver, intestine, testes, blood, and skin averaged less than 1 mg per cent. Of the total nickel ingested, about 95 per cent was excreted in the feces and about 1 per cent in the urine. Other toxicologic studies have been summarized by Schroeder[9]. The present study, started in 1960, describes the results of two year feeding of nickel as nickel sulfate hexahydrate to rats and dogs, and a three generation reproduction study in rats.

Material and Methods

Nickel sulfate hexahydrate fines ($NiSO_4.6H_2O$) containing 22.3 per cent nickel by analysis (theoretically 22.32 per cent) served as the test material in this study.

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Two-year feeding study in rats: Four groups of 25, 28-day old weanling albino rats (Wistar derived[a]) of each sex, culled to a narrow starting weight (56-65g), individually caged, were placed on diets consisting of 0, 100, 1000, and 2500 ppm Ni. Littermate distribution between diets was used within each sex. Finely ground meal[b] served as the basic diet and to this was added with thorough mixing nickel sulfate hexahydrate fines in amounts calculated to yield the above dietary levels of nickel. Body weight was recorded weekly and food consumption was measured over 3-day periods at the end of 1, 3, 6, 12, and 24 months.

Hematologic studies (hematocrit, hemoglobin, and total and differential leukocyte counts) were made on 5 rats of each sex and dietary level at 3-month intervals. Due to poor survival, smaller numbers were used at 24-months for control male and female rats and for male rats on 2500 ppm diet. Semiquantitative tests for urinary reducing substances and protein were made on pooled urine from 5 rats of each sex and dietary level at 3-month intervals through 21 months.

At the end of 23 months, one female rat from each diet was sacrificed. Their gastrointestinal tracts were washed free of contents and small sections of liver and kidney were removed for histopathologic study. The remaining carcasses were homogenized and aliquots were taken for nickel analysis. At sacrifice of two-year survivors, samples of liver, kidney, bone (femur), and fat were taken from each rat. Each tissue was pooled as to sex and diet for subsequent analysis for nickel content.

Nickel analyses on tissues (wet basis) were performed by La Wall and Harrison Research Laboratories using the spectrophotometric method of Alexander *et al.*[10] as modified by the Research Laboratories of the Rohm and Haas Company and identified as their Test Method 200 2-2, second revision, 1956.

At autopsy of 2-year survivors, gross examinations were carried out and absolute and relative organ-to-body weight ratios for heart, spleen, kidneys, liver, and testes were analysed statistically. Tissues preserved in formalin for histopathologic study were in addition to the above: lung, urinary bladder, stomach, small and large intestine, skeletal muscle, brain, skin, bone marrow, pituitary, thyroid, adrenal, pancreas, and gonad.

Body weight changes and organ-to-body weight ratios were evaluated by analysis of variance and Duncan multiple range and multiple F tests.[11]

Two-year feeding study in dogs: Groups of three male and three female purebred beagle dogs of about 6-months of age, individually housed, were maintained for two years on diets providing 0, 100, 1000, and 2500 ppm Ni. Prior to being placed on the above diets, the dogs were immunized against distemper, infectious hepatitis and leptospirosis, and treated as needed for intestinal parasites. Finely ground dog kibbled meal[b] served as the basic diet to which was added with thorough mixing, nickel sulfate hexahydrate in amounts calculated to yield the above diet levels of nickel. Weighed amounts of feed, 400 g/day through the first 22 weeks and 450 g/day thereafter, moistened and thoroughly mixed with an equal weight of water, were offered to the dogs once a day. Feed consumed was recorded daily and body weights were recorded weekly.

Hematologic studies (hematocrit, hemoglobin, and total and differential leukocyte counts) were made prior to placing the dogs on the respective diets, at 2, 4, and 13 weeks, and at 3-month intervals thereafter. Semiquantitative tests for urinary reducing substances and protein concentration were made before placing dogs on respective diets, at one-month, and at 3-month intervals thereafter.

Urine and feces for nickel analysis: At approximately 12 months, one week collections of excreta were made on one dog of each sex on each of the respective diets for analyses of Ni content. During the 23rd and 24th months, one week collections were made on two dogs of each sex and diet level, and three successive weekly collections were made from two other dogs of each sex and diet level. The sixth dog of each sex on each of the Ni-containing diets for 24 months was returned to the control diet. Two successive one-week collections of excreta were made following passage of charcoal-marked feces.

At autopsy, specimens of bone, liver, kidney, lung, skeletal muscle, and fat were taken from each dog for Ni analyses. Nickel analyses for excreta and tissues were conducted by the La Wall and Harrison Research Laboratories, as for rats. Feces were oven-dried and ground and tissue Ni was determined on wet basis.

At sacrifice, organ-to-body weight ratios were determined for heart, spleen, kidneys, liver, and testes and data evaluated by Duncan test[11]. Tissues preserved in formalin for histopathologic study, in addition to the above, included: lung, stomach, large and small intestine, urinary bladder, skeletal muscle, brain, skin, bone marrow, pituitary, thyroid, adrenal, pancreas, and gonad.

Reproduction studies in rats: A three-generation study was undertaken in albino rats (Wistar derived[a]). For this study, 28-day-old littermate rats, within but not between sexes were separated into 4 groups of 30 rats of each sex, to constitute the parent F/0 generation.

(a) Albino Farms, Red Bank, New Jersey.

(b) Radlston Purina, St. Louis, Missouri.

Mean body weight and weight range, in so far as possible were similar for all groups. One group was placed on each of the following dietary concentration of nickel: 0, 250, 500, and 1000 ppm.

Finely ground laboratory chow[(b)] served as the basic diet. Stock aqueous solutions of nickel sulfate hexahydrate were prepared in appropriate concentrations so that the addition of 100 ml for each 6 kg of diet resulted in the desired nickel content, respectively. Additions of nickel solutions were thoroughly blended into the diet by mixing in a rotary-type blender. Diets were prepared fresh each week. Rats were individually caged and had free access to water and diet. Weekly body weight records were obtained, except during mating through weaning of litters.

After 11 weeks on the above dietary regimen, 20 females from each diet were transferred to individual breeding cages and each was mated with a male of the same dietary level of nickel for the F/1a generation. Male rats within each group were rotated to a different female on each of three successive 7-day periods. On the 20th mating day all males were removed. Records were maintained of mating, number of pregnancies, litters cast (alive and dead), pups in litter at 1, 5, and 21 days (weaning), and total weight of the litter at weaning. Litters containing more than 10 offsprings were randomly reduced to 10 on day 5. All surviving F/1a siblings were sacrificed and autopsied at weaning. Approximateyl 10 days after weaning of F/1a litters, F/0 parent generation rats were remated for F/1b litters. Procedure and observations recorded were the same as those described for F/1a litters. Following weaning of F/1b litters, surviving F/0 rats were sacrificed and autopsied.

For the F/2 generation 30 male and 30 female F/1b offsprings from each diet level were continued on their respective parents' diet for 11 weeks at which time 20 of each sex within each group were mated and the same procedure followed as with the F/0 generation through production and weaning of F/2a and F/2b litters. At weaning of F/2b rats, F/1b parents were sacrificed and autopsied. For the F/3 generation, the same procedure as with the previous generations was followed through the production of F/3a and F/3b litters. All matings in each generation were made with rats from different litters. The following indices were calculated for each generation: Fertility (F.I.) = (pregnancies/matings) × 100; Gestation (G. I.) = (litters cast/pregnancies) × 100; Viability (V.I.) = (live pups at day 5/live pups born) × 100; and Lactation (L.I.) = (weaned/live pups-discards on day 5) × 100.

Histopathologic studies were performed on 10 male and 10 female F/3b weanlings from each diet level. Tissues included were the same as those described in the two year rat study.

Results and Discussion

Two-year feeding study in rats: Data on body weight at representative periods and on number of survivors at two-years are summarized in Table 1. Two-year survival was poor, particularly among control rats of both sexes and males on 2500 ppm, but there is no indication of an effect due to nickel. Nickel had a depressant effect on body weight in both sexes on 2500 ppm and sporadically for rats on 1000 ppm diet. Food consumption indicated no consistent trends, but it appeared that the lesser weight gains, particularly on the 2500 ppm diet, may be in part a result of lower food consumption.

Hematologic values for hemoglobin, hematocrit and differential leukocyte counts, obtained at 3-month

TABLE 1. BODY WEIGHT AND MORTALITY DATA ON RATS** RECEIVING NICKEL SULFATE IN THEIR DIET FOR TWO YEARS

Sex	Diet concn (ppm)	Average body wt (g ± S.D.)								
		Start	1 wk	3 wk	6 wk	13 wk	26 wk	52 wk	78 wk	104 wk
Female	0	57	87±14	147±25	205±25	267±31	309±39	369±72(5)	393±115(9)	496±84(21)
	100	57	87±15	144±24	205±16	268±25	307±30	356±59	376±68(7)	409±72(18)
	1000	57	82±16	146±13	191±17*	250±32	277±37*	304±30(4)*	324±54(7)*	325±62(18)*
	2500	57	78±13*	131±13*	180±17*	232±17(1)*	261±20(3)*	269±29(4)*	266±35(6)*	303±36(18)*
Male	0	60	97±13	185±19	285±37	406±45(1)	467±66(2)	551±49(6)	562±58(11)	483(23)
	100	61	98±17	186±27	298±25(1)	425±38	506±51	551±53(2)	537±101(5)	511±81(17)
	1000	61	94±15	176±15	266±30	392±43(3)	459±52	494±69(9)*	519±69(11)	446±61(18)
	2500	60	88±15*	154±11*	241±16*	331±30(2)*	382±38(5)*	389±43(9)*	365±45(9)*	367(23)

*Value differs significantly from control, P = 0.05. Figures within the parentheses indicate cumulative mortality.
**At start 25 rats of each sex/group.

TABLE 2. TISSUE CONCENTRATIONS OF NICKEL IN RATS RECEIVING NICKEL SULFATE IN THEIR DIET FOR TWO YEARS

Sex	Diet concn (ppm)	Tissue concentration of Ni (ppm) on wet wt basis			
		Bone	Liver	Kidney	Fat
Female	0	0.53	0.094	0.14	0.51
	2500	0.82	0.64	3.4	1.0
Male	0	<0.096	0.055	<0.14	<0.055
	2500	0.64	0.68	4.9	1.4

intervals, for rats of all dietary levels of nickel did not depart significantly from those of the controls. Results of tests for urinary reducing substance at three month intervals were negative. Results of semiquantitative tests for urinary protein at the same time intervals were quite variable and inconsistent, with no clear trends.

Results of nickel analyses on individual body tissues (bone, liver, kidney, fat), summarized in Table 2, indicate no important storage sites of nickel. Kidneys showed the highest content (about 4 ppm), fat averaged 1 ppm, bone and liver, about 0.7 ppm. Results on the whole carcasses, on wet basis, on diet levels of 0, 100, 1000, and 2500 ppm for 23 months, are 0.38, 0.33, 1.5, and 3.0 ppm, respectively. The total nickel content of one rat weighing 251 g, on the 2500 ppm diet, was 0.75 mg.

Organ-to-body weight ratios obtained at sacrifice of two-year survivors are summarized in Table 3. A tendency toward increased heart-to-body weight ratios and decreased liver-to-body weight ratios appears in female rats on 1000 and 2500 ppm diets.

Gross pathologic findings on rats sacrificed at term were negative. Histologic findings were essentially negative. The distribution of the lesions found was not indicative of any characteristic effect of nickel in the diet.

Two-year feeding study in dogs: All dogs survived the two year experimental period. During the first 3 days, all six dogs on 2500 ppm Ni diet vomited, usually within an hour. On the fourth day they were returned to the control diet. All but one dog readjusted within 3 days. The one dog readjusted after parenteral feeding and intravenous fluids. At the start of the second week 5 of the dogs were placed on 1500 ppm Ni and the sixth dog was included at the start of the sixth week. This level of Ni apparently was well tolerated, as no emesis, salivation or gastro-intestinal irritation was observed. At two-week intervals the diet level of Ni was raised to 1700, 2100 and 2500 ppm, respectively, with no further evidence of emesis, salivation, or gastrointestinal irritation.

Analyses of body weight data at representative intervals suggest no effect for dogs on 100 and 1000 ppm diets. At 13 weeks dogs on 0, 100, and 1000 ppm diets gained 20 per cent in body weight as compared to 10 per cent for dogs on 2500 ppm. At 52 weeks dogs on the two lower levels of Ni and the controls had gained 39 per cent as compared to 20 per cent for dogs on 2500 ppm. Similar comparison at 65, 78, 91, and 104 weeks showed a gain of about 45 per cent in body weight for dogs on 0, 100, and 1000 ppm as compared to less than 20 per cent for dogs on 2500 ppm.

Cumulative food consumption data for all dogs, except for dogs on 2500 ppm during the terminal weeks when they were caged for collection of excreta, are quite comparable and do not explain the difference in body weight.

Hematology: Hematologic values obtained at three month intervals were quite variable but within normal range. However, the greatest variability appeared in the hematocrit and hemoglobin values with a tendency toward lower values in dogs on 2500 ppm Ni, suggestive of a simple hypochromic anemia.

Urinary tests for reducing substances and protein gave comparable values for all groups of dogs. Urine volume data during the 23rd and 24th month revealed high urine volumes for dogs on 2500 ppm diet. Two weeks of

TABLE 3. ORGAN-TO-BODY WEIGHT RATIO DATA ON RATS RECEIVING NICKEL SULFATE IN THEIR DIET FOR TWO YEARS

Sex	Diet concn (ppm)	Rats No.	Organ-to-body wt ratios (g/kg±SD)				
			Heart	Spleen	Kidney	Liver	Testes
Female	0	4	2.5±0.3	2.5±1.3	6.8±1.1	36.6±5.2	
	100	7	2.8±0.3	2.3±0.5	7.9±1.1	34.5±7.4	
	1000	6	3.8±0.7[a]	2.0±0.4	8.6±1.5	29.0±4.1[a]	
	2500	7	3.2±0.4[a]	1.9±0.2	7.8±0.8	29.1±2.4[a]	
Male	0	2	2.8	1.9	8.2	38.6	7.9
	100	8	3.3±0.5	2.0±0.3	7.6±0.9	25.9±3.6	6.7±1.6
	1000	7	3.3±0.9	2.3±0.6	10.3±5.8	30.4±8.1	7.2±1.1
	2500	2	3.3	1.5	7.1	31.0	9.5

[a]Value differs significantly from control, P = 0.05

TABLE 4. ORGAN-TO-BODY WEIGHT RATIO DATA OBTAINED ON DOGS RECEIVING NICKEL SULFATE IN THEIR DIET FOR TWO YEARS

Diet concn (ppm)	Dogs No.	Organ-to-body wt ratios (g/kg ± SD)				
		Heart	Spleen	Kidney	Liver	Testes
0	6	8.0±1.3	7.4±2.2	5.7±0.9	28.1±2.8[a]	2.4±0.5
100	6	8.0±0.9	6.8±1.8	5.9±0.8	29.5±3.3	2.4±0.3
1000	6	7.1±0.6	6.8±2.3	6.1±0.6	29.2±3.6	2.4±0.4
2500	6	8.4±1.1	6.9±1.8	7.8±0.8[b]	33.1±3.1[b]	3.1±0.5

[a]Average of 5.
[b]Value differs significantly from control, P = 0.05.

additional observations on all dogs on 2500 were made. Urine volumes for two of the dogs (one of each sex) were unusually high. On the eighth day the antidiuretic effect of pitressin tannate (5 and 10 units) by IM injection was tested. In the two dogs with polyuria no effect was observed. Whether these findings bear any relationship to the dietary intake of Ni does not appear to be answerable by this small number of observations.

Data on the excretion of Ni in urine and feces obtained at 12 months and during the 23rd and 24th months showed variable amounts of Ni in the feces, inconsistent with the amounts in the diets and ingested. No explanation is offered on the inconsistancy and variability of the results obtained. However, only approximately 1 to 3 per cent of the ingested Ni was excreted in the urine and tissue levels (below) were small, indicating little body retention of Ni.

Tissue storage: Tissue analyses on bone, liver, kidney, lung, skeletal muscle, and fat obtained at sacrifice indicated limited retention of Ni, highest values found were for kidney (4-7 ppm in dogs receiving 2500 ppm), and in one dog 1.6 ppm was found after withdrawal of Ni diet for two weeks.

Organ-to-body weight data are summarized in Table 4. Statistical by significant higher kidney and liver ratios appeared only in dogs on 2500 ppm for two years.

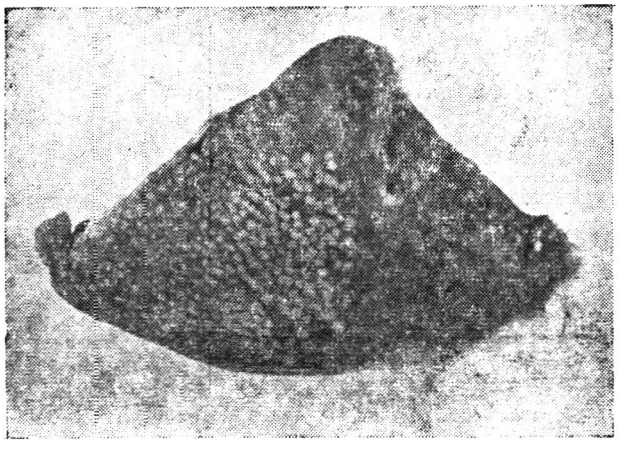

Fig. 1. Formalin-fixed lung from dog on 2500 ppm nickel, showing multiple, subpleural, peripheral cholesterol granulomas.

Histopathologic findings showed no characteristic lesions in dogs receiving 100 and 1000 ppm. All dogs on 2500 ppm showed histologic changes in their lungs. Grossly 5 of 6 dogs displayed multiple subpleural peripheral cholesterol granulomas, (Fig. 1; photomicrographs reproduced elsewhere, Hennigar[12]). The only dog not showing this had been returned to control diet for two weeks prior to sacrifice. Other lung pathology included bronchiolectasis (4 dogs), emphysema (3 dogs), and focal cholesterol pneumonia (4 dogs). The only other change observed consisted of granulocytic hyperplasia of the bone marrow in two dogs on 2500 ppm.

Reproduction studies: Body weights for parent generation rats on 250 and 500 ppm diets, before mating and at weaning of respective litters, were not adversely affected, but rats on 1000 ppm exhibited slightly lower body weights. The average decrease in body weight did not exceed 8 per cent for females and 13 per cent for males.

Data on reproduction through three generations are summarized in Table 5. On fertility, gestation, viability and lactation indices, no adverse effects were noted at any of the dietary levels of nickel. Data on the number of pups born dead showed higher incidence of stillborn in the first generation at all levels of Ni, not observed to any extent in subsequent generations. The number of siblings (alive and dead) cast per litter averaged 10.3, 10.6, 9.8, and 9.0 for 0, 250, 500, and 1000 ppm diets, respectively. The number of siblings weaned per litter were progressively fewer with increasing dietary level of nickel, averaging 8.1, 7.2, 6.8, and 6.4 for 0, 250, 500, and 1000 ppm diets, respectively. On average weaning body weight, a clear-cut adverse effect is only apparent in weanlings of females on 1000 ppm diet, averaging 73 per cent of control. However, offsprings maintained on 1000 ppm diet from weaning to mating of succeeding generations recovered considerably from this deficit, averaging 92 per cent of controls.

TABLE 5. SUMMARY OF REPRODUCTION DATA FOR RATS ON VARIOUS DIETARY LEVELS OF NICKEL[a] THROUGH THREE GENERATIONS

Generation	Diet concn (ppm)	Number of rats			Total number of pups					Weanlings mean body wt. (g)	Indices (%)[b]			
		Mated[c]	Pregnant[d]	Whelped[e]	Born alive	Born dead	Alive[f] day 5	Discarded[g]	Weaned		F.I	G.I.	V.I.	L.I.
F_o	0	20(1)	15(1)	14	113	5	113(10)	2	89	37.5	79	93	100	87
F_{1a}	250	20(1)	11	11(1)	72	17	69	5	60	40.1	58	100	96	94
	500	19	14	14(1)	96	13	95	5	72	34.4	74	100	99	80
	1000	20	12	12(1)	93	16	91	5	83	27.8	60	100	98	97
F_o	0	17	14	14	143	3	139	0(8)	137	33.8	82	100	97	99
F_{1b}	250	19	16	16	164	6	150	0(12)	134	31.6	84	100	91	89
	500	19	14	14(3)	109	27	106	0(9)	98	28.1	74	100	97	93
	1000	20(1)	15	15	93	31	77	0(3)	67	25.1	79	100	83	87
F_{1b}	0	20	15	15	142	0	138	13	115	35.9	75	100	97	92
F_{2a}	250	20	18	18(1)	188	2	181	30	119	31.1	90	100	96	79
	500	20	17	17	171	0	141	10	129	34.6	85	100	82	98
	1000	20	16(2)	14	127	6	120	8	96	25.9	80	88	94	86
F_{1b}	0	20	14	14(1)	149	1	146	30	116	37.9	70	100	98	100
F_{2b}	250	20	17	17(1)	181	16	177	39	130	35.7	85	100	98	94
	500	20	16(1)	15	166	0	160	22	131	36.9	80	94	96	95
	1000	18	11	11(1)	109	3	103	10	90	26.9	61	100	94	97
F_{2b}	0	20	18(1)	17	180	5	175	20	125	36.7	90	94	97	81
F_{3a}	250	20	19	19	207	4	201	21	121	34.5	95	100	97	67
	500	20	19(1)	18(1)	184	5	172	20	102	35.6	95	95	93	67
	1000	20	20(1)	19	168	7	162(10)	6	107	280.	100	95	96	73
F_{2b}	0	18	18	18	216	0	206	38	158	41.9	100	100	95	94
F_{3b}	250	20	18	18	204	2	198	32	161	41.4	90	100	97	97
	500	18	16	16(1)	142	6	138	22	102	41.2	89	100	97	88
	1000	17	17	17	139	7	138	13	114	29.7	100	100	99	91

[a]Nickel sulfate hexahydrate (22.3% Ni).

[b]Indices: F.I., G.I., V.I., and L.I. refer to Fertility, Gestation, Viability, and Lactation Index, respectively, defined in text.

[c]Numbers within parentheses refers to non-pregnant dams found dead during mating period, not included in calculation of F.I.

[d]Within parentheses, dams found dead and autopsied to confirm pregnancies.

[e]Numbers within parentheses refers to litters born dead

[f]Numbers within parentheses refers to number of siblings sacrificed after day 5 and before weaning due to death of dam, not used in calculation of L.I.

[g]To reduce litter size to 10 on day 5; and within parentheses are totals in excess of 10/litter not discarded as planned, due to oversight, and carried to weaning.

Gross observations on siblings cast, at all dietary levels of nickel through three generations showed no teratogenic effects. Histopathologic findings on F/3b weanlings, 10 of each sex on each dietary level, were entirely negative.

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Production of Fungal Rennet Substitute for Cheese Making

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An aqueous extract obtained from the mouldy bran of *Rhizopus oligosporus* grown at 30°C for 72 hr possessed high milk clotting and relatively low proteolytic activity. The preparation could be stored actively under refrigeration. The organism produced less milk clotting enzyme on liquid medium. The enzyme was influenced by Ca in its activity. The concentrate of the enzyme extract was brown in colour, bland in taste with a pleasant vegetable aroma. The enzyme was free from pathogens and aflatoxin.
Cheddar cheese trials involving 50-litres of milk per batch with the enzyme preparation, showed that curd working properties at cutting, cooking, cheddaring and pressing were similar to those with rennet. Fat loss in whey was slighlty higher than that of control. The yields of ripened cheese (30 days) with rennet and fungal enzyme were about the same. Both types developed flavour and aroma without marked differences in body and texture during early ripening (30 days).

Calf-rennet is by far the most common milk clotting enzyme in cheese manufacture. Shortage of rennet for commercial cheese production has led to a world wide search for suitable rennet substitutes. Several proteolytic enzymes of animal, plant and microbial origin have been tried for the purpose. Some of the available bacterial enzymes have their own characteristic differences and limitations for use as rennet substitutes[1-4].

Among the several milk clotting enzymes from fungi "Surecurd" from *Endothia parasitica*[5] and Meito microbial rennet from *Mucor pusillus* Lindt. have been commercially produced for use in cheese manufacture. Many types of cheese have been produced with the above milk clotting enzymes and the qualities of some of these cheese have been even superior to that made with rennet[6,7]. However, development of bitterness and body defects in cheese made with microbial proteases have also been reported. Besides the above commercial preparations enzymes of certain strains of *Rhizopus chinensis* and *Rhizopus oligosporus* have been used as rennet substitutes for cheese manufacture[8,9].

The cheese industry is finding it difficult to get its requirements of rennet though no precise data are available regarding its actual needs. Rennet substitute from microbial sources are not commercially available in the country. There is considerable potential for the development of cheese industry, if suitable rennet substitutes can be made available.

This paper discusses the production of a rennet substitute from *Rhizopus oligosporus* (CFTRI 1104) for use in cheese manufacture.

Materials and Methods

Organisms: Fungal cultures of *Aspergillus*, *Penicillium*, *Rhizopus* and a few other important genera known to produce proteolytic enzymes were selected from culture collections maintained at the CFTRI, Mysore, for use in these trials.

Media: Four types of media were used for screening the organisms. (*i*) The liquid medium consisting of 10 per cent each of corn-starch and wheat bran prepared on the formulae of Arima[10]. The inoculated

*Southern Regional Station. National Dairy Research Institute, Bangalore.

flasks were incubated on a rotary shaker (230 rpm with a stroke of 5 cm) for 96 hr at 28-30°C. (*ii*) Wheat bran moistened to 70 per cent level with 0.2 N HCl. The autoclaved medium had a pH of 4.5. (*iii*) Same as (*ii*) but fortified with defatted soybean flour at 10 per cent level. (*iv*) Same as (*ii*) but fortified with defatted peanut flour at 10 per cent level.

Inoculum: Mold cultures maintained on potato dextrose agar for 4 to 7 days were used for inoculating the wheat bran semisolid or liquid medium. After a week's growth, a spore suspension or the whole material in the starter flasks were used for directly inoculating bran medium in trays.

Enzyme source: Mould bran obtained after 72 hr growth of the fungus at 30°C was air dried and the enzyme extracted in tapwater. The extract was concentrated 5-6 fold in a forced circulation evaporator under a vacuum of 15-60 mm Hg at a temperature below 40°C. The concentrate was used in these studies. In the case of liquid cultures, the contents of the flasks were filtered through Whatman No. 1 filter paper and the clear filtrate was used as enzyme source.

Milk clotting activity: This was determined by the procedure of Berridge as modified by Arima[11]. Time required to clot 5 ml of 10 per cent milk (containing 0.01 M $CaCl_2$) prepared from Difco skim milk powder, by 0.5 ml of enzyme solution (of suitable dilution) was recorded. Soxhlet units were determined by the formula

$$U = \frac{M\ (ml)}{E\ (ml)} \times \frac{35°C}{t(°C)} \times \frac{2400\ (sec)}{T\ (sec)}$$

where, U = Soxhlet units
M = Milk volume
E = Enzyme quantity
t = Temperature of reaction
T = Time required for curd formation

Proteolytic activity: This was estimated by a modified colorimetric procedure of Anson[12] employing 1 per cent casein as a substrate. Colour developed by TCA soluble products released during the hydrolysis with the Folin-Ciocalteau reagent was read at 660 nm and the enzyme activity calculated as μg tyrosine with a standard curve.

Protein determination: This was done by Biuret method as of Chaykin[13]. Lipase activity was determined by the method of Somkuti and Babel[14].

Fractionation of enzyme into milk clotting and proteolytic components by column chromatography: Ten grams of carboxymethyl cellulose was soaked in acetate buffer (0.1 M; pH 4.5) overnight. This was regenerated by washing with 1 N HCl, 1 N NaOH and again with 1 N HCl. After washing twice with the above buffer, the material was soaked in it overnight. It was packed in a glass column (30 × 2.5 cm). The enzyme to be fractionated was loaded on this column and eluted with the same buffer.

Cheese making trials: Cheddar cheese were prepared in pilot plant cheese vats at the Southern Regional Station of National Dairy Research Institute, Bangalore, using farm produced cow milk. Milk was standardised for fat and casein contents to a ratio of 1:0.7. Each batch of cheese was made with 50 lit. of freshly pasteurised milk tempered to 30°C and inoculated with 1 per cent of an active lactic culture containing a combination of *Streptococcus thermophilus*, *S. lactis*, *Lactobacillus helveticus* and *L. casei* in equal proportions. Calcium chloride was added at the rate of 10 g/50 lit. of milk. As soon as there was an increase of 0.01 to 0.02 per cent in acidity (lactic acid) the milk was coloured with annato cheese colour (1.5 ml). Four batches of cheese were prepared using 50, 45, 40 and 35 ml each respectively of *R. oligosporus* enzyme*. Cheese prepared with Hansen's rennet extract** (10 ml per batch) served as control. The rest of the cheese making was according to accepted industrial procedures with minor changes to suit the nature of the fungal rennet. Cheese have been stored at 10°C with an RH of 85-90 per cent for ripening. The biochemical as well as organoleptic changes taking place are being studied at monthly intervals and these results shall be reported later. The comparative manufacturing data are given in Table 3.

Results

Selection of a potent strain: Fifty cultures of fungi known to elaborate proteolytic enzymes were screened

TABLE 1. MILK CLOTTING ACTIVITY OF SELECTED STRAINS OF FUNGI GROWN ON DIFFERENT MEDIA

Culture	Soxhlet units/ml		
	Wheat bran	Wheat bran + soybean meal	Wheat bran + peanut meal
A. niger (1)	26.6	66	100
A. niger (coffee)	40.0	80	100
A. awamori	Nil	Nil	36
A. carbonarius	13.0	18	44
A. niger T (1)	Nil	Nil	133
A. niger T(2)	,,	,,	133
A. niger C (4)	,,	,,	200
R. chinensis	,,	,,	200
R. niveus	133	133	200
R. oligosporus	200	200	200

*3600 SU/ml and 1440 PU/ml of milk clotting and proteolytic activities.

**8000 SU/ml and 800 PU/ml of milk clotting and proteolytic activities.

TABLE 2. EFFECT OF PERIOD OF GROWTH ON THE RELATIVE PRODUCTION OF MILK CLOTTING AND PROTEOLYTIC ENZYMES OF SELECTED STRAINS OF FUNGI

Culture	72 hr		96 hr		120 hr	
	Soxhlet units/ml	Protease units/ml	Soxhlet units/ml	Protease units/ml	Soxhlet units/ml	Protease units/ml
R. chinensis	400	240	200	300	200	280
R. oligosporus	400	20	200	60	200	20
A. niger (coffee)	400	840	400	200	400	40

for milk clotting activity according to method described earlier. Out of these, 10 cultures showed milk clotting activities when their culture filtrates or mold bran extracts were assayed for enzyme activity as given in Table 1.

Based on the above studies, 3 cultures giving high yields of milk clotting activities were selected and grown on wheat bran semi solid medium for different periods. The proteolytic activities of the extracts are given in Table 2.

Based on these studies, *R. oligosporus* was selected as the most potent culture for the following studies.

Effect of pH on enzyme activity: The skim milk substrate was adjusted to different pH levels and the assay performed as shown in Fig. 1.

Effect of temperature on enzyme activity: The assay was performed at test temperatures of 55, 65, and 70°C and the results are presented in Fig. 2.

Effect of calcium on enzyme activity: The addition of Ca to milk on clotting was studied by incorporating varying amounts of $CaCl_2$ into the substrate and performing the assay. The data are presented in Fig. 3.

Temperature tolerance: Aliquots of the enzyme were held at 55, 65 and 70°C respectively for 15 min and the residual activities in the samples estimated as under.

Initial activity: 200 Soxhlet units/ml.

Test temp (°C)	Residual activity (Soxhlet units/ml)	Per cent inactivation
55	171	15.0
60	165	17.5
65	150	25.0
70	Nil	100.00

Microbiological examination: The enzyme concentrate stored in the refrigerator was examined for standard plate count, yeasts and molds and coliforms as per standard methods. The enzyme was also screened for aflatoxin by accepted procedures.

Fractionation of the enzyme: One of the important factors in a milk clotting enzyme preparation is its low

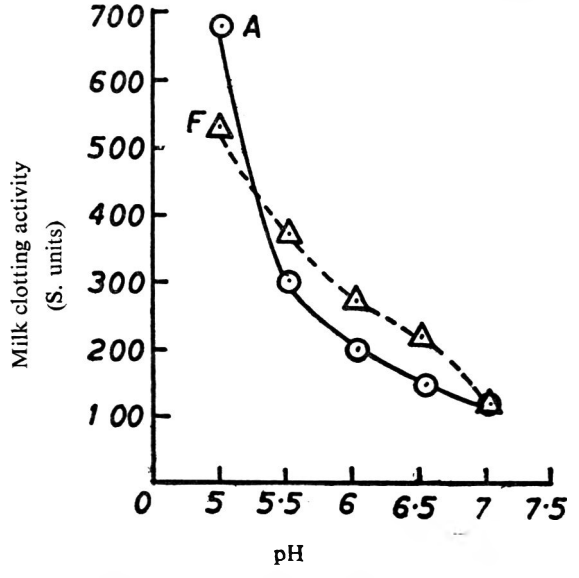

Fig. 1. Effect of pH on enzyme activity
A, animal; F, fungal

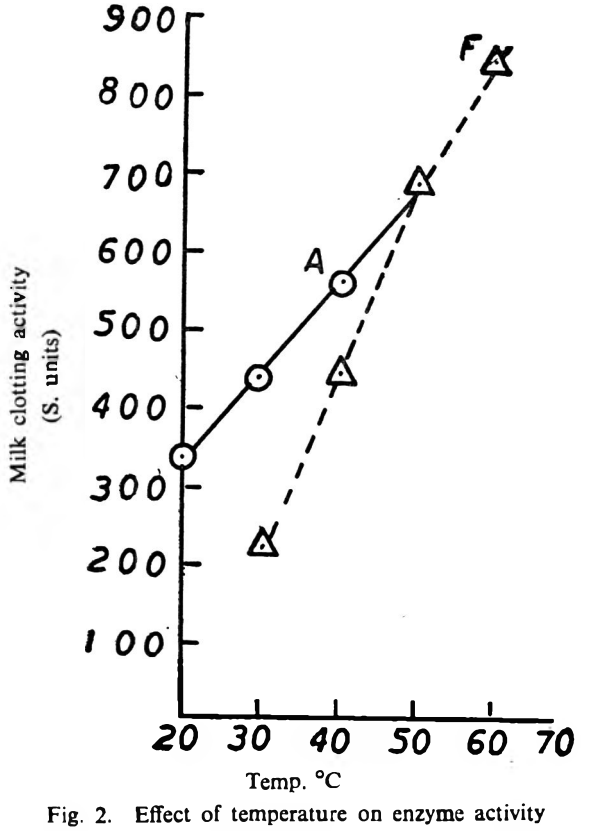

Fig. 2. Effect of temperature on enzyme activity
A, animal; F, fungal

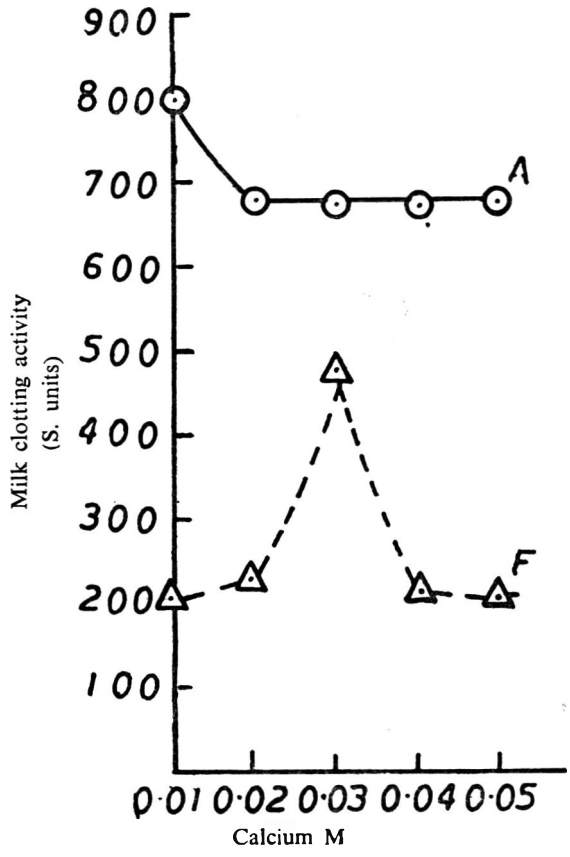

Fig. 3. Effect of calcium level on enzyme activity
A, animal; F, fungal

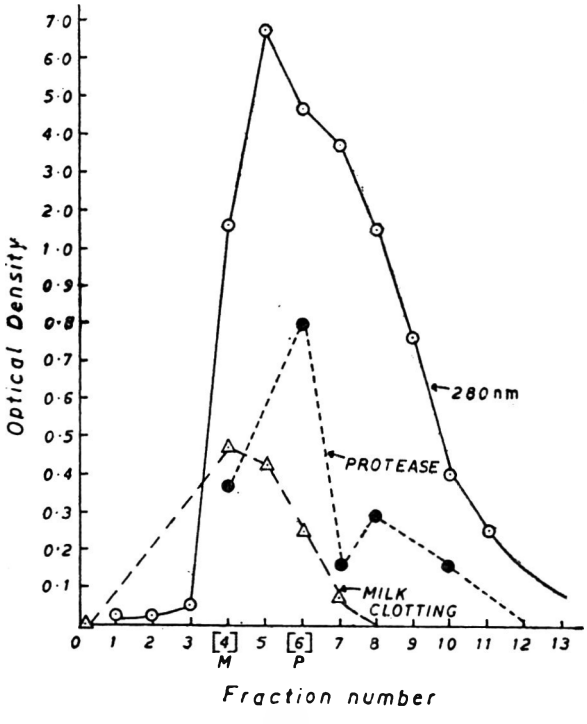

Fig. 4. Fraction of *R. oligosporus* enzyme on carboxymethyl cellulose

proteolytic activity. With a view to see whether the milk clotting and proteolytic components in the enzyme exist as separate entities, and whether a preparation having an optimum ratio of milk clotting to proteolytic activity can be developed, the enzyme was fractionated as follows:

Ten ml of *R. oligosporus* concentrate was dialyzed overnight against acetate buffer pH 4.5 (0.05 M). This was concentrated to 4 ml having a protein content of 400 μg/ml by exposing the enzyme to a blast of cool air (5°C). Three ml of this material having a protein equivalent of 1.2 mg was loaded on a CMC column as described earlier. Fractions were eluted with acetate buffer pH 4.5 (0.1 M). The optical density of the fraction at 280 nm was recorded and those having high optical densities were assayed for milk clotting and protease activities as shown in Fig. 4.

Discussion

The screening experiments showed that none of the cultures tested gave high milk clotting titres in liquid medium. Two species of *Rhizopus* and one *Aspergillus* gave very high milk clotting activity on wheat bran medium.

The comparative results for both calf and fungal rennets given in Fig. 1 show that enzyme activity was maximum at acid pH for both milk coagulants, the milk clotting activity decreasing with increase in pH. It is seen from Fig. 2 that milk clotting activity of both rennets increased with temperature, fungal enzyme being more stable towards heat. Fig. 3 shows that fungal rennet was more influenced by addition of calcium than rennet. Inactivation of the enzyme at 55, 65 and 70°C for 15 min were 15, 25 and 100 per cent respectively. The standard plate count was about 3000/g and coliform were absent. Yeasts and molds were less than 10/g. *Salmonella* was absent in 50-g sample of the enzyme. Aflatoxin was not present.

Since O.D. of animal rennet was more than that of fungal rennet, fungal enzyme was less dark than rennet which generally is coloured with caramel. Colour of fungal enzyme will not be a problem in cheese making since it gets further diluted during manufacture of cheese.

The presence of lipase (52 μM/ml/120 min) in fungal enzyme is considered to be potentially advantageous in accelerating ripening of certain types of cheese without development of off-flavours due to lipolysis[15].

CMC chromatography of the enzyme solution showed that the enzyme protein was distributed between fractions 3 to 9 (Fig. 4). The milk clotting activity got separated out in a single peak in fraction No. 4 and the proteolytic activity emerged as two peaks, the major peak appearing in fraction No. 6. Since the proteolytic and milk clotting activities are obtained in different peaks,

work on development of an ideal enzyme preparation for cheese manufacture is underway. (The total protein recovery was 94 per cent. The recovery of milk clotting activity was 59 per cent while that of protease activity was 25 per cent). Earlier workers,[9,17] have obtained 1 to 4 components from fungal rennets.

From Table 3, it is seen that curd working properties were more or less the same for rennet as well as fungal enzyme at cutting, cooking, cheddaring and pressing of cheese. Higher amounts of fungal enzymes had to be used to obtain curd of required firmness. The use of fungal rennet can be minimised by its further concentration/purification. Fat losses in whey were 0.3 and 0.4 per cent for rennet and fungal enzymes respectively. Apparently, this may be due to slow set and fragility of the fungal curd. There were no marked variations in the overall time of manufacture of the two types of cheese.

The yields of green cheese were 6.5 and 6.6 kg for rennet and fungal enzyme, respectively. The yields of ripened cheese (3 weeks) with rennet and fungal enzymes were 4.1 and 4.2 kg, respectively. The two types of cheese developed mild flavour and aroma without marked differences in texture and taste in 30 days. It has been reported[18] that cheddar cheese prepared with Meito rennet appeared to be normal at 2 to 5 months, but became more bitter than control cheese in 14 months.

It may be concluded that *R. oligosporus* CFTRI 1104 merits further study as a potential replacement for rennet or as an adjunct to be incorporated into rennet for manufacture of cheese.

TABLE 3. CHEESE MANUFACTURING DATA

Steps in manufacture	Calf rennet[a]	Fungal rennet[b] (*R. oligosporus*)
Milk*		
Acidity, %	0.16	0.16
Quantity (lit)	50.00	50.00
S.N.F.%	8.73	8.57
Fat, %	4.35	4.33
Total protein, %	3.39	3.48
Casein, %	2.73	2.80
Fat-casein ratio	1:0.7	1:0.70
Starter culture		
Acidity, %	0.64	0.60
Quantity, (ml)	500.00	500.00
Renneting		
Acidity, %	0.17	0.17
Quantity, (ml)	10.0	42.00
Cutting		
Acidity, %	0.12	0.12
Cooking (°C)	38.0	38.00
Dipping acidity, %	0.145	0.147
Cheddaring		
Acidity, %	0.30	0.24
Time taken (hr-min)	1-22	1-30
Milling acidity, %	0.30	0.24
Green cheese yield (kg)	6.5	6.60
Fat loss in whey, %	0.3	0.40
Ripened cheese yield (kg) (3 weeks)	4.1	4.20

[a]Mean of 2 batches [b]Mean of 4 batches

*10 g of calcium chloride added to cheese milk.

Acknowledgement

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Studies on Composition, Storage and Acceptability of Sunflower Oil

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The chemical composition of sunflower oil produced in Hyderabad was different from sunflower oil of Canada in respect of essential fatty acids. Heating resulted in a decrease in linoleic and increase in oleic acid fraction. Studies on storage behaviour indicated that sunflower oil became rancid earlier than groundnut oil. Both prolonged heating and storage, accelerated rancidity in oils, the effect being more pronounced in sunflower oil. Consumers' acceptability revealed that most consumers felt that flavour quality of sunflower oil was similar to other vegetable oils.

Sunflower (*Helianthus annus*) seed as a source of oil though recognised fifteen years back in India did not gain much importance. Recently with the introduction of four varieties of Russian origin seeds with high oil (50 per cent) content interest on sunflower oil was revived. These varieties along with Canadian origin 'Sunrise' were tried by Indian Council of Agricultural Research in 31 centres in eleven States during Kharif 1970–71. As a result, All India Coordinated Research Project on Sunflower considered extensive potentialities for increasing sunflower areas to about 930.000 hectares by 1978–79 as it had the advantage of high level of production of oil per unit area and per unit time[1].

In India, the per capita availability of fats and oils is only 12 g day as compared to the ICMR recommendation of 40 g/day[2]. World production of sunflower seed oil has increased from 2741 thousand metric tonnes to 4558 thousand metric tonnes from 1964 to 1974[3]. In the present study composition, storage and acceptability of sunflower oil (*var. Aarmaveric E.C.*—68415) produced at Hyderabad was compared with sunflower oil (Safflo) produced at Canada. Groundnut oil was used as standard for comparison as it is well accepted and widely studied.

The fat content of sunflower seed ranges from 22 to 36 per cent as reported by several investigators[4]. Variation in the linoleic acid (44-72 per cent) and oleic acid (14-34 per cent) was reported[5] and it was mainly attributed to varietal differences and environmental conditions during the ripening of seeds. Roberts on *et al.*[6] reported that sunflower grown at warmer locations and at lower latitudes had a lower linoleic acid than those grown in cooler locations and higher latitudes.

Materials and Methods

Sunflower seeds were procured from the dry land project, Indian Council of Agricultural Research, Hyderabad. Oil was extracted in the local *ghani* (Screw-press) by the usual procedure applied to any edible oilseeds. Unshelled seeds were used for extracting oil. A sample of sunflower oil (Safflo, brand name) was obtained from Canada. Expeller pressed groundnut oil was obtained from the local market.

Fatty acid composition was determined by gas liquid chromatography.[7] Iodine number,[8] refractive index,[9] peroxide value[10] and acid number[10] were estimated for all the three samples of oil. Schall test[11] was conducted to determine the accelerated oxidation of oils. Absorption of oil by some of the deep fried foods used in India was determined by soxhlet method.[12] A sample of the sunflower oil was also hydrogenated with the assistance of Regional Research Laboratory, Hyderabad.

Sensory evaluation of food products prepared with sunflower oil and groundnut oil was carried out using a trained panel of eight judges selected from the Institute staff. They were selected on the basis of their sensitivity and recognition thresholds and their performance in duo-trio tests. Judges were trained for a fortnight using both triangle tests and duo-trio tests. Since the objective of final evaluation was to test the acceptability of oil even during training fried and baked foods prepared with different vegetable oils were used as test products. They were trained to differentiate as well as assign suitable scores for the samples by descriptive scoring and successive scoring. Products tested and methods of cooking adapted are shown below:

Product	*Method of cooking*
Poorie, Boondi	Deep fat frying
Chapati, Dosa	Shallow frying
Sambhar, Vegetable curry	Seasoning
Mayonnaise, vinegrette	Salad dressing
Plain sponge cake & biscuits	Baking

Hydrogenated fat was used for baked products. Colour, flavour, texture and taste of all products were evaluated first by descriptive scoring. Subsequently the same products were tested by successive scoring based on the principle of hedonic scale for testing the like-dislike effect and quality judgement. An interval of at least 3 to 4 days was maintained between the two tests for each product.

Consumer acceptance of sunflower oil (Hyderabad) was evaluated in comparison with the commonly used vegetable oils. Eighty families of the college students representing the high and middle income groups were selected for consumer acceptance study. These 80 housewives were asked to prepare *Poorie* (a deep fat fried product of wheat flour) *Papads* (a deep fat fried product of pulses), chapaties (a shallow fried wheat product) and a vegetable curry with the oil they usually use and sunflower oil supplied to them on the same day. Opinion of the consumers was collected through a personal interview schedule.

Results and Discussion

Remarkable difference in the composition particularly in the oleic and linoleic fraction was observed between Hyderabad and Canada sample (Table 1). Linoleic fraction was much higher in Canadian sample. But compared to groundnut oil, sunflower oil (Hyderabad) had higher linoleic acid. Eric *et al.*[13] and Cummins[14] also reported higher values of 71.56 and 61.8 per cent of linoleic respectively for sunflower oil samples from Canada. Cummins[14] reported 43.5 per cent linoleic acid for a sample of sunflower oil from Russia. The lower values of linoleic acid in Hyderabad sample may be due to warm climatic conditions under which sunflower seeds were grown.

Sunflower oil being more unsaturated had a higher iodine value particularly the oil from Canada (Table 2).

Though fat contributes to the satiety value, excess of absorption of fat is undesirable. Results of the absorption of sunflower oil and groundnut oil by certain deep fried foods are presented in Table 3. The product was allowed to drain by itself in a wire basket for 15 min after frying and the fat absorbed was determined.

TABLE 1. PERCENTAGE OF FATTY ACID COMPOSITION OF SUNFLOWER OIL AND GROUNDNUT OIL SAMPLES

Fatty acid	Sunflower oil			Groundnut oil
	Hyderabad	Canada	Hydrogenated (Hyderabad)	
Palmitic acid	5.70	8.20	0.70	14.81
Stearic acid	2.70	1.17	2.20	0.65
Oleic acid	50.70	15.30	87.15*	51.86
Linoleic acid	40.90	74.83	9.90	31.71
Arachadic acid	—	—	—	0.69
Behenic acid	—	—	—	0.19

*Includes isomers of oleic acid.

TABLE 2. IODINE VALUE AND REFRACTIVE INDEX OF SUNFLOWER OIL AND GROUNDNUT OIL SAMPLES

Sample	I.V.	R.I.(40°C)
Sunflower oil (Hyderabad)	119.5	1.4656
,, (Canada)	126.0	1.4685
Groundnut oil	89.0	1.4640

I.V. = Iodine value; R.I. = Refractive index

TABLE 3. ABSORPTION OF FAT BY DEEP FRIED FOODS

Food sample	*Sunflower oil* Fat content (%)	*Groundnut oil* Fat content (%)
Poorie	10.00	8.75
Murukku	20.00	15.00
Boondi	25.00	20.00
Potato chips	35.30	0.203

Changes in composition of oils on heating: In the process of deep fat frying oils are heated in open pans for one to two hours at high temperature (160 to 200°C). Residual oil is supplemented with fresh oil and reheated for subsequent cooking. Such a procedure is likely to accelerate oxidative changes. Heating, and reheating changes the chemical composition, and also the viscosity of oil. Changes in fatty acid composition and refractive index (index of viscosity) are presented in Table 4.

A major change observed due to heating was reduction in linoleic acid and elevation in oleic acid fraction. Nearly 66 per cent of linoleic acid is reduced due to heating in groundnut oil and 50 per cent in the case of sunflower oil. Nevertheless the major reduction occurred after one hour of heating in both oils which shows the effect of prolonged heating. Fleischnan *et al.*[15] also reported similar observations in groundnut oil and sunflower oil.

Changes on storage of oils: Peroxide value and acid number were determined to assess the extent of rancidity in oil on prolonged storage. Results of accelerated rancidity test on periodical testing are presented in Table 5.

Rancidity of oil judged by the flavour changes was detected in all the three samples on the 7th day and it was pronounced by the 10th day. The relative velocity of rancidity changes was slightly higher in sunflower oil compared to groundnut oil. It is possible that the level of antioxidants was very low to protect them from rancidity changes.

TABLE 4. FATTY ACID COMPOSITION AND REFRACTIVE INDEX OF FRESH AND HEATED OILS

Oil samples	Heating time min	R.I. 40°C	Palmitic %	Stearic %	Oleic %	Linoleic %
Sunflower oil (Hyderabad)	Unheated	1.4656	5.70	2.70	50.70	40.90
,,	30	1.4659	3.54	1.29	59.42	35.73
,,	60	1.4661	5.18	1.58	57.27	35.63
,,	90	1.4663	6.87	1.70	68.45	22.47
Groundnut oil	Unheated	1.4640	14.81	0.65	51.86	31.77
,,	30	1.4647	19.89	0.31	48.11	29.40
,,	60	1.4647	18.82	0.79	57.64	21.56
,,	90	1.4650	27.65	0.27	59.62	11.05

TABLE 5. PEROXIDE VALUE AS DETERMINED BY SCHALL TEST

Storage period (days)	meq peroxide/kg oil		
	Groundnut oil	Sunflower oil (Hyderabad)	Sunflower oil (Canada)
3	25	30	18
5	35	44	32
7	40	55	50
9	55	69	75
10	62	80	80
12	70	98	100
13	85	102	105
14	100	106	110

Effect of heat treatment on shelf-life of sunflower oil and groundnut oil: It is customary to re-use heated oil in food preparation. Potato chips were fried continuously for one and half hours at 180°C. Samples of heated oil were removed from the pan at intervals of 30, 60 and 90 min to determine the peroxide value and acid number. Samples of heated oil were stored at room temperature (27°C) for 60 days. Peroxide values of heated oil during storage are shown in Table 6.

No peroxide value was observed in fresh oil. Prolonged exposure to heat and prolonged storage increased the peroxide value which was expected. The peroxide value of sunflower oil was nearly double that of groundnut oil at all periods of storage. Sunflower sample being more unsaturated, the degree of rancidity might have been more. Groundnut oil was comparatively more resistant to rancidity.

Acid number: it is a measure of hydrolytic rancidity. Heating and subsequent storage had a marked influence on the acid number of the oils. In 60 days of storage, the acid number increased from 0.44 to 1.47 and from 0.36 to 1.20 in unheated samples of sunflower and groundnut oils respectively. In the case of heated samples of sunflower oil, the acid number after 60 days of storage was 1.55, 1.67 and 1.71 respectively for 30, 60 and 90 min of heating. Corresponding values for groundnut oil were 1.32, 1.40 and 1.49 which were not much different from sunflower oil values though slighty lower. Since the onset of hydrolytic rancidity mostly depends on the external factors such as heat and storage than the fatty acid composition, perhaps the difference in the acid number of groundnut and sunflower oil was not much.

Sensory evaluation: Results of descriptive scoring were found to coincide with the results of successive

TABLE 6. CHANGES IN PEROXIDE VALUE ON HEATING AND STORAGE OF OILS

Storage period (days)	Fresh oil		Heating time (min) 30		60		90	
	SF	GN	SF	GN	SF	GN	SF	GN
	←——— meq peroxide/100 g ———→							
30	0.48	0.18	0.54	0.23	0.57	0.31	0.59	0.35
50	0.79	0.33	0.89	0.39	0.91	0.42	0.96	0.46
60	1.10	0.52	1.12	0.66	1.21	0.84	1.23	0.92

SF – Sunflower oil (Hyderabad); GN – Groundnut oil

TABLE 7. MEAN SCORES OBTAINED BY THE DESCRIPTIVE SCORING FOR OVERALL ACCEPTABILITY OF CONTROL AND TEST PRODUCTS

Food product	Control	Test	't' value
Poorie	96.50	95.00	0.90
Boondi	94.08	91.73	0.32
Parathas	94.61	96.13	0.36
Dosa	85.40	89.40	0.36
Vegetable curry	87.60	91.60	0.91
Sambhar	93.60	96.20	0.47
Cakes	85.30	85.00	0.21
Biscuits	91.63	90.12	0.75
Mayonnaise sauce	67.34	70.50	0.82
Vinegrette dressing	67.34	70.50	0.82

rating inspite of the interval maintained between the two tests, indicating the consistency in the scoring. Statistical analysis of the overall acceptability or total scores for colour, flavour, texture and taste of all products showed no statistically significant differences between the sunflower oil products and groundnut oil products.

All the values were not significant. Since results of sensory evaluation indicated acceptance by the judges a large scale consumer trial was also carried out.

Consumer opinion of sunflower oil as cooking medium: Consumers were asked to indicate their opinion over a scale of values for colour, appearance, flavour and taste and whether the foods prepared with sunflower oil was similar, better or worse than the same preparations made with the other vegetable oils to which they were already used to. Majority of the consumers (80 per cent) expressed that *poorie*, *papads*, chapathies and beans curry prepared with sunflower oil was similar in colour. appearance, flavour and taste to the same foods prepared in groundnut oil (Table 7). Tweleve to fifteen per cent felt that sunflower oil products were better whereas 2 to 5 per cent felt sunflower oil products to be worse than those of groundnut oil.

Results of consumer evaluation indicate that sunflower oil imparts flavour qualities to foods similar to that of groundnut oil though a small percentage of consumers expressed it to be better. Majority of the consumers were willing to change over to sunflower oil provided that it is available at a cheaper rate than other vegetable oils in the market. In developing countries where tradition is deeply ingrained, people will not buy a new food product simply on the strength of its low cost and better nutritional qualities unless they are convinced of the sensory qualities of a food.

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Studies on Chemical Composition of Twenty Strawberry *(Fragaria ananasa)* Varieties

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Twenty strawberry varieties were examined for their physical characteristics such as colour, firmness, flavour and size and chemical compositions like total soluble solids, acidity, alcohol insolubles, starch, sugars, anthocyanins, Vitamin C, dry matter, iron, phosphorus, potassium, calcium and crude protein. Wide variations in these constituents were observed. The use of such information as a possible index of quality in starwberry varieties used for processing is discussed.

Strawberry, a soft textured aggregate fruit with highly perishable character is a potential raw material for canning, freezing, jam, jelly and juice preparations in India. Its wide utility in processing industry underlines the scope for its commercial cultivation. In an attempt to select and breed varieties suitable for sub-tropical conditions, a large number of indigenous and exotic varieties have been introduced under the strawberry improvement project of this Institute. The practical utility of nutritional evaluation of germplasm of fruits

and vegetables in identifying superior genotypes has been reported earlier[1-4]. In the absence of such information in strawberry fruit, a study was undertaken with twenty promising varieties and the results are reported in this communication.

Materials and Methods

Fruits (500 g) harvested at the optimum stage of maturity were used for analysis. Physical characters like colour, flavour, firmness and size were recorded by visual observations. The average weight of single fruit, per cent edible matter and per cent calyx were determined gravimetrically. Fruits free from calyx were crushed in a pestle and mortar and pressed through muslin cloth. Total soluble solids in these juice samples were measured using Erma refractometer at 20°C. For the estimation of acidity 25 g fruits were blended with distilled water in a waring blender and the representative sample of the filtered water extract was titrated against standard alkali. Acidity was expressed as per cent citric acid (W/W). Dry matter, alcohol insolubles, starch, total and reducing sugars and ascorbic acid content were estimated by standard AOAC methods[5]. Sucrose was calculated by multiplying the difference between total and reducing sugars by 0.95. Fructose was estimated by resorcinol-thiourea method[6]. Glucose values were obtained by subtracting fructose values from reducing sugars. Estimation of starch in alcohol insolubles was done by colorimetric method[7]. Anthocyanins in fruit pulp was estimated from 1 g of pulp using 100 ml acidic methanol as extractant overnight and the optical density was measured at 540 mμ using spectronic 20. The oven dried material was used for nitrogen estimation and the values were multiplied by 6.25 to get crude protein content. A known weight of oven dried material was digested with tri-acid mixture (nitric acid: sulphuric acid: perchloric acid in the ratio of 10: 1:1) and the digested material was used for the estimation of phosphorus, potassium, calcium and iron[8,9].

Results and Discussion

The fruit characteristics and the analytical data of twenty strawberry varieties grown under the agroclimatic conditions of Hessaraghatta, Bangalore are presented in Tables 1 and 2. Visual parameters like size, colour, firmness and flavour are some important attributes used as criteria of quality in strawberry fruits for dessert as well as processing industry. Observations recorded on these physical characters are of paramount importance in pinpointing superior varieties. Easy removal of calyx and core from the fruits is a desirable character in commercial varieties. Skin abrasion and tissue bruising during handling causes cell breakdown and accelerates the deterioration. Therefore, varieties with firm flesh and tough skin are recommended as superior for commercial utilization.[10] Observations recorded on the textural properties of different strawberry varieties indicated that a few varieties like 'Early Cambridge' 'Fresno', 'Ranadier,' 'Midway,' 'Northwest', 'Shasta', 'Sunrise' and 'Torrey' are hard and firm in texture. Strawberry for canning purposes should be firm in texture, of good colour, flavour and of large size[11]. The present study revealed that a few varieties have favourable fruit size, colour and texture thereby suggesting their superiority over the other varieties. The variations in texture, richness of flavour, colour depths observed amongst different varieties studied suggest their wider scope of utilization.

Fruit size is not only an important attribute in grading strawberry for processing but also is a significant component in reducing the harvest cost. High graded commodity can be expected from varieties of large size. Among the twenty varieties studied, the fruit size as indicated by average fruit weight ranged from 1.85 to 6.10 g. The edible portion after removing calyx ranged from 94.6 to 98.2 per cent. The weight loss by calyx removal is minimum in 'Blakemore' variety.

The colour is an important character to be considered in strawberry fruits for canning, freezing, preparation of jam, jelly, and preserves. The anthocyanins impart red colour to the fruits and hence, its estimation serves as an indicator of colour values. Varieties like 'Midway', 'Fresno', 'Selection IHR 19' and 'Surprise-De-Halls' are comparatively rich in anthocyanin pigments. These could be employed in breeding varieties with superior colour attribute. Although high anthocyanin content varieties are preferred in freezing industry, it is reported that low value is a positive character for canning purposes as they show little tendency to give non-enzymatic browning discolouration.[12]

Ascorbic acid is the predominant vitamin of soft fruits and its estimation serves as guideline in suggesting varieties of high vitamin C content. In our present study, ascorbic acid content ranged from 29.2 to 89.1 mg per 100 g of fruits. Similar variations have been observed by earlier workers[13-16].

The range of values for other constituents studied are: total soluble solids, 5.3 to 14.0; acidity, 0.7 to 1.76 per cent; Brix acid ratio, 3.06 to 12.04; alcohol insolubles, 2.20 to 4.38; starch, 0.27 to 1.00; total sugar, 3.54 to 5.9 per cent; sugar–acid ratio, 2.45 to 6.0; sucrose, 0.06 to 1.53; glucose, 2.12 to 3.99; fructose, 0.7 to 1.39 per cent and glucose–fructose ratio, 2.21 to 3.97. Total soluble solids is a common factor looked in most of the fruits. The wide variations in total soluble solids content observed indicate the possibility of variations in the concentration of sugars, organic acids, mineral salts nitrogenous compounds, pectic substances, tannins and

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF STRAWBERRY VARIETIES

Variety	Colour*	Flavour	Firmness	Size	Av wt of fruit (g)	Edible matter %	Total soluble solids	% acidity (W/W)	Brix acid ratio	Total sugars (%)	Sugar acid ratio	Anthocyanins**
Albriton	Signal Red	Medium	Med hard	Fairly uniform	4.58	98.0	14.0	1.59	8.80	5.27	3.31	0.09
Bangalore local	Currant Red	V mild	Med soft	,,	3.68	94.6	6.3	1.27	4.96	5.04	3.97	0.02
Blakemore	Signal Red	Mild	Med hard	Uniform	2.79	98.2	7.7	1.72	4.47	4.72	2.75	0.09
CH-40	Guardsman Red	V. mild	Med hard	Non-uniform	3.40	97.6	8.8	1.36	6.47	5.09	3.74	0.04
Early Cmabridge	Cherry Red	Medium	Hard	Uniform	3.07	94.8	8.0	1.50	5.33	3.70	2.46	0.05
Fresno	Union Jack Red	V mild	Hard	Non-uniform	4.95	96.6	10.3	1.48	6.95	5.30	3.58	0.10
Granadier	Union Jack Red	V. mild	Hard	Fiarly uniform	6.10	97.5	8.5	1.22	6.96	3.54	2.90	0.09
Midway	Post Office Red	Medium	Hard	,,	2.93	98.0	5.3	1.73	3.06	5.15	2.98	0.17
North west	Current Red	Mild	Hard	Uniform	4.90	96.8	7.8	1.04	7.50	4.02	3.87	0.04
Rear Gourd	Guardsman Red	Medium	Med hard	Non-uniform	3.38	96.7	10.5	1.37	7.66	5.33	3.89	0.06
Red Gauntlet	Union Jack Red	Rich	Med hard	,,	5.03	96.4	8.8	1.76	5.00	4.48	2.55	0.04
Robinson	Post Office Red	Rich	Med hard	Uniform	5.76	97.7	8.6	1.17	7.50	5.59	5.00	0.09
Selection IHR 16	Scarlet	Rich	Med soft	,,	2.94	95.0	8.6	0.81	10.61	5.90	7.29	0.05
Selection IHR 16	Union Jack Red	Rich	Med soft	,,	1.85	96.2	8.1	1.54	5.25	4.07	2.64	0.15
Senga Sengana	Current Red	Medium	Med soft	Fairly uniform	3.13	95.7	10.0	0.83	12.04	5.54	6.83	0.07
Shasta	Turkey Red	Medium	Hard	,,	3.55	95.5	11.0	1.46	7.53	4.39	3.01	0.05
Sunrise	Union Jack Red	Mild	Hard	Non-uniform	3.40	95.3	7.2	1.18	6.10	4.51	3.82	0.03
Surprise-De-Halls	Union Jack Red	Mild	Med soft	Fairly uniform	3.75	95.2	9.0	1.37	6.56	5.44	3.97	0.22
Swiss	Union Jack Red	Medium	Med hard	Uniform	2.70	96.0	8.4	1.29	6.51	4.56	3.54	0.09
Torrey	Current Red	Mild	Hard	Non-uniform	5.94	97.3	3.3	0.72	11.52	4.32	6.70	0.06

*R.H.S. colour chart, The Royal Horticultural Society, London. **Determined by optical density at 540 m μ.

pigments. Presence of large quantity of sugars is an useful attribute for dessert and juice quality. Varieties like 'Albriton', 'Bangalore Local', 'CH 40', 'Fresno', 'Midway', 'Rear Gourd', 'Robinson', 'Selection IHR 16', 'Senga Sangana' and 'Surprise-De-Halls' contained more than 5 per cent total sugars and 1 per cent fructose. The edible quality is determined in part by the brix acid ratio. The suitability of a particular variety for juice making also depends on the balance of acid and sugar, the amount of phenolic constituents, aroma constituents and the amount of vitamins present especially ascorbic acid. Deficiency or excess of one or more components may be overcome by blending, but it is desirable that bulk fruit for manufacture should be of suitable uniform composition.

The concentration of dry matter and mineral constituents ranged as follows: dry matter, 8.7 to 13.0 per cent; Iron 0.39 to 2.55; phosphorus, 14.0 to 23.5; calcium, 7.0 to 35.0; potassium, 320 to 660 mg and crude protein, 0.48 to 0.80 per cent. Although the nutritional constituents available in strawberry fruit are relatively less, more emphasis could be given to varieties with high ascorbic acid, carbohydrate and mineral contents.

The composition in relation to major organoleptic quality of flavour, texture, colour and nutritional constituents is of paramount importance in evaluating strawberry varieties with regard to their relative merits in processing industry. A variety with high quality in fresh state will naturally give a best quality processed product. The analytical data of twenty strawberry

TABLE 2. CHEMICAL COMPOSITION OF STRAWBERRY VARIETIES

Variety	Alcohol insolubles(%)	Starch (%)	Sucrose (%)	Glucose (%)	Fructose (%)	Glucose fructose ratio	Vitamin C (mg %)	Dry matter (g %)	Crude protein (g %)	Iron (mg %)	Phosphorus (mg %)	Potassium (g %)	Calcium (mg %)
Albriton	3.48	0.32	0.73	3.40	1.10	3.09	48.4	11.5	0.66	0.69	19.6	0.66	33.6
Bangalore local	4.12	0.44	0.93	3.11	0.95	3.27	29.2	11.2	0.69	0.78	15.7	0.44	11.9
Blakemore	3.23	0.50	0.23	3.57	0.98	3.57	62.9	10.8	0.50	1.30	14.0	0.47	7.7
CH-40	4.38	0.82	0.88	3.06	1.10	2.78	84.5	11.1	0.49	2.55	15.5	0.50	7.7
Early Cambridge	4.20	0.36	0.63	2.12	0.92	2.30	74.3	11.6	0.59	1.86	17.4	0.58	12.6
Fresno	2.96	0.54	0.12	3.98	1.19	3.34	89.1	8.9	0.67	0.64	19.0	0.36	7.2
Granadier	2.20	0.30	0.06	2.78	0.70	3.97	68.8	9.4	0.61	0.61	23.5	0.39	7.7
Midway	2.96	0.51	1.29	2.61	1.18	2.21	84.7	11.9	0.65	0.60	17.9	0.53	12.6
Northwest	2.32	0.41	0.35	2.76	0.89	3.10	59.3	8.7	0.59	0.76	17.4	0.34	7.2
Rear Gourd	3.32	0.27	0.88	3.33	1.07	3.08	61.1	9.8	0.60	0.39	16.7	0.50	35.0
Red Gauntlet	3.72	0.64	0.35	3.10	1.01	3.06	64.0	9.8	0.54	0.85	18.6	0.35	9.2
Robinson	4.28	0.54	0.97	3.18	1.39	2.28	57.1	10.1	0.60	0.61	16.2	0.52	31.5
Selection IHR 16	3.01	0.52	1.53	3.17	1.12	3.83	68.7	9.7	0.48	1.08	16.5	0.32	7.0
Selection IHR 19	4.12	1.00	0.33	2.97	0.75	3.96	56.6	11.7	0.80	2.34	18.7	0.48	12.6
Senga Sengana	3.65	0.62	0.95	3.41	1.14	3.00	55.0	11.1	0.69	0.87	20.5	0.41	11.9
Shasta	3.85	0.48	0.88	2.48	0.98	2.53	73.1	12.5	0.77	0.63	16.3	0.56	18.2
Sunrise	3.12	0.55	0.55	2.97	0.96	3.09	82.6	9.0	0.50	0.69	21.6	0.35	7.2
Surprise-De-Halls	4.12	0.68	0.22	3.99	1.22	3.27	56.5	10.6	0.53	1.22	21.5	0.45	7.7
Swiss	3.64	0.77	0.29	3.34	0.92	3.69	71.5	13.0	0.63	0.48	22.6	0.49	9.2
Torry	3.40	0.80	0.67	2.79	0.82	3.40	71.0	10.8	0.71	0.62	22.8	0.41	11.9

varieties as reported inthis paper provide a guideline for suggesting varietal superiority in relation to their compositional quality. The present information is also useful for breeding varieties with chemical characteristics which would make them particularly suitable for specific processing purposes.

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Utilization of Filled Milk in Flavoured Milk Preparation

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Flavoured filled milk having fat, 3.5 per cent and SNF, 8.5 per cent was prepared by using coconut oil and low heat spray dried skim milk powder as major ingredients. Acceptability studies indicated high preference for rose and pineapple flavoured milk. Rose flavoured milk prepared from admixture of 50 per cent filled milk and 50 per cent standardised milk was highly acceptable and had a keeping quality of one week.

In urban areas, the flavoured milk is catching up quite fast as is evident from the opening of chain of milk parlours in several states of India. The cost of flavoured milk can be reduced by utilizing coconut oil instead of milk fat which fetches high price in the northern parts of the country. Already considerable quantities of filled milk and filled milk products are on sale in some of the far eastern countries.[1] The objectionable flavour of coconut oil can be masked to some extent by the use of synthetic flavours and sugar.

This paper reports the acceptability and keeping quality of rose flavoured milk prepared from filled milk replaced at different levels with standardised milk.

Materials and Methods

Tinned coconut oil (Tata brand) locally purchased was used. Skim milk powder (low heat spray dried) was obtained from experimental dairy, N.D.R.I., Karnal. Sugar, emulsifier, stabilizer and synthetic flavours were purchased locally.

The amount of coconut oil, skim milk powder, sugar, emulsifier (glycerol monostearate, 0.4 per cent) stabiliser (sodium alginate, 0.02 per cent) and water required for the preparation of flavoured filled milk having fat, 3.5 per cent; SNF, 8.5 per cent; and sugar, 6.0 per cent was determined by calculation and the ingredients were mixed as follows:

Skim milk powder was first thoroughly mixed with sugar, glycerol monostearate and sodium alginate. The dry mixture was then dispersed in water at about 37°C and vigorously agitated so that the powder and sugar were completely dissolved. The mixture was then filtered through a muslin cloth.

Addition of coconut oil: Required quantity of coconut oil was melted and added to the reconstituted skim milk and vigorously stirred.

Holder pasteurisation: The mixture was pasteurised at 71°C for 30 min in a vat containing water.

Homogenization: After pasteurization, the mixture was homogenised at pasteurisation temperature with Manton Gaulin Piston type homogenizer, the pressure on the first and second stage being 175 kg/cm^2 and 35 kg/cm^2 respectively. Filled milk was then stored at 4.0 to 7.0°C for about 3 hr prior to addition of synthetic flavours.

Addition of flavours: To each of these lots, different synthetic flavours like Rose, Cherry, Lemon, Pineapple, Mango (Alphonso), Strawberry and Almond were added at the rate of 0.4 to 0.8 ml/kg of filled milk.

Rose flavoured milk was prepared separately from the admixture of filled milk and standardised milk (fat, 3.5 and SNF, 8.5 per cent). Standardised milk was replaced by the filled milk at 0, 10, 20 and 50 per cent levels.

Eight such trials were conducted in each of the above experiments. Flavoured milk was offered to a panel of 8 judges drawn from various categories of the Dairy Technology Division, staff for determining the acceptability of the product. Different attributes examined were (*i*) flavour, (*ii*) body and homogeniety, (*iii*) sedimentation and (*iv*) acceptability.

Fat and moisture of skim milk powder were determined as per American Dry Milk Institute (ADMI) method[2], fat and SNF of milk were determined as per ISI method[3].

Moisture, fat and free fatty acids of coconut oil were determined as per ISI methods as applied to ghee[4].

Results and Discussion

The low heat spray dried powder used had a slightly cooked flavour and its moisture and fat contents were 4 and 1.4 per cent respectively. The moisture, fat and free fatty acids (expressed as per cent oleic acid) were 0.3, 99.7 and 0.95 respectively.

The extent of preference is shown by the number of positive signs.

TABLE 1. ORGANOLEPTIC EVALUATION OF FLAVOURED FILLED MILK PREPARED FROM DIFFERENT SYNTHETIC FLAVOURS (8 TRIALS)

Flavour added	Preference ratings
Control	—
Cherry	+
Mango-Alphonso	+
Pineapple	+++
Almond	—
Lemon	++
Strawberry	+
Rose	+++

—, Not acceptable; +, Acceptable.

Only control samples showed distinct coconut flavour. Other samples had slight coconut flavour but were acceptable. The body was good for all the samples including control; no fat separation or sedimentation was found in any of the samples (Table I). The study revealed that the addition of sugar and flavour improved the flavour of flavoured filled milk. The technological procedure adopted in the study resulted in a product which showed semblence with natural flavoured milk in terms of homogeneity and body. Amongst the synthetic flavours used in the study, rose and pineapple were highly effective in masking the coconut flavour. Prakash *et al*[5] also reported the high preference for rose flavour as it masked the beany flavour of soy milk preparation. Almond flavour was found to be least effective. Study pertaining to the masking off of the flavouring compounds from coconut oil by the addition of sugar and synthetic flavour was not carried out.

At all levels of addition of standardised milk, the samples had acceptable flavour and good body. Neither fat separation nor sedimentation was observed in any of the samples. The acceptability and keeping quality studies of rose flavoured milk prepared from admixtures at various levels of filled milk and standardised milk are given in Table 2. It is obvious that the acceptability level increased with the increased level of the admixture of natural standardised milk. The keeping quality of such milk was observed to be on an average of over 7 days at 4°C.

TABLE 2. ORGANOLEPTIC EVALUATION AND KEEPING QUALITY OF ROSE FLAVOURED FILLED MILK PARTLY REPLACED BY ROSE FLAVOURED MILK

Standardised milk in filled milk (%)	Preference ratings	Keeping quality at 4°C (days)
0	+	>7
10	++	,,
20	+++	,,
50	++++	,,

+, Acceptable.

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Studies on Preparation, Standardization and Organoleptic Scoring of Soymilk

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An improved method of preparation of soymilk has been evolved after critical evaluation of the effect of mild alkali or metabisulphite treatment, dilution factor, soaking time and steaming upon palatability and protein extraction. Optimal results were obtained when steam was passed immediately after slurrying, metabisulphite was added at 0.05% concentration, dilution was kept at 1 part soybean: 6 parts water (W/V), and soak water was used for homogenization. Palatability of soymilk and blend with cowmilk was, on the average, rated as good.

Soymilk, being cheaper, holds out a considerable promise as an effective substitute for cow's milk[1-3]. It promoted growth in infants allergic to cowmilk[4]. However, the typical 'beany' flavour which develops due to a volatile compound, 1-octen-3-ol following activation of lipoxidase enzyme[6] constitutes the most serious impediment against popular acceptability of soymilk.

Poineering studies of De and Subrahmanyan[7] and Desikachar *et al.*[1] have helped in standardizing the conditions for preparation of soymilk. The high temperature rapid hydration-cum-grinding process is stated to ensure optimal protein extraction from the seeds and also minimize the chances of development of off-flavour.[8,9] It was, therefore, considered worthwhile to examine the possibility of obtaining a better product by introducing simple modifications in the technique. The results of an improved method of small scale soymilk preparation are described in this paper.

Materials and Methods

The required amount of soybean seeds were, soaked, boiled for 10 min and dehulled, were homogenized with hot water and boiled for 10 min with constant stirring. Two extracts were taken by straining the slurry through the muslin cloth and resuspending the residue in hot water and straining again. The combined extracts were strained through 8 folds of muslin. The final volume was adjusted as required, one teaspoonful of sugar was added per 350 ml and soymilk was gently heated for 30 min. The different treatments tried included: (*i*) soaking in 0.03 per cent sodium bicarbonate solution; (*ii*) different dilutions, *viz.* 1:5, 1:6 and 1:7; (*iii*) variation in soaking time, *viz.* 3, 4, 6, 12 and 18 hr (overnight); (*iv*) cooking the soybeans in the domestic pressure cooker for 5 and 10 min before dehulling; (*v*) steaming the slurry and the final milk instead of boiling; (*vi*) adding the soaked soybean to boiling water; (*vii*) using the soak water from boiling-dehulling for slurrying; and (*viii*) boiling the soaked soybean in 0.03, 0.05 and 0.07 per cent sodium metabisulphite solution. The soymilk obtained, was rated for general acceptability by laboratory personnel against cowmilk and blends (soymilk admixed with cowmilk, 2:1 and 4:1) on a 10 point scale.

Results and Discussion

On the basis of a series of trials, designed to evaluate the extent of influence of various factors upon the acceptability and protein content of the resultant product, the conditions and procedures were standardized:

Weighed quantity of soybean was soaked for 3 hr and cooked for 10 min in the preboiled soak water to which sodium metabisulphite (175 mg/350 ml) was added. The seeds were manually dehulled and homogenized in an electric blender using soak water from the boiling-dehulling process; the slurry was steamed for 10 min in the from a fine jet. Two extracts were collected by straining the slurry through 4 folds of muslin, resuspending the residue in hot water (2 min) and straining again. The combined extract, strained through 8 folds of muslin, was gently heated for 30 min. A teaspoonful (approx. 2-3 g) of sugar was added per 350 ml of soymilk and the volume was made upto the required extent to give the final ratio of soybean 1 part water 6 parts (W/V).

Plain water was preferred over sodium bicarbonate

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TABLE 1. AVERAGE PROTEIN CONTENT AND GENERAL ACCEPTABILITY OF SOYMILK *VS* COWMILK AND THEIR BLENDS

	Protein content (g/100ml)	Appearance	Consistancy	Palatability	Flavour
Cowmilk	3.28	9.7E	9.5E	9.7E	9.6E
Soymilk	3.96	7.6G	7.9G	7.0G	7.3G
Blended milk (a) soymilk (2:1, V/V)	3.76	8.3VG	8.4VG	7.8G	7.9G
Blended milk (b) soymilk (4:1, V/V)	3.85	8.0VG	8.0VG	7.4G	7.7G
Significance**		**	**	**	**
S.E.		0.074	0.07	0.103	0.094
C.D. 5%		0.2	0.196	0.287	0.261
C.D. 1%		0.27	0.26	0.378	0.345

**$P < 0.01$ @, Average of 10 replicates
VG, Very good; E, Excellent; FG, Fairly good; G, Good

solution because the latter failed to improve the palatability. Acceptability was best when the ratio of soybean and water (W/V) was kept 1:6, and soaking time was reduced to 3 hr only. Pressure cooking prior to dehulling was abandoned because it has an adverse effect upon palatability.

Protein extraction as well as palatability were found to improve appreciably by slurring in pre-boiled soak water and steaming. A significant fact which has come to light is that the protein content of soymilk can be increased to a level which matches cow's milk with only simple modifications in the technique outlined above. (Table 1). Soak water contains a considerable proportion of soluble protein (albumin) present in soybean. At an optimum concentration (0.05 per cent) metabisulphite also improves the appearance, possibly through prevention of browning during heating[10] and also the palatability.

The results of the present study clearly show that soymilk prepared under carefully controlled conditions could be popularized. Studies in the Philippines[11] have also demonstrated that the boiling water grinding technique could be easily adopted to cater to local demand with limited initial capital investment. Soymilk can meet a substantial part of the protein requirement of school children. The present study also indicates that while soymilk as such is quite acceptable, its palatability could be further improved by blending with cowmilk at 1:4 proportion; the cost of the blend is estimated to be less than one third of cowmilk.

Acknowledgement

Encouragement received from Dr. D. P. Motiramani, Director of Research Services, J. N. Krishi Vishwa Vidyalaya, Jabalpur is gratefully acknowledged. We also thank shri Kripashankar for statistical analysis of the data.

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Comparative Antimicrobial Action of Certain Antioxidants and Preservatives

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The antimicrobial effect of three antioxidants viz. BHA, BHT and PG were studied against *Bacillus subtilis*, *Saccharomyces cerevisiae* and compared with a few other antimicrobial agents. BHA was found to have inhibitory effect against all the test organisms. The activity was highest against *Saccharomyces cerevisiae*. At lower concentrations, it delays germination and appears to interfere with conidial formation and colour development in fungi.

The antioxidant propoerties of BHA, BHT, gallates etc have been known for a long time and have been commonly used in reducing the rancidity development in meat, fish, fish byproducts, oils etc[1,3]. However, information regarding the effect of these antioxidants on microorganisms is fragmentary. Kaufmann and Ahmad[1] found an inhibitory effect of nordihydroguaiaretic acid (NDGA) on *Saccharomyces cerevisiae*. It has been reported that 1 per cent BHT is slightly inhibitory towards *Salmonella senftenberg*[5]. More recently, Chang and Branon[6] found BHA to be inhibitory towards *Aspergillus parasiticus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimurium*. The present study is an attempt at finding out their role in microbial growth inhibition.

Materials and Methods

Three commercially available antioxidants namely butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) commonly used in foods and a few antimicrobial agents used in foods viz. calcium gluconate, sodium sorbate, nisin, sorbic acid and orange oil were obtained from trade (Orange oil was very kindly supplied by Nagpur Orange Growers' Association). The test organisms were, a grampositive aerobic spore former *Bacillus subtilis*, a fermentative yeast—*Saccharomyces cerevisiae* and two toxigenic moulds, *Aspergillus flavus* and *A. fumigatus* which thus represented a heterogenous group. Such a heterogenous group was chosen to know whether the antioxidants exerted any selective inhibition. The test organisms had been isolated from various food materials in the laboratory and maintained on specific media. Fresh transfers were made from stock cultures, *Bacillus subtilis* being grown on nutrient agar slant at 37°C for 24 hr, *Saccharomyces cerevisiae*, *Aspergillus flavus* and *A. fumigatus* on potato dextrose agar slants at 32°C, the former for 24 hr and the latter two for 4 days as per standard methods[7].

The inhibitory action of the antioxidants and the antimicrobial agents were initially assayed by the disc diffusion method using Whatman No 1 filter paper discs of 15 mm diameter (Table 1). However, as plate count had to be done each time to know the exact cell concentration in the seeding medium, disc diffusion assay technique was abandoned and further studies were done by the plate count method. For this, the antioxidants and the antimicrobial agents were added in the appropriate concentrations into the culture media before sterilisation. The orange oil was transferred aseptically to the sterilized media to eliminate the

TABLE 1. EFFECT OF ANTIMICROBIAL AGENTS AND ANTIOXIDANTS ON THE TEST ORGANISMS BY THE DISC DIFFUSION METHOD

Test material	*B. subtilis*	*S. cerevisiae*	*A. flavus*
1. Orange oil	Nil	35 mm	28 mm
2. Calcium gluconate	Nil	Very slight	Very slight
3. Sodium sorbate	,,	,,	,,
4. Nisin	,,	Nil	Nil
5. Sorbic acid	,,	24 mm	22 mm
6. BHA	Very slight	Slight	Slight
7. BHT	No inhibition	Nil	Nil
8. PG	,,	,,	,,

TABLE 2. EFFECT OF VARIOUS PRESERVATIVES AND ANTIOXIDANT AT A CONCENTRATION OF 2500 PPM ON THE PLATE COUNT OF THE TEST ORGANISM

Name of preservative/ antioxidant	*B. subtilis*	*S. cerevisiae*	*A. flavus*
1. Orange oil	—	15×10	27×10^2
2. Nisin	20×10^4	16×10^4	17×10^4
3. Sorbic acid	27×10^3	20×10	30×10^2
4. Sodium sorbate	27×10^4	80×10^2	10×10^4
5. BHA	No visible growth		
6. BHT	26×10^4	18×10^4	17×10^4
7. PG	26.5×10^4	17.5×10^4	17.5×10^4
8. Calcium gluconate	26×10^4	32×10^2	40×10^2
9. Control	27×10^4	18×10^4	17.5×10^4

TABLE 3. EFFECT OF BHA AND CITRUS OIL AT VARIOUS CONCENTRATIONS ON THE TEST MICROORGANISMS

Test material	Concentration ppm	*B. subtilis*	*S. cerevisiae*	*A. flavus* (5 days reading)	(12 days reading)
BHA	2000	30×10^5	20×10^4	Nil	32×10^4*
	1500	10×10^6	48×10^4	,,	40×10^5*
	1000	40×10^6	31×10^5	,,	15×10^6*
	750	85×10^6	72×10^5	,,	2×10^6*
Orange oil	2000	—	12×10^3	18×10^3	—
	1500	—	25×10^3	31×10^3	—
	1000	—	20×10^4	42×10^5	—
	750	—	80×10^4	80×10^5	—
Control		73×10^7	48×10^6	83×10^6	—

—, Not done; *White buttony structures.

possibility of any loss of oil due to sterilisation. Serial dilutions of the test organisms were made in Ringer's solution and grown on culture media in petri dishes. controls were run in all cases for initial viable numbers. Since nisin did not exhibit inhibitory effect against *B. subtilis* and *S. cerevisiae*, its further use in the study was abandoned.

Results and Discussion

The average results of 5 replicates of the effect of various preservatives and antioxidants at a concentration of 2500 ppm on the three test organisms are given in Table 2. As the inhibitory effect was highest in the case of sorbic acid and orange oil among preservatives and as there was no inhibition in the case of antioxidants except BHA, lower concentrations were studied only in the case of orange oil and BHA. Nisin did not show any inhibitory effect against *B. subtilis* or *S. cerevisiae*. Perhaps its activity is purely heat dependent[8]. BHA was effective in completely inhibiting growth at a concentration of 2500 ppm at a cell concentration of 10^{4-5}/ml. As seen from Table 3, lower concentrations delayed the germination of spores by 6-12 days and the appearance was very different from normal colonies. They were white buttony structures with no formation of colour or conidia. To ascertain if this effect was specific towards *A. flavus* only, similar studies were carried out with *Aspergillus fumigatus* and another unidentified *Aspergillus* sp. The effect was found to be similar.

BHA thus exhibits a fairly high antimicrobial effect towards certain bacteria, yeasts and moulds. However, this was low compared to the reported inhibition[6] and may be due to the method of dispersion. The activity was highest in the case of *S. cerevisiae*. Chang and Branon[6] observed that BHA is less effective against gram negative bacteria. In view of the phenolic nature of these antioxidants, microbial inhibition is probably expected. However, it was interesting to note that in the concentrations studied i.e. upto 2500 ppm, the other antioxidants had no inhibitory effect. The effect of BHA at lower concentrations was more interesting. The germination of spores was delayed by 5-12 days and when the spores germinated and grew, no conidia were formed and even the hyphae did not develop any colour. The exact mechanism of this non-development of colour is not known. A possible explanation may be that since all filamentous fungi are obligate aerobes, even the very little oxygen required for colour development or reproduction may not be available. In the case of many filamentous fungi the actual concentration of oxygen needed has been observed to be higher for reproduction than vegetative growth[9]. Even the delay in spore germination may be perhaps due to the same reason.

It thus appears that in addition to acting as an antioxidant in controlling the rancidity development in foods in which it is used, BHA may also aid in delaying the germination and growth of spoilage microorganisms at lower concentrations and inhibiting them at higher concentrations. Its activity against sporulation of toxigenic fungi like *A. flavus* and *A. fumigatus* is of particular significance in cured meat, fish, etc where it is used.

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A Study on the Proteolysis of Fat Globule Membrane Proteins Isolated from Buffalo Milk*

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Fat globule membrane proteins (FGMP) of buffalo milk exhibited time and concentration dependent hydrolysis by trypsin. Except protein components V and VI all other components were almost completely cleaved at higher concentration of trypsin. Glycoproteins were relatively more resistant to proteolysis than other protein components. Proteins of ultracentrifugally classified membrane pellets exhibited similar behaviour towards proteolysis indicating apparent similarities between these fractions. The rate of proteolysis was higher in FGMP from cow milk than from buffalo milk. However, buffalo milk FGMP and its fractions showed similarities in the rate of proteolysis. Sterilization of milk retarded proteolysis of FGMP, whereas chilling accelerated such enzymatic cleavage.

The lipoprotein envelope around the fat dropet is a complex system oriented at the fat/plasma interface and plays and important role in stabilizing fat emulsion in milk and milk based preparations. Morphological and biochemical observations have shown fat globule membrane to be a true biological membrane[1,3]. However, Wooding[4] through electron microscopic observations expressed his doubt regarding the intactness of the milk fat globule membrane. In a more recent study Kobylka and Carraway[5] observed that trypsin or pronase could cleave all the major associated membrane proteins in intact fat globules or in isolated membrane thereby demonstrating that membrane surrounding the fat globule does not represent a significant permeability barrier to proteolytic enzymes. However, their studies were confined to intact lipoprotein complex only. Hence, the present study was undertaken to investigate the mode of trypsin action on delipidated FGMP and its various fractions. This paper also reports some preliminary observations on the rennet action on FGMP.

Materials and Methods

Buffalo milk samples (Murrah breed) were collected from Institute's herd. Trypsin (Lot 62C-2040) was purchased from Sigma Chemical Company, USA. Rennet was purchased from Hansen Laboratory, Copenhagen, Denmark. Acrylamide and N N'-Methylenediacrylamide were the products of E. Merck, Germany. N, N, N', N'-Tetramethylethylenediamine was obtained from Koch-Light Laboratories, England. All other chemicals were of analytical grade.

FGMP and its various fractions were prepared following the methods reported earlier[6]. The procedures used for pasteurization, boiling, sterilization and chilling milk have been described elsewhere[7].

Assay system for trypsin action: In all the assay systems substrate concentration was kept constant (10 mg of FGMP sample in 2 ml of phosphate buffer, pH 7.2 containing 15 mg sodium dodecyl sulphate). The contents were then maintained in a water bath at 37°C and after 5 min a definite aliquot of the trypsin solution was added. The reaction was terminated after definite intervals by adding 0.02 ml 2-mercaptoethanol and placing the tube in boiling water bath for 5 min. Polyacrylamide gel electrophoresis in SDS-system was carried out according to Weber and Osborn[8]. Glycoprotein components in the gel were detected by the method of Fairbanks *et al*[9]

Assay system for rennet action: Butter-milk obtained by churning four times washed cream was exhaustively dialysed against distilled water. To 10 ml of the butter milk (protein concentration 50 mg/ml), 0.3 ml of rennet solution (50 mg/ml) was added and the contents incubated in a water bath at 30°C for 1 hr. The reaction was terminated by adding 1.6 ml of 80 per cent TCA and the contents were filtered through Whatman No. 42 filter paper. Two ml of the clear filtrate was used for determining the released sialic acid by the method of Warren[10]

Results and Discussion

Time dependent hydrolysis of FGMP: The FGMP solubilized in phosphate buffer containg SDS was

*Part of the paper was presented at the 44th Annual reeting of the Society of Biological Chemists (India) held at University of Calcutta on October 27–29, 1975.

N.D.R.I. Publication No. 76–53.

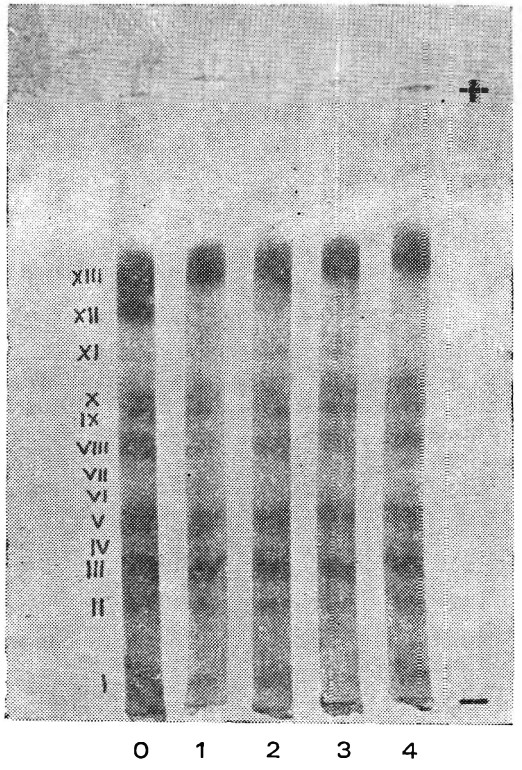

Fig. 1. Time dependent hydrolysis of fat globule membrane protein by tryps in (200 μg/ml). 1, 2, 3 and 4 indicate incubation period (hr) and 0 indicates the control.

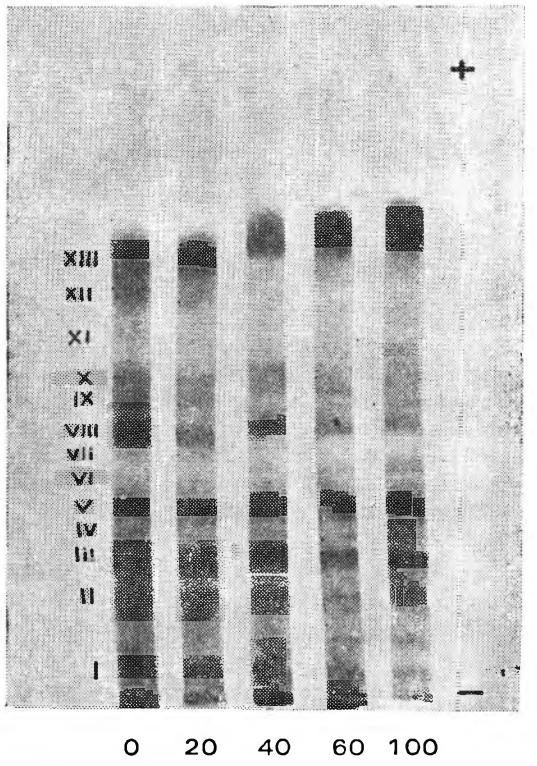

Fig. 2. Rate of proteolysis of fat globule membrane protein with increasing concentration of trypsin. 20, 40, 60 and 100 indicate concentration of trypsin (μg).

treated with 0.5 ml of trypsin (200 μg/ml). The reaction was terminated after 0, 1, 2 and 3 hr of incubation periods. Fig. 1 depicts the resolution patterns of the trypsin treated FGMP specimens by SDS-polyacrylamide gel electrophoresis. It is evident from the electropherogram that component XII was most susceptible to trypsin which disappeared after one hour of the reaction. Bands V and VI appear to be more resistant to proteolysis as compared to other bands. It may be worthwhile to mention that band XIII was mainly contributed by trypsin. From this experiment it is clear that after one hour of incubation no further changes appeared in the electropherogram. Hence, in all subsequent studies the reactions were terminated after two hours.

Proteolysis with increasing concentration of enzyme: The solubilized FGMP was incubated with different concentrations of trypsin and the reactions were stopped after 2 hrs. It is again interesting to note that components XII and VII gradually disappeared from the electropherogram as the concentration of trypsin increased from 20 to 40 μg suggesting their accessibility to trypsin (Fig. 2). Component VIII and V underwent a concentration dependent cleavage to give polypeptides of slightly lower molecular weight. However, these polypeptides were also completely cleaved at a higher trypsin concentration in the system (Fig. 3). Kobylka and Carraway[5] also reported similar phenomena in bovine milk FGMP. From the electropherogram it is further evident that trypsin could act on all the protein components in buaffalo milk FGMP indicating that none of these components is inherently resistant to proteolysis.

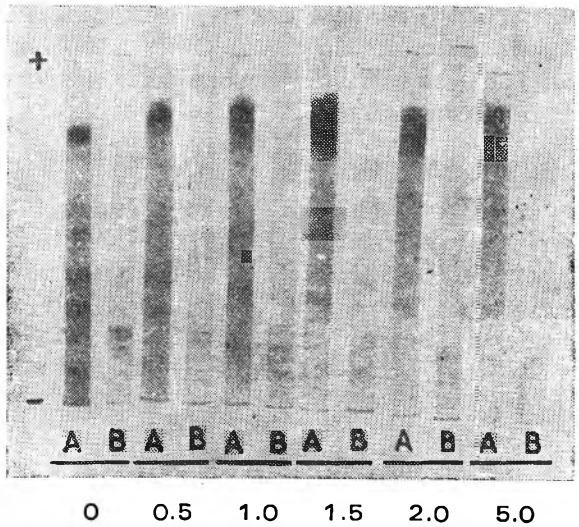

Fig. 3. Rate of proteolysis of fat globule membrane protein with increasing concentration of trypsin. 0.5, 1.0, 1.5, 2.0 and 5.0 indicate concentration of trypsin (mg).

A. Protein; B. Glycoprotein.

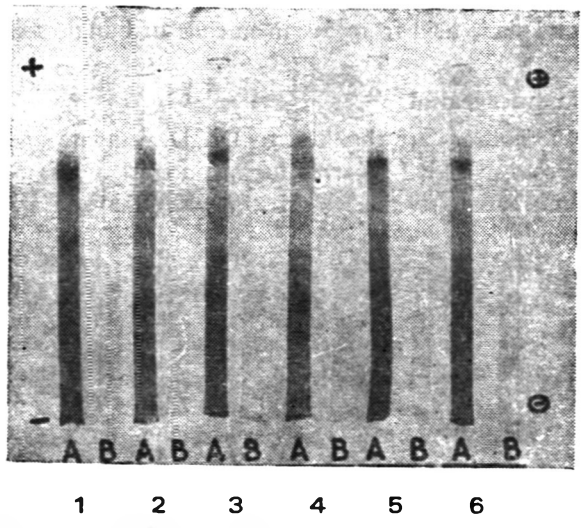

Fig. 4. Proteolysis of fat globule membrane protein fractions by trypsin (200 μg/ml).

Untreated:—1. Whole; 3. Butter serum; 5. Insoluble.
Treated:—2. Whole; 4. Butter serum; 6. Insoluble.

Fate of glycoproteins in FGMP on proteolysis: Electrophoretic resolution patterns of FGMP treated with 0.5 1.0, 1.5, 2.0 and 5.0 mg of trypsin are shown in Fig. 3. The gels were stained for both protein and glycoprotein. Thirteen protein and five glycoprotein components could be detected in the electropherogram. Fig. 3 shows that except components V and VI all other components almost completely disappeared from the electrophero. gram at trypsin concentration of 1.5 mg in the system-

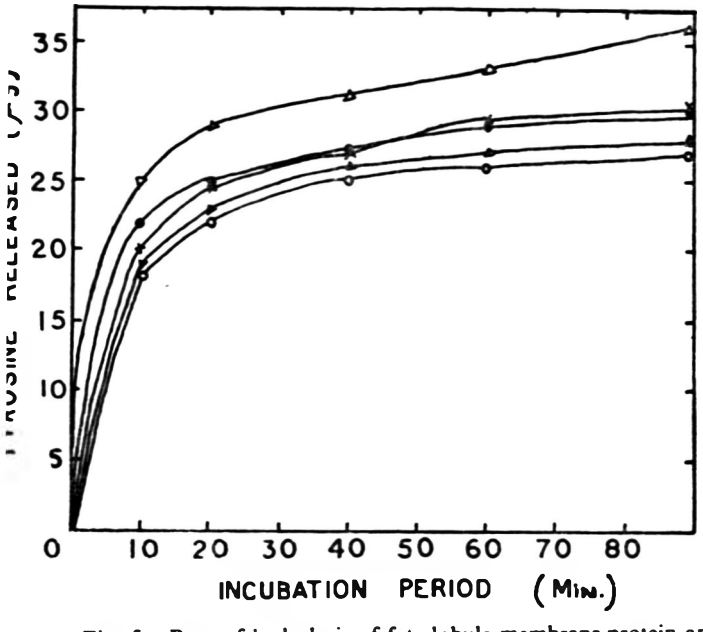

Fig. 5. Rate of hydrolysis of fat globule membrane protein and its fractions by trypsin.

×—×—×, Cow; ●—●—●, buffalo; O—O—O, inseluble fraction; △—△—△ Butter serum.

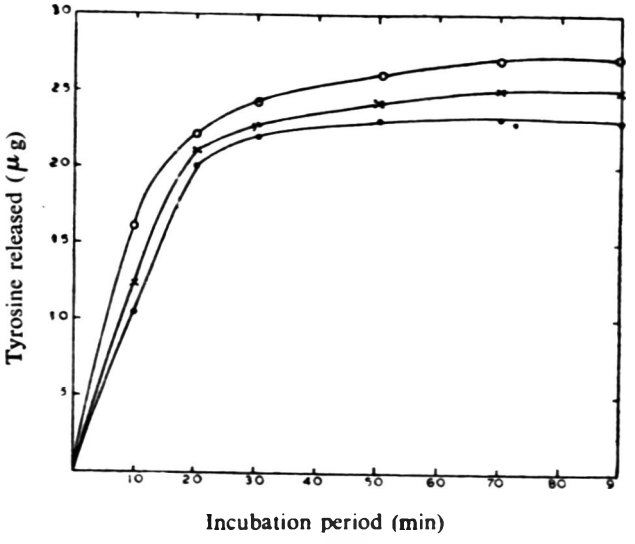

Fig. 6. Rate of hydrolysis of ultracentrifugally classified fat globule membrane protein fractions by trypsin.

These observations reveal that protein components V and VI are most resistant towards trypsin action. However, glycoprotein components were found to be more resistant to proteolysis than other protein components.

Proteolysis of ultracentrifugally classified FGMP, fractions: Fig. 4 shows the digestion patterns of FGMP and its fractions by trypsin. It is evident from the figure that digestion patterns were more or less similar in all the cases. It may be of interest to mention that the FGMP specimens isolated from ultracentrifugally classified membrane pellets also behaved similarly towards proteolysis revealing their similar characteristics. These observations are in agreement with our earlier electro-

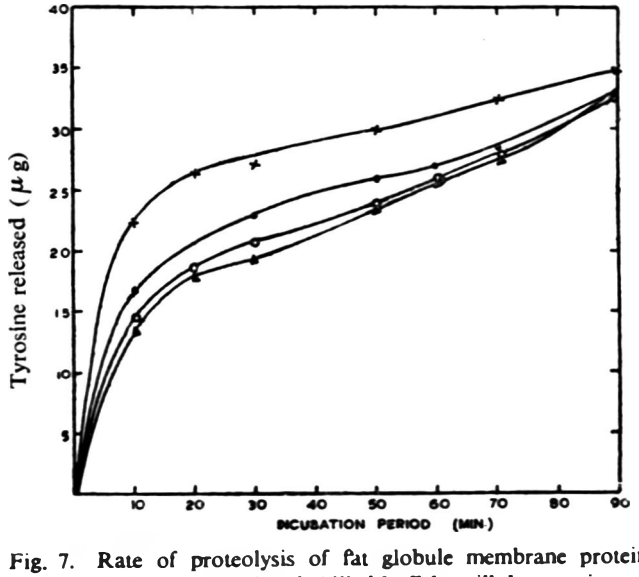

Fig. 7. Rate of proteolysis of fat globule membrane protein isolated from heat treated and chilled buffalo milk by trypsin.

●—●—●, Unprocessed; ×—×—×, Pasteurized; △—△—△ Boiled; O—O—O, Sterilized; △—△—△ Chilled.

phoretic data on these proteins[11]. Rate of proteolysis of FGMP and its fractions by trypsin (5 mg) was also investigated (Fig. 5). Protein specimen from cow milk exhibited higher rate of proteolysis than that obtained from buffalo milk. Buffalo milk FGMP and its fractions did not show any appreciable differences in the rate of proteolysis.

Studies on the proteolysis of ultracentrifugally classified FGMP fractions revealed a slight increase in the rate of proteolysis from FGMP obtained from heavier membrane pellet to that obtained from lighter membrane pellets (Fig. 6).

Effect of heating and chilling milk on FGMP proteolysis: Proteolysis of FGMP by trypsin was affected when milk was heat treated or chilled (Fig. 7). It is obvious from the figure that sterilization retarded proteolysis, whereas chilling accelerated the process. These observations could be correlated with tyrosine and phenylalanine levels in these specimens[7]. Paucity of available literature makes it difficult to offer a suitable explanation for the observed differences in the rate of proteolysis. However, Stephen[12] observed a slight lower rate of proteolysis in casein micelle obtained from heated milk than that isolated from chilled milk.

Rennet action on FGMP isolated from buffalo and cow milk was evaluated by measuring the release of sialic acid. It has been observed that during 90 min reaction period rennin could release 26.65 and 24.24 per cent sialic acid from FGMP of buafflo and cow milk, respectively. Further increase in the reaction time had no significant impact on such process. During the reaction, there was no release of free sialic acid. No information is available on the action of rennet on FGMP. It is of interest that rennin could release about 60-90 per cent bound sialic acid from casein micelle of buffalo milk[13].

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Nutritive Value of Weaning Foods and Malted Milk Powder

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Roller-drying of weaning foods in the presence of malt extract lowered protein quality (PER) while processing with sugar in the place of malt extract had no effect on the PER; however, marked reductions in chemically available lysine occurred in both cases. Plasma threonine level was found to serve as a better indicator of lysine damage in weaning foods than available lysine or plasma lysine levels.

Development of processed weaning foods prepared by roller-drying of blends of cereals, legumes and groundnut flour has been reported from this Institute[1]. Specifications for these have been recently drawn up by the Indian Standards Institution[2] and the Protein Advisory Group of United Nations[3]. In these specifications, emphasis has been laid on the protein quality in terms of PER determination. Apart from the PER determination, the only other chemical method available for assessing the loss of nutritive value of proteins during processing is the 'available lysine' (FDNB) method[4]. Although good correlation has been reported between available lysine values and growth response of animals for animal proteins and cottonseed meals, there are other reports indicating discrepancy between the values obtained by FDNB method and growth assay procedures using rat or chick for heat-treated foods[5-8]. No chemical methods are available for measuring the nonavailability of other amino acids which may become unavailable during processing[9].

Measurement of plasma amino acid (PAA) concentrations has been recommended for predicting the limiting amino acids in diets[10]. Narayana Rao and McLaughlan[11] have shown that this technique can provide useful information on the effect of processing on amino acid availability. The present communication deals with the effect of processing on the nutritive value of weaning foods as measured by PER and PAA methods.

Materials and Methods

The composition of the weaning foods perpared with added sugar or malt extract is shown in Table 1. The different samples used in the experiements are as follows: (*a*) the raw blend containing sugar without any processing, (*b*) raw blend as in '*a*' cooked in water (1:4) and dried on a laboratory roller drier, and (*c*) sample '*a*' cooked and dried on a roller drier after addition of malt extract instead of sugar. A properietary malted milk product obtained from the local market was used for comparison.

The protein content of the samples was determined by the micro-kjeldahl method. The essential amino acid contents in the various samples were determined according to methods described earlier[12]. Available lysine was estimated by Carpenter's method[4]. The results are shown in Table 2.

Determination of PAA level: Feeding experiments to determine the PAA levels were carried out as follows:

Table 1. Composition of the blend used for preparing weaning foods

	Parts
Roasted groundnut cake flour ..	15
Roasted Bengal gram flour ..	12
Roasted green gram flour ..	13
Sesame flour (solvent extracted) ..	15
Rice flour ..	10
Barley flour ..	10
Hydrogenated groundnut oil (Vanaspati) ..	4
Sugar or malt extract ..	7
Common salt ..	3
Calcium carbonate ..	1
Tricalcium phosphate ..	1
Vitamin premix* ..	1
Protein content ..	25.6–27.9%

*Contained 3000 I. U. of vitamin A, 300 I. U. of vitamin D 0.7 mg of thiamine hydrochloride, 1.1 mg of riboflavin, 7.0 mg of niacin and 30.0 mg of vitamin C.

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TABLE 2. ESSENTIAL AMINO ACIDS AND AVAILABLE LYSINE IN WEANING FOODS AND MALTED MILK POWDER

	Lysine	Available lysine	Methionine	Cystine	Threonine	Tryptophan
			g/16gN			
Weaning food (raw blend)	4.0	3.6	1.2	1.0	2.9	1.5
Weaning food (with sugar)	4.3	2.1	1.3	1.1	3.0	1.5
Weaning food (with malt extract)	4.3	1.4	1.4	1.0	3.0	1.5
Malted milk powder	3.6	1.9	1.6	0.6	3.2	1.9

Male weanling rats belonging to the Wistar strain, 8 per group, were housed individually in cages with wire-mesh bottom and were given diets containing the various food products for one week. The diets contained (in per cent) the following ingredients: groundnut oil 10, salt mixture[132], vitamin mixture[141], weaning foods or malted milk powder to provide 10 per cent protein in the diet and corn starch to make to 100 per cent. The diets were also supplemented with lysine, methionine and threonine to raise their levels to those of human milk proteins. Food intake was restricted by removal of diets from the cages for 6 hours daily. This feeding regimen was followed to induce the rats to start eating as soon as the diets were placed. The rats were killed 6 hours after replacing the diet, as in this period, the limiting amino acid falls to a low level in the plasma. To obtain fasting PAA levels, a group of 8 rats fed on skim milk powder diet was fasted for 16 hours, killed and blood was taken for analysis. PAA scores were calculated as described previously[15]. The concentrations of free amino acids in the plasma of individual rats were determined by microbiological methods[16]. The results are presented in Tables 3 and 4.

Protein efficiency ratio: This was determined by the standard procedure[14]. Male weanling rats 21 days old, were allotted to comparable groups and the animals were kept in individual cages. Weighed quantities of diets were served daily and the diet was moistened with hot water and placed in the cages. The residual diet was dried in hot air and the food consumption of each rat calculated. Weekly weight gains of rats were recorded and the protein efficiency ratio was calculated at the end of 4 weeks. The results are shown in Tables 5 and 6.

Results and Discussion

Amino acid contents: The results in Table 2 indicate that there are no marked variations in the contents of

TABLE 3. PLASMA AMINO ACID LEVELS IN RATS FED MALTED MILK POWDER

Group No.	Diet	Concentration in plasma (μg/ml) Lysine	Methionine	Threonine	Tryptophan
I.	Malted milk powder	28.1(33*)	3.7(60*)	55.1(82*)	14.1(99*)
II.	Malted milk powder + L-lysine (2.0 g/16 g N)	56.6(67*)	4.2(68*)	35.7(53*)	13.8(97*)
III.	Malted milk powder + L-lysine (2.0 g/16 g N) + L-methionine (1.5 g/16 g N)	55.2(66*)	5.4(87*)	41.4(62*)	14.8(104*)
IV.	Malted milk powder + L-lysine (2.0 g/16 g N) + L-methionine (1.5 g/16 g N) + L-threonine (1.0 g/16 g N)	63.0(75*)	7.0(113*)	77.6(116*)	13.8(97*)
V.	None (fasted)	84.1	6.2	66.8	14.2

(Experimental period, 1 week; 8 male rats per group; Protein level 10%)

Results of statistical analysis by Wilcoxon-Mann-Whitney test at 5% level

Lysine
I II, III, IV V

Threonine
I II, III IV V

Methionine
I, II II, III IV, V

Tryptophan
I, II, III, IV, V

*PAA Scores; *No significant difference.*

TABLE 4. PLASMA AMINO ACID LEVELS IN RATS FED WEANING FOODS

Group No.	Diet	Concentration in plasma (μg/ml) Lysine	Methionine	Threonine	Tryptophan
I.	Raw Blend	24.8(37*)	4.3(83*)	16.4(43*)	11.4(109*)
II.	Weaning food (with sugar)	29.9(45*)	4.4(85*)	17.5(46*)	12.3(117*)
III.	Weaning food (with malt extract, W.F.)	27.9(42*)	3.8(73*)	28.3(74*)	11.2(107*)
IV.	W.F. + L-lysine (2 g/16 g N)	74.6(112*)	4.0(77*)	13.6(36*)	10.3(98*)
V.	W.F. + L-lysine (2 g/16 g N) + L-methionine (1.5 g/16 g N)	74.6(112*)	6.0(115*)	14.8(39*)	10.7(102*)
VI.	None (fasted)	66.9	5.2	38.2	10.5

(Experimental period, 1 week; 8 male rats per group; Protein level 10%)

Results of statistical analysis by Wilcoxon-Mann-Whitney test at 5% level

Lysine

I, II, III IV, V, VI

Methionine

I, II, III, IV V, VI II, VI

Threonine

I, II, V I, IV, V II, III VI

Tryptophan

I, II, III, V, VI I, III, IV, V, VI

*PAA Scores; *No significant difference.*

total lysine, methionine, cystine, threonine and tryptophan of the unprocessed or processed samples. While the content of available lysine in the raw mix was very nearly equal to that of total lysine, marked reductions were observed in the available lysine values in the processed samples of weaning foods containing sugar or malt extract. The commercial sample of malted milk powder gave values of 3.6 and 1.9 (g)/16gN) for total and 'available lysine' respectively.

Free amino acid levels in the blood. The level of lysine in the blood was low and PAA scores also indicated that lysine is the limiting amino acid in malted milk powder (Table 3). In the case of weaning food samples, the palsma levels as well as scores were low for lysine and threonine in the unprocessed mix and the sample processed with sugar. For the weaning food processed with malt extract, the plasma level and score for lysine was low, while these values were higher for threonine as compared

TABLE 5. PER OF MALTED MILK POWDER

Group No.	Diet	Protein in diet (N ×6.25%) on dry basis	Food intake (g)	Protein intake (g)	Gain in weight	PER
I.	Malted milk powder (MM)	10.1	113	11.5	9.9	0.85
II.	MM + L-lysine (2.0 g/16 g N)	10.2	194	18.8	42.0	2.11
III.	MM + L-lysine (2.0 g/16 g N) + L-methionine (1.5 g/16 g N)	10.5	199	20.9	48.4	2.31
IV.	MM + L-lysine (2.0 g/16 g N) + L-methionine (1.5 g/16 g N) + L-threonine (1.0 g/16 g N)	10.2	199	20.2	51.3	2.52
V.	Skim milk powder	11.1	253	28.2	100.6	3.57
	Standard error of the mean (24 df)					±0.10

(Experimental period, 4 weeks; 7 male rats per group)

Results of statistical analysis of PER by Duncan's multiple range test at 5% level

I II III IV V

No significant difference.

TABLE 6. PER OF WEANING FOODS

Group No.	Diet	Protein*	Food intake (g)	Protein intake (g)	Gain in weight	PER
I.	Raw blend	11.5	241	27.7	65.9	2.35
II.	Weaning food (with sugar)	11.2	229	25.6	60.7	2.37
III.	Weaning food (with malt extract, W.F.)	11.2	200	22.4	44.6	1.98
IV.	W.F.+L-lysine (2 g/16 g N)	11.2	236	26.4	69.6	2.60
V.	W.F.+L-lysine (2 g/16 g N)+ L-methionine (1.5 g/16 g N)	11.2	226	25.4	67.4	2.64
VI.	Skim milk powder	11.4	204	23.3	79.4	3.42
	S.E. of mean (30 df)					±0.09

(Experimental period: 4 weeks; 7 male rats per group).

Results of statistical analysis of PER by Duncan's multiple range test at 5% level

III I II IV V VI

No significant difference.

*Protein in diet (N×6.25%) on dry basis.

with the raw mix. Similarly, the plasma level as well as score for threonine were moderately high in the case of malted milk powder. These observations are in conformity with those of Morrison *et al.*[17] and Gray *et al.*[18] who observed elevated threonine levels in the plasma of rats and chicks fed lysine deficient diets. Threonine may be deaminated relatively slowly as compared with other amino acids and hence may accumulate in the blood as a consequence of reduced availability of lysine. Supplementation of the food products with amino acids in general resulted in increases in the blood levels as well as scores of the amino acids. No marked changes were observed in the blood levels of methionine and tryptophan of animals fed on raw, processed or lysine-supplemented weaning foods.

Protein efficiency ratio: Supplementation with lysine resulted in marked increases in the PER of weaning food containing malt extract in the formulation and malted milk powder (Tables 5 and 6). These results and the data on available lysine contents and PAA scores for lysine show that destruction of lysine during processing is primarily responsible for lowering the nutritive value of these products. The PER of the weaning food processed with cane sugar was similar to that of the unprocessed raw blend indicating that sucrose can be added safely to weaning food formulations to ensure retention of protein quality during roller-drying. Addition of methionine to lysine-supplemented weaning food did not result in any further improvement in PER. Addition of methionine and threonine to the lysine-supplemented malted milk powder was also not beneficial.

Plasma lysine levels and scores for weaning foods and malted milk powder were found similar; but marked differences were observed in the gain in weight of rats as well as PER on these two foods. These data indicate that as long as lysine is the limiting factor in the diet, plasma lysine levels are less influenced by dietary concentrations of lysine than weight gains or PER. Because of this reason, measurement of plasma lysine alone may provide a misleading estimate of the adequacy of dietary lysine content. Similar observations have also been made by Morrison *et al.*[17] in rats. In the present studies, the plasma threonine level in the case of weaning food containing malt extract was significantly higher and the PER significantly lower as compared with the values for the raw mix. The available lysine content of the processed weaning food containing sugar was considerably lower than that of the raw mix although the PER was not affected. Similar discrepancies between the values obtained by FDNB method and data obtained by chick or rat assays have also been observed by other workers[7,8]. The data obtained in the present investigation on the available lysine contents and PAA levels of lysine and threonine and PER values for the raw mix and roller-dried weaning food indicate that increase in the plasma threonine level is a better index of processing damage to lysine than available lysine content or plasma lysine level. The results also indicate that while some reduction in the protein quality of weaning food occurs during roller-drying in the presence of malt extract, a greater damage to the quality of protein results in the case of malted milk powder.

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PROTEOLYSIS OF FORTIFIED BUFFALO SKIM MILK BY TRYPSIN*

The rate of proteolysis of buffalo skim milk by trypsin was studied after adequate dilution of the milk to a solids level as in human milk. Dilution of milk samples with water (1:3) reduced the proteolytic rate to almost half its original rate. Addition of either lactose, whole casein, α-casein, β-casein, α_s-casein or β-k-casein to the diluted skim milk system, stimulated proteolysis. Of the casein samples tested, whole acid casein increased the proteolytic rate relatively more than the casein fractions. Human milk samples exhibited a proteolytic rate almost that obtained with diluted skim milk (1:3); Whereas human colostrum appeared to undergo faster proteolysis comparable with the casein fortified skim milk system.

Attempts are being made in this laboratory[1,2] to find a suitable substitute for infant feeding by humanization of buffalo milk. Any such attempt should fulfil the digestibility requirements as a major parameter to assess its wholesomeness. Mellander[3] observed a difference in the peptic digestion behaviour between caseins from cow and human milk. Kinoshita[4] studied the digestibility of milk proteins by proteolytic enzymes. Ganguli *et al.*[5] observed that buffalo milk casein is hydrolysed at a slower rate than cow milk casein by proteolytic enzymes like trypsin, pepsin and pancreatic enzymes. Ganguli *et al.*[6] further observed that the number of peptides released from buffalo casein by trypsin was less than from cow casein. The present paper reports on the proteolysis of diluted and modified buffalo milk by trypsin with a view to humanize buffalo milk from proteolytic standpoint.

Buffalo milk samples (Murrah breed) were from the Institutes herd and human milk samples were collected from the local Civil Hospital. Acid casein was isolated from buffalo skim milk by isoelectric precipitation according to Gupta and Ganguli[7]. α-casein and β-casein were prepared from buffalo skim milk by the procedures of Hipp *et al*[8] and Aschaffenburg[9], respectively. α_s-casein was isolated following the method of Zittle *et al*[10]. β-k-casein was prepared by the procedure of El-Negoumy[11], with necessary modification. Buffalo skim milk (having 3.5 to 3.8 per cent protein) was diluted to three volumes of water. Lactose (4g/100 ml) and casein fractions (0.5 g/100 ml) were added to this diluted milk system and homogenized using a Potter-Elvehjem homogenizer.

The trypsin action on the milk system was conducted as follows: 1.0 ml of the milk sample in a test tube was kept in a water bath maintained at 37°C. 1.0 ml of 0.1 M sodium phosphate buffer (pH 7.0) and 0.5 ml of trypsin (purchased from Sigma Chemical Co., U S.A.) solution were added (3.0 mg/ml). The reaction was terminated after an interval of 10, 20, 30, 40, 50 and 60 min by adding 10 ml of 10 per cent trichloroacetic acid. Tubes were further incubated for half an hour and the contents were filtered through Whatman filter paper No. 1. Blanks were also run simultaneously by terminating the reaction with 10 per cent TCA (trichloroacetic acid) at zero minute initially. The released peptide in the filtrate was estimated by the method of Lowry *et al*[12]. and proteolytic rate was expressed as μg tyrosine released.

Data on the effect of milk constituents on proteolysis of buffalo skim milk are given in Table 1. Considerable reduction in the proteolysis was observed on dilution of milk sample. Among the milk constituents added, acid casein had a maximum stimulating effect on proteolysis followed by α-casein, β-casein and a mixture of β-k-casein. It was further observed that lactose and α_s-casein stimulated proteolysis to a lesser extent as compared with other milk fractionsl The rate of proteolysis of buffalo skim milk (before and after dilution) and human milk are depicted in Fig. 1(A). Human colostrum was observed to be more susceptible to trypsin action than normal human milk. However, no marked difference in the proteolysis was noticed between individual and pooled human milk samples. These data further show that the diluted buffalo milk was more susceptible towards trypsin action than human milk.

TABLE 1. EFFECT OF ADDITION OF MILK CONSTITUENTS ON THE PROTEOLYSIS OF BUFFALO SKIM MILK

Milk type	Constituents added proportion	Protein concn (mg/ml)	Tyrosine released (μg) at 60 min proteolysis Range	Mean±SE
Skim milk		0.038	28.0 – 31.0	29.8±0.59
Diluted milk	Water 1:3	0.009	10.3 – 13.0	12.6±0.61
,,	Lactose 4 g/100 ml	0.009	10.3 – 14.6	14.3±0.13
,,	Acid casein 50 mg/100 ml	0.015	18.2 – 21.3	18.9±0.60
,,	α_s-casein 50 mg/100 ml	0.015	13.0 – 15.1	14.4±0.41
,,	β-k-casein 50 mg/100 ml	0.015	13.8 – 18.0	16.3±0.71
,,	α-casein 50 mg/100 ml	0.015	17.3 – 18.6	17.8±0.25
,,	β-casein 50 mg/100 ml	0.015	17.3 – 18.2	17.9±0.16

*Results are on 5 samples analysed.

*N.D.R.I. Publication No. 75-184.

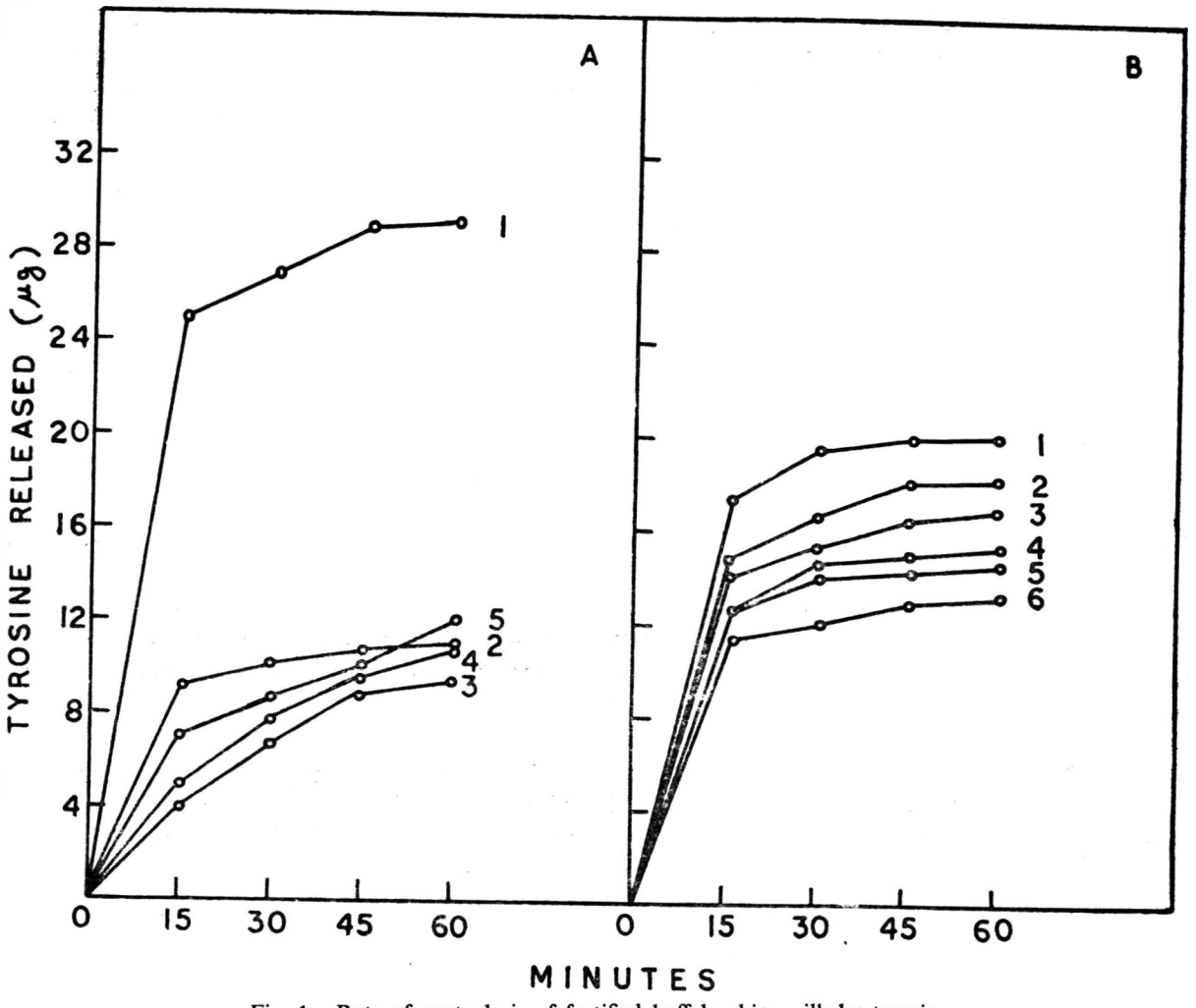

Fig. 1. Rate of proteolysis of fortified buffalo skim milk by trypsin.

A = 1) skim milk; 2) diluted skim milk (DMS); 3) individual human milk; 4) pooled human milk; 5) human colostrum.
B = 1) DMS+Acid casein; 2) DMS+ α-casein; 3) DMS+ β-casein; 4) DMS+ β-k-casein; 5) DMS+ α_s-casein; 6) DMS+lactose.

A sharp increase in the release of peptide in case of buffalo milk was noticed during the first fifteen minutes minutes of the reaction after which the rate of proteolysis slowed down considerably. From Fig. 1(B) it is evident that throughout the course of reaction milk fortified with acid casein exhibited highest rate of proteolysis followed by that of fortified with α-casein, β-casein and a mixture of β-k-casein. This could be due to higher substrate levels in these systems, when compared with dilute milk system. Milk fortification with casein and its fraction appears to improve the digestible quality of such preparations.

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BOOK REVIEWS

Dough and Baked Goods-Chemical, Air, Non-Leavened Edited by D. J. De Renzo, Noyes Data Corporation, Park Ridge, New Jersey, London, England-1975, pp. 429

Noyes Data Corporation in its series on Food Technology Review has brought out this publication No. 26 in the area of Doughs and Baked Foods. This review gives detailed technical information on several U.S. patents on commercial baking techniques, where yeast is not used. The Book definitely serves as a guide to US Patent Literature in this field, after avoiding the details regarding legal aspects in which R & D workers are not likely to be interested. The publication gives reliable and comprehensive coverage on more practical commercial and timely process information that has accumulated since 1960. Often, such information is not available in all the related periodicals and journals.

As the Book brings out a number of technical possibilities available, it may prove immensely useful to R & D workers of the developing countries in launching or altering programmes of work in their relevant areas of product and process development. Till recently users of NDC Publication were not so happy about the loose binding of their earlier publications. The get up and bound form of this publication is a welcome change adopted by NDC.

To begin with, chemical leavened products are discussed with special reference to manufacturing processes, cake mixes including substitutes, ingredients and additives, cookies and biscuits, bread and pan cakes etc. The nutritional enrichment processes of such products is also covered.

Air leavened products without any use of chemical leaveners have been discussed next and cover Angel cakes and pre-mixes, puffs, egg white substitutes etc. Non-leavened products cover information on pie dough, pies, puff pastries, batter and bread composition etc. The coverage of the tortillas is likely to prove useful to the Indian sub-continent where similar food preparations are made as a part of their staple food and consumed by millions of people. A Chapter on refrigerated and frozen products includes information on refrigerated biscuit dough and cake batters, frozen non-leavened pastry and cakes, baking adjuncts.

Useful information on: (*i*) leavening agents like baking powder, baking acids, carbon dioxide sources etc., (*ii*) shortening compositions for cakes, pie etc., and (*iii*) mono-glyceride based and other emulsifiers is presented in the latter chapters. Different preservation methods have been covered on retardation of staling and mold inhibition, combination of anti-stalent mold inhibitor, heat sterilisation etc. Flavouring processes and compositions with special reference to leavened products have also been covered. Fillings, toppings and coatings form the last chapter of the review.

At the end of the Book are enlisted Company index, Inventor index and US Patent Number Index and are likely to prove handy to R & D workers and several commercial entrepreneurs of other countries for seeking further details in their interested areas. The present compilation by D. J. De Renzo will be useful to workers in the field of bakery products and more so as it completes coverage on bakery products as the same author had earlier written 'Bakery Products—Yeast Leavened' for NDC.

S. R. SHURPALEKAR

"*Basic Food Chemistry*" by Frank A. Lee. The AVI Publishing Company Inc., Connecticut, U.S.A., 1975, pp. 430; 25 U.S. $

This is another attempt to give the chemistry aspects of food in a single book form. It has 18 chapters covering most of the aspects of food chemistry.

The first 6 chapters give the basic constituents of the food, namely carbohydrates, proteins, lipids and vitamins together with a chapter each on phytosynthesis and enzymes. A detailed treatise on phytosynthesis is appropriate in view of the basic nature of the reaction. Similarly descriptive coverage of enzymes emphasizing on the latest classification is relevant. However, descriptions about the nature of moisture, structure of major minerals and plant acids would have enhanced the value of the book.

Chapter 7 to 10 cover subjects like flavour and colour. Compared to most other chemistry books the coverage on flavour is in fair depth. The coverage on colour is detailed, with a chapter on browning. However, it is felt that food chemists should lay greater emphasis on minor browning reactions, like metal-polyphenol reaction, conversion of proanthocyanidins under acid condition to anthocyanidins, etc.

Chapters 11 to 18 specialises on various categories of foods. Coffee. tea and cocoa have been each allotted a chapter, which has enabled the author to give full justice to their chemistry with reference to the technology involved. However, the chemistry and technology of spices are equally well developed, and therefore, on the above score a chapter on spices would have been justified.

Similarly, chapters on fish and fish products, cereals and oil-seeds, etc. would have given a greater balanced treatment since detailed chapters are given for subjects like meat, milk and fruits and vegetables. It must be emphasised here that the subjects covered including fermentation and alcoholic beverages and baked products are well dealt both in breadth and depth.

Bibliography given at the end of each chapter will assist the reader in finding required references for additional details. There is also a well-compiled index in the end.

The book on the whole is a good compilation on food chemistry and would be a welcome addition in the library of any teaching or research and development institution concerned with food technology. The printing and get up are in the usually high standard of the Avi Publishing Company Inc.

A. G. MATHEW

Proceedings of an International Symposium held in Glasgow, September 1974: Edited by Dr R B. Duckworth, Academic Press Inc. (London) Ltd., 1975. Pages 716. Price: £ 17.50, $ 45.25.

Water Relations of Foods, one of the series of monographs in food science and technology, edited by Dr RB Duckworth is proceedings of an international symposium held on the subject in Glasgow in September 1974. This symposium was organised to bring together leading academic and industrial food scientists and also a few more accredited guests from other disciplines from various parts of the world to encompass in one meeting, chemical and biological aspects of interaction between water and other constitutents of biological system as well as the behaviour of water in food. In all 35 papers were presented in this symposium, organised into 7 sections, viz., (1) water and its molecular interaction with other constituents of biological systems, (2) methods and criteria used in the study on water in foods, (3) water in relation to the behaviour of microorganisms, (4) influence of water on enzyme action in foods, (5) non-enzymic chemical changes at low and intermediate moisture contents, (6) effects associated with freezing and thawing and (7) aspects of condition and properties of water in relatively moist food materials.

The first two sections were devoted to the physical chemistry of water in simple systems as well as in the more complex food component systems (carbohydrates, lipids and proteins) and methods and techniques for their presence. Emphasis was placed on the nature of the intermolecular forces involved, e.g., electrostatic, hydrogen bonding and hydrophobic forces. Papers were also presented on the various techniques in use to measure state of water input and model systems made up of food components. Compared to the early days when water was measured by crude and empirical methods, currently there are wide array of thermodynamic, hydrodynamic and structural methods for measuring the state of water sorption isotherms, dielectric properties, calorimetric and differential thermal measurements, nuclear magnetic resonance spectra and electron microscopy. Thus these papers reflect remarkable recent growth of knowledge in this field which has resulted largely from successful application of these relatively new techniques but also in part some improvements in more traditional methods.

Section 3 dealt with water activity and the growth of food spoilage and pathogenic organisms. It is realised only recently that water activity is the basic controlling factor in the preservation of foods against both chemical and microbiological deterioration. Water activity (a_w) and physically related properties such as the equilibrium relative humidity (ERH) and water sorption isotherms have significance in most phases of food processing, specially the process and products characterised by the terms dried and intermediate moisture foods. Special mention is made of the paper on the 'Significance of water activity for microorganisms in meat' by L Leistner and W Rodel in which a classification of meat products based on a_w and pH of the product with corresponding storage temperature has been advocated and is thus an improvement on the usually followed practice to consider meat as perishable if the pH is above 4.5 and at the same time a_w higher than 0.90. The authors have concluded that these concepts which are based on measurable factors important for food poisoning and spoilage bacteria and not on empirical experience alone would probably be helpful for meat manufacturers as well as food inspection services and would thereby improve the appropriate storage of meat.

Section 4 centres on water relations of enzymatic and non-enzymatic deteriorative reactions in foods. It was most interesting and informative to relate how much the rate of the reactions may be accelerated and in some cases even depressed by increased water activity. Potthast and Hamm presented data on the influence of water activity on the composition of lipids of the muscle in freeze dried beef during storage, and observed that below 25 per cent relative humidity there was no breakdown of ATP and that at about 40 per cent relative humidity the velocity of breakdown increased with increasing water acitivity. It may be mentioned here that authors believed that glycogen is not broken down even at high water activity because of the lack of water to dissolve the glycogen, its molecule being too large to

be dissolved in the small water pocket. It has also been stated that even at very low a_w chemical and enzymatic changes of the carbohydrates occur which do not happen in untreated and freeze dried samples.

Section 5 deals with topics like influence of water content on non-enzymatic browning reactions in dehydrated foods and model systems, oxidative changes in foods at low and intermediate moisture levels etc. Labuja gives an overview of the oxidative deterioration of foods and in that connection describes various oxidative reactions including lipid oxidation, carotenoid degradation, oxidation of myoglobin causing discolouration in meat, oxidation of other food components such as protein and oxidation of ascorbic acid.

Section 6 deals with effects associated with freezing and thawing of food quality, reaction kinetics and chemical alternations in the cell constituents including enzymes of the food.

In six papers of the last section data have been presented on aspects of the condition and properties of water in relatively moist food materials, e.g., meat, wheat flour dough and intermediate moisture foods.

A perusal of the topics discussed in the symposium indicates that subjects were very well chosen and programme very well structured. The presentation of various papers flowed smoothly, thanks to highly qualified and well prepared speakers. It is understood that the papers were followed by lively discussions characterised by many enlightened questions and answers which un fortunately have not been published as part of the proceedings. The value of the book would have been much enhanced if the discussions were also included in the proceedings.

Lastly, as has been hinted in the preface to this publication, the subject of Water Relations of Foods had its origin in food dehydration studies which is primarily defence oriented and were instituted because of the peculiar value of food dehydration as a means of conserving storage, shipping space and of reducing weight for transport. However, a perusal of the papers presented in the symposium indicate that excepting one contributor none other represented any institute actively involved in research on foods pertaining to defence from any of the countries.

The monograph, Water Relations of Foods, has been a very informative and well documented publication and it is hoped that the basic information given therein will be of great help to all research workers interested in food science and technology.

H. NATH

'*Plants consumed by Man*' by B. Brouk (1975), 479 pages, Academic Press, London New York, San Francisco-£ 14.80.

'Plants consumed by man' is a comprehensive book set out in 10 chapters and with a series of appendices. As the author states in the preface there is no other single book, within the covers of which one can get ready information on the etymology, history, geography, chemistry, morphology, physiology of plants which are commonly consumed by man and which therefore have an economic bearing. The word consumption is used in its broadest sense and include not only primary food plants, but also those plants which yield flavours, colors, thickening agents and which could be used for making beverages or used as masticatories and fumitories.

The introductory chapter explains the various types of enconomic plants and then lists the various categories of plants consumed. The remaining 9 chapters deal successively with cereals and pseudocereals, vegetables, fruits, nuts, plant extracts, flavouring plants, beverage plants, fumitories and masticatories, and lastly fermentative microorganisms. In these 10 chapters, 366 plants are described, and are well illustrated with line drawings which very clearly indicate the general appearance and in many cases the internal structure of the plant part consumed, so much so, anyone encountering one of these plants for the first time would have a fair visual knowledge of the usable plant part. The appendices include a morphological survey of the plants which sets out taxonomically the plant parts consumed, a glossary of botanical terms (illustrated), a bibliography, a list of the 366 plants described in the book and an index of scientific and common plant names.

For a book of this size, however it is just not possible to include *all* the plants consumed by man and the author himself states this in the book. Indian (and possibly, other Asian) readers would find several plants missing. To name of few: several Cucurbitaceous vegetables, greens of *Amaranthaceae*, curry leaf (*Muraya koenigi*), asafoetida (*Ferula asafoetida*), Indian sarsaparilla (*Hemidesmus indicus*), tulasi (*Ocimum sanctum*)

Such 'regional' deficiencies however do not detract the general usefulness of this reference book which should find a place not only in all reference libraries for use by food scientists, agriculturists and botanists but also in private homes where the culinary art is taken seriously.

S. MAHADEVAN

NOTES AND NEWS

Research programmes supported by the IDRC, CANADA

The International Development Research Centre (IDRC), is a public corporation, created in 1970, by Act of Canadian Parliament, to support research designed to adapt science and technology to the specific needs of developing countries. The Centre is unique in that, while it is financed by the Parliament of Canada, it is governed by an international Board of Governors who independently set its policies and priorities. Dr. W. David Hopper is the President of the IDRC.

Post-production losses in rice

Losses in the rice crop in handling, threshing, drying and storing can be 20 per cent of production, according to studies done in the Philippines by staff of the International Rice Research Institute (IRRI). Losses are probably considerably higher in other Asian countries, where techniques are less advanced.

The IDRC sponsored a survey of post-harvest rice technology in Indonesia, Malaysia, the Philippines and Thailand, during 1974.

The survey report by Dr. de Padua, drew attention to a variety of defects in the post-production systems; introduction of high yielding varieties of rice demanded mechanical harvesters, grain driers and storage silos, which often turned out to be ill-designed for conditions in these countries. The technology imported from industrialized countries was in particular unsuited to the high moisture rice crops harvested during rainy season.

Three complementary projects designed to improve the post-harvest processes and technology for rice have begun in Indonesia, Thailand and Singapore. It is hoped that techniques and machinery tested during this research will reduce the heavy losses often suffered after harvest.

Research on Sorghum

Sorghum is the main food crop for some 400 million people in the semi-arid tropical regions, very little research was done upon it until recent years to increase its yield per hectare and to improve its nutritional content.

The Prairie Research Laboratory (PRL) at Saskatoon has pioneered a technique for producing intergeneric somatic hybrids by cell fusion. Using tissue culture methods and fusing plant protoplasts from different plant families by enzymatic removal of cell walls and the use of polyethylene glycol to effect fusion, hybrid cells of soybean-barley, pea-carrot and rape seed-soybean have all been obtained on an experimental scale.

The CIMMYT in Mexico and ICRISAT in India, are exploring the feasibility to creat a viable fertile hybrid of maize and sorghum. If the high photosynthetic efficiency, high yield capability and good protection of the grain by the sorrounding sheath of maize can be married to the best qualities of sorghum it would make an exceedingly valuable addition to the world's cereal grains.

Increasing the availability of fish and fish products in Southeast Asia.

Fish and other marine animals have long been an important and inexpensive source of protein for Southeast Asia's vast population. Three grants totalling £ 312, 200 in support of research aimed at increasing the availability of these resources were announced by Dr. W. David Hopper, President of IDRC.

A grant of £ 105, 600, will assist the Department of Fisheries of the Ministry of Agriculture of Malaysia, in developing practical and economical methods of oyster culture in Sabah, Malaysia.

A second grant of £ 128, 200 will enable B.C. Research in British Columbia, Canada to develop an efficient commercial system for the bulk collection and preparation of gonadotropin from salmon spawning in British Columbia waters. Gonadotropin has been used to induce breeding in fish for aqua culture programs. A constant and inexpensive supply of the extract would help meet the growing demand for fish seed in Asia and other developing regions.

Mexican scientists work to improve pasture management, cut milk and meat costs

In an effort to cut the cost of milk and meat to Mexican households scientists in the government's Pasture Research Department are launching a three year program to increase forage production in an experimental area, which can serve as a production model for the whole country.

B. C. Roy National Award Fund

The Management Committee of Dr. B. C. Roy National Award Fund under the auspices of the Medical Council of India, has sanctioned a grant of Rs. 50,000 to the Nutrition Rehabilitation Centre, Govt. Erskine Hospital, for the puchase of Mini-bus to be named after "Dr. B. C. Roy".

The objectives of the programme launched by the Nutrition Rehabilitation Centre, Govt. Erskine Hospital, since 1971 under the direction of Dr. S. A. Kabir, former Dean of the Medical College are:

(1) To correct the Nutritional Status of the under-five children with locally available food at cheap cost, with no involvement of costly animal proteins; (2) to reduce the morbidity and thereby the mortality rates in the under-fives; (3) to educate the mother and the village people to safegaurd the growth of the children; (4) considering that most of the morbidity in the villages in pre school children is due to simple ailments like diarrhoea, respiratory infection, intestinal worms, simple fevers, scabies, etc., these centres provide also a medical coverage for treatment of these ailments; (5) to train all categories of officers and staff of the Applied Nutrition Programme of the Govt of Tamilnadu (6) to provide immunisation and (7) to take care of the expectant and lactating mothers.

The Centre has prepared films, slides, guide books, for educating the public on care of children, immunisation of diseases and treatment of common diseases, etc., has trained a number of staff in several cadres for the Applied Nutrition Programme. The Govt of Tamilnadu have recognised this as an Apex Training Institute, in applied Nutrition Programme.

Symposium on Fats and Oils in relation to Food Products and their Preparations.

An "All India Symposium on Fats and Oils in relation to Food products and their preparations", was held on 3rd & 4th June 1976, at CFTRI, Mysore, with the following objectives:

(*i*) To provide a common platform for the processors, planners, government executives and scientists connected with R & D problems, (*ii*) To focus attention to the problems of Lipid Technology connected with the usage of fats and oils in Indian Food items. (*iii*) To discuss and identify areas for future research and developmental activities relating to (*a*) raw-material survey of resources and newer sources of fats and oils, (*b*) processing, hydrogenation, emulsification, interesterification, refining and modification of fats and oils, (*c*) fat based food products—Indian confectionery, deep fat fried products, bakery products, margarine, chocolate, pickles, salad cream, mayonnaise, butter, cheese and ghee, (*d*) nutrition and toxicity, (*e*) auto-oxidation, antioxidants and storage characteristics of fats and oils, and (*f*) chemistry, analytical techniques, adulteration and quality control.

Dr. B. L. Amla, Director, Central Food Technological Research Institute, Mysore, presided over the function. The Symposium was inaugurated by Shri N. Chikke Gowda, Minister for Agriculture, Animal Husbandary and Veterinary Services, Government of Karnataka. Dr. P. K. Kymal President of the Association, welcomed the members. Dr. H. Nath, Director, Defence Food Research Laboratory, Mysore, presented the theme of the Symposium. The Inaugural Session ended with vote of thanks, by Shri S. Venkoba Rao, President, Oil Technologists Association of India (Southern Regional Branch), Regional Research Laboratory, Hyderabad. Over 100 delegates from Research Institutions, Universities, Government Departments and Industries, took part in the deliberations. Special lectures by Dr. K. T. Achaya, Executive Director, Protein Foods and Nutrition Development Association of India, Bombay on "Producing and marketing a low cost volume food," and Dr. J. Ganguli, Professor, Biochemistry Department, Indian Institute of Science, Bangalore, on "The mechanism of cholesterol lowering effects of polyunsaturated fatty acids", were also arranged during the symposium. The Symposium was spread over to six technical sessions and a plenary session for framing the recommendations. The recommendations are as follows:

Raw Material Survey of Resources and Newer Sources of Fats and Oils

1. During 1959, the Natural Resources Committee of the Planning Commission while conducting survey of Utilization of Agricultural and Industrial By—products and Wastes have assessed the potentiality of non-edible oilseed at 6.67 million tonnes. Considering this, it is necessary that further intensive survey should be undertaken to find out newer sources.

2. Considering the very low per-capita consumption of fats and oils in India it is necessary to lay down a specific national policy for increasing the production and conservation of the major/lesser known oil and fat resources for adequately meeting the requirements of the population.

3. While evolving high yielding varieties of cotton-seeds, research may be carried out for having seeds with low gossypol content.

4. In view of 5 to 15 per cent of losses of oilseeds that occur during post-harvest handling and storage before it reaches the millers, it is necessary to carry out systematic investigations for evolving proper storage, handling and transportation procedures of oilseeds and other oil-bearing materials and also for obtaining good quality oil in tropical countries.

Processing, Hydrogenation, Emulsification, Interesterification, Refining, and Modification of Fats and Oils

5. Economics, must more than ever dictate industrial development. Lowering of production cost can perhaps be achieved by decentralisation; in addition reduction of packing, marketing and distribution costs are essential if such products as peanut butter and defatted, shaped groundnuts are to have a meaningful future. How these cost reductions are to be achieved in practice need to be vigorously pursued.

6. Non-traditional indigenous fats have started to swell the national economy. Last year, something like 70,000 tonnes of such fats have been put to use namely for soap-making and thus sparing the traditional oil resources for use for edible purposes. The three keys to this happy state of affairs are:

(*a*) Upgrading techniques at a laboratory level;
(*b*) Commercialisation of such upgrading; and
(*c*) Organisations for collection of fat bearing materials.

These conditions also apply to the fats described today, like rice bran oil, goat tallow and sheep tallow. The first step has been taken and the subsequent stages must be pursued in equal measure by research, technology and commercial interests.

7. It is recommended that the entire concept including relevant regulations of straight hydrogenation be

examined critically bearing in mind the needs and aspirations of the common people, nutritional quality, marketability and acceptability.

8. The products and processes described are based on a renewable resource, namely fats. Products like surface-active agents, bakery adjuncts and small scale deodorisation units, are all welcome, and further studies to extend this range are desirable. There is a need to carry such studies to an industrial realisation, which implies rigorous laboratory evaluation, testing for industrial utility, and finally industrial production. A positive attitude especially on the part of industry towards these new products would be essential for success.

Fat Based Food Products—Indian Confectionery, Deep Fat Fried Products, Bakery Products, Margarine, Chocolate, Pickles, Salad Cream, Mayonnaise, Butter, Cheese and Ghee

9. Deep fat fried foods are important in Indian dietary. Therefore there is need to stimulate research investigations to (*i*) find the optimum quantity of fat required in the fried product to impart the required sensory attributes, (*ii*) standardise the optimum and appropriate conditions of frying and (*iii*) find out the appropriate packing and storage conditions.

10. In view of the increasing use of bread as a mass consumption item, there is need for a bread-spread within the reach of larger sections of the population. Margarine, with added colour and flavour can aesthetically and nutritionally fill this need and its development as a commercial product should be encouraged.

Nutrition and Toxicity

11. Studies on short term feeding of sal fat have shown it to be non-toxic in rats. Thus there is a case for utilization of this source of oil for edible purposes especially in confectionery products replacing cocoa fat. Intensive studies may be undertaken in long term feeding programmes and also on upgrading the fat. It will be worthwhile to investigate the toxicity of sal fat by conducting long term feeding studies.

12. Polyunsaturated fatty acids are known to bring down the serum cholesterol level and thus minimise the risks of ischaemic heart diceases. Studies have indicated that intake of vegetable oils lead to increased synthesis of unsaturated phospholipids which in turn solubilise more of cholesterol in the miscellar form for excretion. Walnut oil being rich source of linoleic acid and linoleneic acid has good hypocholesterolemic properties. Work in this area should be intensified. Also special fat based products may be developed for this purpose.

13. There is a scope for the development of cheap methods for destruction of aflatoxin in raw groundnut oil. However, more work is needed to establish that there is no flavour deterioration by exposure to sunlight.

Autoxidation, Antioxidants and Storage Characteristics of Fats and Oils

14. Antioxidant materials developed during various kinds of processing of vegetable and animal sources are of intrinsic interest and their study should be pursued. They are also of potential application because of the swing away from synthetic antioxidants.

15. Feasibility of trace hydrogenation of oils should be examined to improve the keeping quality of oils without significantly affecting the essential fatty acids like linoleic acid.

16. Public should be educated of the hazards of using excessively recycled oil for deep fat frying.

17. Plastic containers have certain advantages over metal containers for transportation and storage of edible fats. Further studies should be conducted with reference to the migration of component from the containers to the oil and their toxicological and organoleptic effects and keeping quality of oils.

Chemistry, Analytical Techniques, Adulteration and Quality Control

18. The detailed physico-chemical studies on the role of lipids and added lipids, in particular phospholipids, be carried out with a view to improving the texture and flavour of chapaties. Such a study should also be extended to other food products.

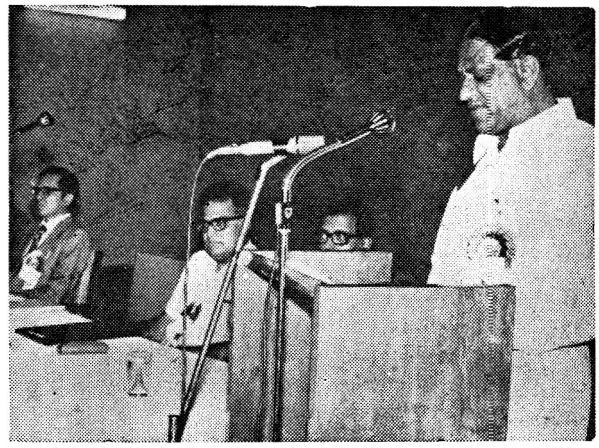
19. Chemical, pharmaceutical and clinical studies on the polymers from sesame and other unsaturated oils, show promise and should be continued.

20. Vigorous and sustained efforts to standardise and improve the existing methods and develop newer and sensitive and specific methods should be made to detect adulteration in oils and fats.

The symposium was followed by a panel discussion on 5th June 1976 on "Integration of food production, processing and marketing". Sri D. V. Urs, Vice chancellor, university of Mysore, Presided over the function and Moderated the Panel Discussion. Dr. M. S. Swaminathan, FRS, Director General, Indian Council of Agricultural Research, New Delhi, in his inaugural address, stressed the urgent need for the concerted effort for an integrated approach of food production, processing and marketing.

The following speakers took part in the discussion:

Dr. P. K. Kymal, Mr. D. J. Balraj, Dr. R. Dwarakinath, Mr. Rao Dr. B. L. Amla and Dr. H. Nath.



Inaugural Address by:

Shri N. Chikke Gowda, Minister for Agriculture & Veterinary Services, Government of Karnataka

Lt. to Rt. Dr. P. K. Kymal, President AFST, Exec. Director, Dept of Food, New Delhi, Dr. B.L. Amla, Director, CFTRI, Mysore, Shri S. Venkoba Rao, President, OTAI, Hyderabad.

Panel Discussion:

Dr. M. S. Swaminathan, FRS, Director General, Indian Council of Agricultural Research, New Delhi at the Panel Discussion

Lt. to Rt. Shri D. V. Urs, Vice-Chancellor, University of Mysore, Dr. P. K. Kymal, President-AFST Executive Director, Department of Food, New Delhi and Dr. B. L. Amla Director, CFTRI, Mysore.

Proceedings of the above symposium and panel discussion are under publication.

Annual General Body Meeting

The Annual General Body Meeting of the Association, was held on 5th June 1976, at the Central Food Technological Research Institute, Mysore. Dr. P. K. Kymal, President of the Association, presided over the meeting. The President welcomed the members and the visitors and reviewed the work done during the year. The minutes of the last Annual General Body Meeting, was read by the Secretary, Shri M. V. Sastry. They were adopted unanimously, proposed for adoption by Shri K.C.De', and seconded by Dr. T. N. Ramachndra Rao.

The report of the Secretary, was circulated to all the members before the start of the meeting. President requested for the comments from the floor, if any, on the Secretary's report. Dr. A. G. Naik Kurade, asked about the follow up action taken for collecting the names of the technical and scientific personnel, engaged in the food science and technology for which the secretary explained that the matter was taken up with the Council of Scientific and Industrial Research, who are collecting the census of scientific and technical personnel, working in different fields. The anomalies noticed in the report regarding the membership was clarified by the President.

To a question by a member on Secretary's tour, the Secretary explained about the usefulness of his tour to Calcutta, New Delhi and Bombay, in strengthening the Association and he appealed to continue this in future also. Regarding collection of funds for Prof. V. Subrahmanyar Award, it was decided that an appeal will be sent round to collect funds from students, colleagues, friends and other well wishers and admirors. This work was entrusted to the next Executive Committee. The question raised regarding holding of the General Body as late as June was observed to be not a serious things as in the last year also it was held-only in May.

The proposal to hold the next General Body Meeting in Trivandrum was deferred and the matter was left to the decision of the Executive Committee.

The Secretary's report was proposed for adoption by Dr. A. G. Naik Kurade and seconded by Shri M. K. Panduranga Setty, which was adopted unanimously. The Treasurer's report containing the Audited Statement of Accounts and Budget proposals was taken up for discussion. With minor suggestions, and clarifications, it was proposed for adoption by Shri S. C. Bhattacharjya and seconded by Dr. J. C. Anand.

The Gardner's Award was presented to Shri R. K. Baisya and Dr. A. N. Bose of Jadavpur University, Calcutta for their paper entitled "Studies on Dehydration of Dahi", published in 1974, Volume 11 No. 3 p. 128 of the Journal of Food Science and Technology. President thanked the members of the expert panel and also the Gardner's Corporation for contributing for this award.

The Suman Food Consultants Travel Award, was given to Shri V. Manavendra Rao, an M.Sc., student in the Food Science and Technology course CFTRI, Mysore, to enable him to attend the present symposium and Annual General Body Meeting, as a delegate. The award was presented by the President and he thanked the donors and the panel of judges.

The Secretary announced the results of election held to the different posts of office bearers of the Association in 1976.

President:

Shri M. R. Chandrashekara

President elect:

Shri C. P. Natarajan

Vice-presidents:

Dr. S. P. Manjrekar (Head quarters)
Shri N. N. Dastur (Southern Zone)
Shri R. N. Ghosh (Eastern Zone)
Dr. D. V. Thamane (Western Zone)
Shri Y. K. Kapoor (Northern Zone)

Hon. Exec. Secretary:

Shri M. M. Krishnaiah

Hon. Joint Secretary:

Shri A. M. Nanjundaswamy

Hon. Treasurer:

Shri T. S. Satyanarayana Rao

Councillors:

Shri Nagendra Sharma (Central Zone)
Miss M. C. Madhura (Bangalore Chapter)
Lft. Conl. O. P. Kapoor (Head quarters)
Dr. G. C. Bhattacharya (Eastern Zone)
Shri Laljeet Singh (Northern Zone)

As there was no proposal for Vice-president's post from the Central Zone, Hon. Exec. Secretary, proposed Dr. B. Panda, for the above vacancy and it was unanimously accepted by the General Body.

The Councillor for Western Zone, will be proposed by the next E. C. Meeting of the Zone, and will be intimated to the E. C. at the Head quarters as per the provisions in the Bye-law.

The Hon. Exec. Secretary introduced the amendments proposed for the Bye-laws for consideration of the General Body. As these resolutions demanded drastic changes in the Bye-law of the Association, it was felt that the Bye-law itself could be redrafted instead of effecting all these amendments for which the General Body

accepted the proposal with majority vote. The task of redrafting the Bye-law was entrusted to Dr. K. T. Achaya, who will take into consideration, while drafting the bye-law all the amendments proposed and any other suggestion that would be proposed by the zones, chapters or members of the Association. Dr. K. T. Achaya, agreed to prepare the draft Bye-law for circulation well in advance of the next Annual General Body Meeting.

The Chairman introduced the next president, Shri M. R. Chandrashekara to the house. He recalled the achievements of Shri M. R. Chandrashekara for the advancement of food science and technology, particularly to the development of Baby Food Industry. After the induction of the new president, the other office bearers Shri M. M. Krishnaiah and Shri T. S. Satyanarayana Rao were introduced to the house. The new President thanked the outgoing Executive Committee for the best work they have turned out during the course of the year.

Activities of the Trivandrum Chapter

The first meeting was convened by Dr. J. S. Pruthi on 20th February 1976 at the Community Canning Centre, Trivandrum-3. About 20 members were present. At the outset, while congratulating the member for evincing keen interest in the starting of the Chapter, Dr. Pruthi highlighted the objectives, activities and achievements of the Association. He also briefly traced the history of the Association since 1945. It was unanimously decided to start the activities of the AFST Chapter at Trivandrum. Pending the completion of formalities at the Head quarters, a Managing Committee/Working Group with the following members was set to look after the functions:

Dr. J. S. Pruthi	*President*
Sri V. V. Nair	*Secretary*
Sri A. V. Bhat	*Treasurer*
Sri R. Hariharan	*Representative in the Southern Zone, Madras.*

It was also decided to hold technical meetings once in a month by inviting scientists and technologists as well as the members of the Association. Dr. Pruthi outlined the tentative future programme of the Chapter.

Dr. Pruthi, in his talk highlighted the latest developments in the processing of spices at CFTRI, with particular reference to processing of green pepper, which has attracted the attention of the industry. Processed green pepper worth Rs. 12 lakh is being exported annually. He also highlighted the main findings regarding the parameters of canning and bottling of green pepper as well as the bulk preservation of green pepper in brine and vinegar.

He invited the members to visit the CFTRI Laboratory at the CSIR Complex at their convenience and thanked all of them for their excellent co-operation.

The Second meeting of the Trivandrum Chapter was held on 28th March 1976.

Sri R. Hariharan, an Exporter of green pepper, spoke on the Practical Problems in the export of processed green pepper. Dr. Pruthi presided.

Introducing the subject, Sri Hariharan stated that he started processing of green pepper in 1975 due to encouragement received from officials of the Food Department and CFTRI Unit at Trivandrum. The different operations involved in the export of green pepper are:

(*i*) preparing the material for shipment as stipulated under Fruit Products Order (FPO); (*ii*) informing the FPO authorities in the region for drawing the samples at random. Ten per cent of the samples are generally drawn and sent for analysis; (*iii*) after getting the analysis report the FPO authorities issue "No Objection Certificate" (NOC) to enable the Customs Authority to pass the goods for shipment abroad.

He further narrated the various steps and organisational difficulties involved in getting place in the ship and despatch of the consignment. The exhorbitant charges levied by the foreign bankers is also another serious problem.

He suggested for the consideration of the FPO authorities and CFTRI to provide facilities for inspection/analysis at Trivandrum and/or Cochin so that the exports of green pepper are not hampered.

The talk was followed by a very interesting discussion, wherein several technical questions relating to the Export Quality Control and Quality Standards for green pepper were raised. In the concluding remarks, the President felt the urgent necessity of fixing appropriate quality standards for different categories of processed green pepper. Sri H. Sreemulanathan proposed a vote of thanks.

The third meeting was held on 23rd April 1976. Dr. R. C. Mandel, Central Tuber Crops Research Institute, Trivandrum gave talk on: Post-harvest Studies on Export Quality of Bananas in Fiji Island. In the South Pacific Region, Fiji Island is one of the producer and exporter of Robusta Banana. Due to tough competition offered by Equador and high labour and consequent high cost of production of banana Fiji was unable to compete effectively.

Efforts were made to improve the preservation and marketing aspects of Robusta Banana of Fiji, which possess many good qualities, but the major defects are (*i*) mixed maturity, (*ii*) bruising and damage (*iii*) mouldy

stems and (*iv*) poor shelf-life as compared to good marketable quality of Equador banana. Following measures were tried to overcome these problems.

(*a*) Suitable proping was done to prevent bunch-twisting, stembending and sometimes loss of heavy bunches, weighing 35 to 40 kg each; (*b*)Attempts were made to protect the bunches against constant rubbing with dry leaves causing 'bruising' and damage by birds. Covering the bunches by perforated alkathene bags could prevent the bruising on the fruit surface, as is being followed in Equador and other countries; (*c*) The selection and grading of bunches at harvest is very essential. Bunches of three fourths maturity were ideal for export purposes and this stage of maturity was confirmed by breaking the finger and observing for light yellow colour. However, maturity record sheets and the conventional clipper system followed in Equador are better methods in this respect; (*d*) Mixed maturity is minimised by putting hands 1, 2 and 3 in one group and hands, 4, 5, 6, and 7 in another group. (*e*) Bruising normally occurs while transporting and handling of raw foods and it can be prevented by (*i*) careful handling from field to packing site (*ii*) pad cushioning by the workers while carrying bunches from the hill-slopes to the roadside, (*iii*) providing sponge cushioning in different layers of banana when transported from a long distance by truck (*iv*) taking precautions while breaking hands into clusters and packing them in cartons, (*v*) thorough washing of fruits in running water and dipping the fruits in benlate solution (0.1 per cent) for half-an-hour.; (*vi*) shelf-life of the fruits is increased by (*a*) dropping the fruits in 1 per cent alum solution followed by (*b*) further dipping in 10 ppm gibberlic acid (GA3) or 100 ppm gibberlines or Barelex (Ga/7) solution. The usual system was to pack the individual fingers of 14 cm or more length, tightly in the wooden cases of 27 kg capacity.

The recent trend is to pack banana in cartons in the form of hands or clusters to save time, avoid bruising and reduce labour and other costs.

At the end, the talk was followed by interesting discussion.

The house unanimously approved for administrative convenience, that Sri S. K. Jain and in his absence Sri V. V. Nair, to act as Secretary of Trivandrum Chapter.

The 4th technical meeting of the chapter was held under the chairmanship of Dr. Pruthi at Community Canning and Preservation Centre, Trivandrum on 27th May at 5.30 p.m.

Sri P. Janardhana Aiyar, retired chief analyst, Govt. of Kerala, gave a talk on "Challenges of Food Adulteration". He stated that one out of every three or four food articles sold in the country is adulterated. The speaker traced the history of food adulteration and pointed the adulteration prevalent in the various countries like Greece, Arabia, France, Germany and England and the punishments given for such offence in those days. The enactment of the Prevention of Food Adulteration Act in 1954 and the amendments made in 1964 and in 1976 were then dealt with, highlighting the main features of the report of the Joint Select Committee of Parliament, which has made some very important recommendations for eradicating this social evil.

The speaker then gave an interesting account of the various types of adulteration met within the country, covering a cross section of the food articles available to the public, and explained how some of these adulterations posed a great threat to the health of the public. While concluding, the speaker emphasised the need for consumer awareness, consumer organisation and consumer resistance to meet this great challenge.

The was talk followed by a documentary film entitled "Criminals at large". This was followed by a very lively discussion where members sought clarifications and cleared their doubts.

Dr. J. S. Pruthi, Scientist-in-charge of CFTRI Unit, Trivandrum, who is responsible for initiating the Trivandrum Chapter was accorded a cordial farewell by all the members on his transfer to CFTRI Experiment Station, Ludhiana. His dynamism and keen interest in developing this chapter won the admiration of one and all Dr. Pruthi cordially thanked all the members for the affection, respect and regard shown to him and for their excellent cooperation during the 5 months of his tenure.

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ANNOUNCEMENT

Proceedings of the International Symposium on Protein Foods and Concentrates: 27th June to 4th July 1967 at CFTRI, Mysore, 1974. pp. 450+vii; Price Rs. 25.00

The 41 papers presented at this Symposium, held at CFTRI in the middle of 1967, provide a comprehensive survey of the technology and economics of production of high protein foods from a wide range of raw materials, such as soyabeans, groundnuts, cottonseed, coconut and sesame, as well as from fish, green leaves and petroleum. The formulation of protein-rich blends, amino-acid supplementation, and consumer acceptance and marketing have been covered quite adequately. There are also separate sections on the protein requirements and sources, as well as on the public health and clinical aspects and the technological risks involved in the production and use of such foods.

The problem of the economically under-developed parts of the world have been well covered by contributions from Guatemala, Hong Kong, India, Indonesia, Japan, Mexico, Peru, the Philippines, Venezuela and Vietnam. The U. K. and the U.S.A. have also made major contributions to this symposium.

Most of the papers review the existing position and indicate the possible lines of development in the future. The discussion that took place at the end of each Session has been competently summarised and the recommendations made in the final, plenary session have been reported *in extenso*.

Although other symposia on protein foods have been held after this one, none of them has achieved such a wide coverage by so many internationally known experts. Hence, the contents of this book will be of permanent interest to food scientists and technologists, and will continue to have relevance in the field of nutritional sociology so long as there exist populations that suffer from a lack of nutritive foods.

Subscribers and Members who wish to have this publication may write to: The Director, C.F.T.R.I., Mysore-570013 (India).

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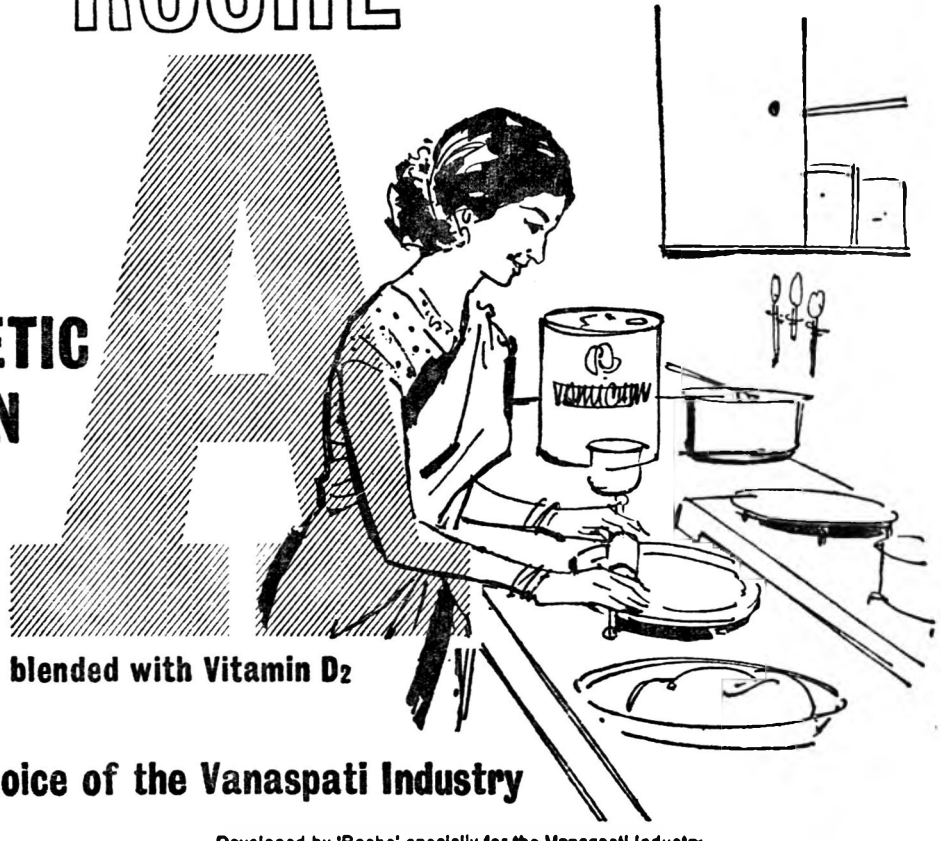

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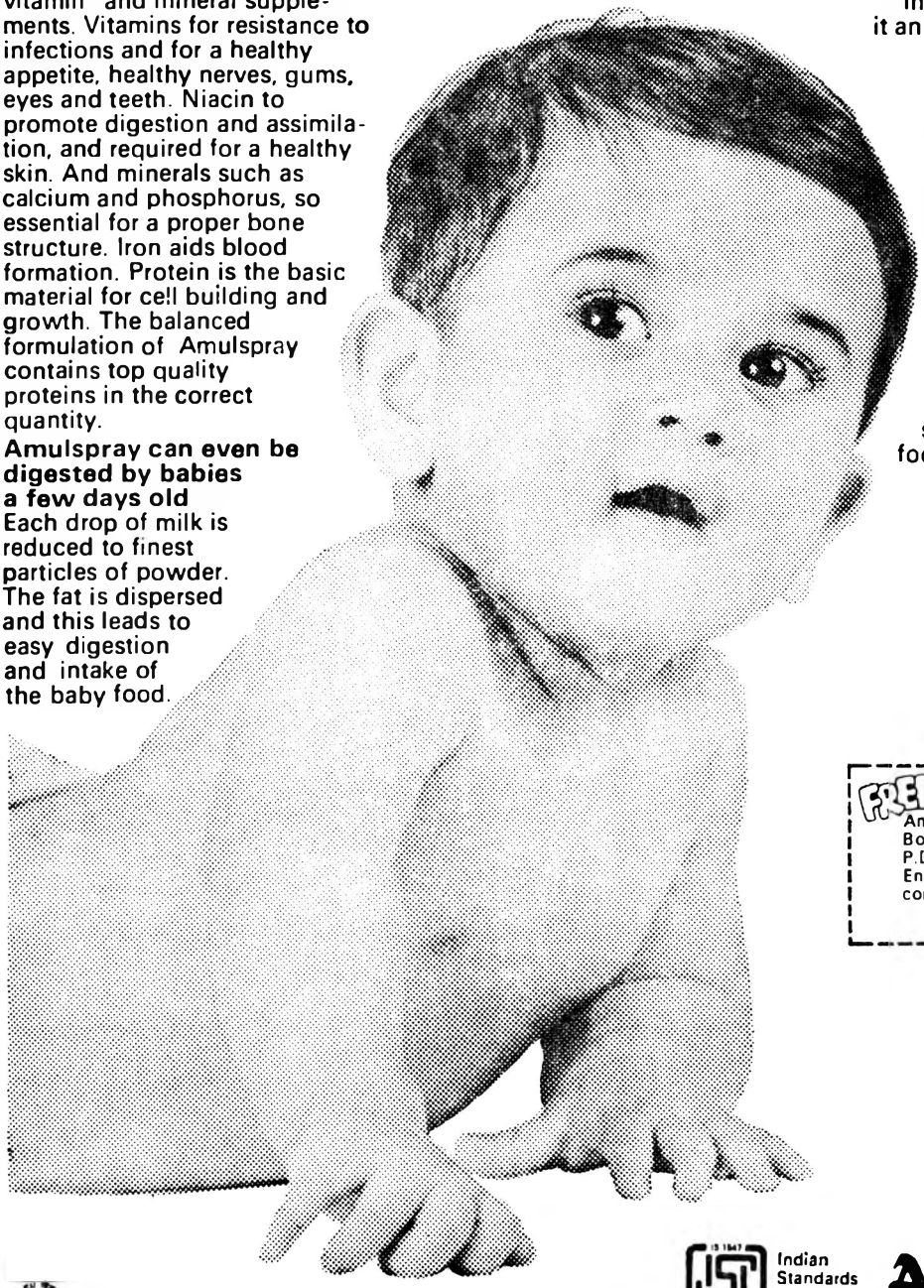

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