Twenty-five Years of Flavor Research in a Food Industry[1]

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ABSTRACT AND SUMMARY

In the last few decades, flavor research has developed along different main routes. The first step was the discovery of the complex mixtures of volatile components that together build the particular flavors. Apart from this, interest became focused on the taste substances which, through interaction with the flavor carriers, impart aroma to a food. The third main route was the investigation of the deterioration in odor and taste due to oxidative or microbial spoilage. As these investigations progressed, it became clear that these flavor areas show considerable interaction and that, in addition, other sensory impressions of a product as regards shape, texture, color, etc., play a role in the ultimate subjective perception by the consumer. In this way, flavor research has widened into a complex branch of science, and it is becoming more and more apparent that consumer testing that encompasses all the elements mentioned above will have to indicate the real preferences of the public.

A short review is given of the main lines of flavor research performed by Unilever in The Netherlands over the past twenty-five years.

RESEARCH ON FATTY FOOD FLAVORS: LACTONES

Margarine producers started flavoring their products a considerable time ago. They applied such techniques as using soured milk, adding butyric acid or diacetyl, or applying starter cultures. After 1945, investigations were started to find other suitable components (1). With Unilever Research in Vlaardingen, The Netherlands, we concentrated our work on lactones (2,3), resulting in a patent on butter flavor in 1952 (4).

While we appreciated lactones as favorable flavor substances, Patton et al. (5) referred to them as off-flavors developing in milk that had deteriorated. They thought that lactones are formed during heating of milk fat and butter (6), but our analyses soon showed that butter triglycerides contain slight amounts of hydroxy fatty acids, more than other bovine fats do, e.g., tallow, which is one of the reasons that the latter fats do not smell of butter. Fresh cream contains hardly any free lactones, but when the product is stored, traces of hydroxy fatty acids are split off by hydrolysis; β-, γ- and δ-homologues are present, but the δ-hydroxy fatty acids are preponderant. They are easily converted into lactones by ring closure. Because the hydroxy acids are formed naturally by biological pathways, we assumed that the lactones would be optically active and dextro-orientation was indeed found (7).

To apply lactones as flavor substances, some countries legally require them to be "nature-identical" products. Chemical synthesis gives a dextro- and levorotatory racemic mixture. To produce hundreds of kilograms of optically active lactone in its purest form, the help of microbiologists was of great value. Microorganisms can produce all sorts of substances, and efforts were made to try and find organisms which smell of lactones. Tuynenburg Muys et al. (8,9) succeeded in finding some organisms producing dextro- or levorotatory lactones. They were yeasts and bacteria which, during incubation with keto acids in aqueous medium at 30 C, reduced these acids to hydroxy acids and ultimately lactones were produced.

Particularly *Saccharomyces cerevisiae* (baker's yeast) appeared to be able to produce dextrorotatory δ-lactones. We therefore decided to start the production of 5-decanolides and 5-dodecanolides in our factory at Maarssen in Holland. With charges of 30 m^3 aqueous suspension of 3,000 kg wet yeast to which 60 kg purified keto acid (0.2% solution) and 600 kg glucose had been added, the process was started at pH 6.5 and a temperature of 17 C. The reductase of the multiplying yeast brought about the conversion in 18 to 24 hr; during this process, the pH decreased to 4.5 and the temperature rose to 20 C. Naturally, the quantity of yeast mass had greatly increased and was hard to get rid of. After acidification to pH 2, the hydroxy acid was extracted continuously from the centrifuged wort with pure toluene in an all-glass 10-meter-high rotating-disc contactor. Foaming constituted a great problem here. The yield of extraction was 95%. Working up was via the sodium salt, and after purification, a lactone layer was obtained. Washing three times and rectification gave a product of 99.9% purity (GLC) (J. Strobos, personal communication). The overall yield was 70% and the rotation $[\alpha]^{20}_{D}$ = 55.9. The captive price of this optically active lactone was at least 5 to 6 times higher than that of the racemic product; nevertheless, many tons of lactones were produced over the years.

Meanwhile, in the early sixties, in a French Unilever margarine factory, a brownish decoloration was observed in coconut fatty acids distilled from soapstock. A concentrate of the brownish substance had a very strong, pungent, sweet smell and, on dilution, a clear coconut-lactone smell could be detected. In the literature, particularly 4-nonalide is reported as the principle of the natural coconut flavor and is given the fancy name of "coconut aldehyde" (10). Spectroscopic examination of the French factory sample showed that it was a mixture of 5-octanolide, 5-decanolide, and 5-dodecanolide. Only small amounts of γ-lactones were present. Since these lactones were of biological origin, it was interesting to determine their optical rotation. We were much surprised to see that both dextrorotatory and levorotatory types were present (Table I).

TABLE I

Optical Rotation (degrees) of Some Individual Lactones Isolated from Coconut Oil (CNO) and Butterfat (BF) (measured in benzene)

5-Alkanolides		α		Concentration (mg/ml)	
CNO	BF	CNO	BF	CNO	BF
	C16		(+) 36		3.6
C14	C14	(+) 11	(+) 37.5	7.5	11.8
C12	C12	(--) 8	(+) 44.5	19.7	22.4
C10	C10	(+) 28	(+) 44	33.3	12.3
C8		(+) 51		40.9	

Resuming our investigations into the degree of optical rotation of the δ-lactones in butterfat, we first checked carefully whether the methods of isolating the lactones did not affect the rotation. We used the dextrorotatory 5-decanolide and 5-dodecanolide produced by the yeast method with optimal $[\alpha]^{20}_{D}$ rotations. After back conversion in the hydroxy acid glyceride form and dissolution in peanut oil, the normal isolation was practised. It appeared that the rotation had remained the same, namely 60 and 53

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[1] Presented at the AOCS Meeting, New Orleans, April 1976.

degrees on an average. It was therefore certain that during our process of isolating lactones from butterfat, no racemization occurred. Samples of butterfat investigated for lactones displayed a considerably lower specific rotation than the δ-lactone from the Maarssen factory. The conclusion was therefore that both dextro- and levorotatory lactones are present in butter, side by side (Table I).

The moment that definitive proof was obtained for the presence of both types of lactones in butter coincided with the commissioning of an extended and modified factory equipped with modernized rotating-disc contactor columns. After some months it was decided to stop the production of the optically active lactones – which are 5 times more expensive – and to use the racemate (99.9% pure) instead.

To guarantee the safety of all the additives – some of which are added in amounts down to only some mg/kg – we have performed extensive long-term feeding experiments with test animals over the years. The lactones, lower fatty acids, methyl ketones, etc., were found to be very safe, which safety, therefore, equally applies to the flavor mixture found in other dairy products.

Besides butter flavor, we investigated heated butter, cream, cheese, yoghurt, fresh bread, crust and crumb, chicken and meat, roasted seeds, tea, coffee, chocolate, fruits, and some perfumes. Also off-flavors developing in oils and fats were investigated with a view to prevent the process of reversion.

OFF-FLAVORS

The major factors causing off-flavors in foods are oxidation and microbial deterioration. Factors such as temperature and light also play a part in the formation of these off-flavors, and it is beyond any doubt that the oxygen in the cell has a certain effect as well. This effect is less pronounced in a plant cell in a state of rest than in a germinating fruit, in which the lipids are greatly modified. In an oil or fat separated from other materials of the cell or the tissue, the oxidation process proceeds in a different way. Practice shows that crude oil or fat can be kept for a reasonably long period when stored properly, despite the fact that the product comes into contact with moist air, phosphatides, proteins, peptides, mucilages, wax, and metals. We tried to elucidate why in refined oils oxidation products are formed, particularly hydroperoxides, followed by oxy-products and finally, as a result of further decomposition, volatile aldehydes, the off-flavors.

The theory of autoxidation and the reaction mechanism playing a role is now well-established (Fig. 1). The question with which reviewers such as Parsons (11), Waters (12), Forss (13), Grosch (14) and the SIOL conference of Marcuse (15) are concerned is: "In what way is the first fat radical RH formed?" In this connection I would like to point out the following: As chlorophyll is absent from refined oils, it cannot play a role in the initiation of the fat radical. Sunlight and fluorescent light can initiate the formation, but during storage in factories and transport, light is not always involved under normal conditions (12). It is sometimes claimed that in polyunsaturated fatty acids, a shift to the conjugated diene system of double bonds takes place and that the conjugated system is more reactive to oxygen, but this has never been proved. Rather the opposite has been shown.

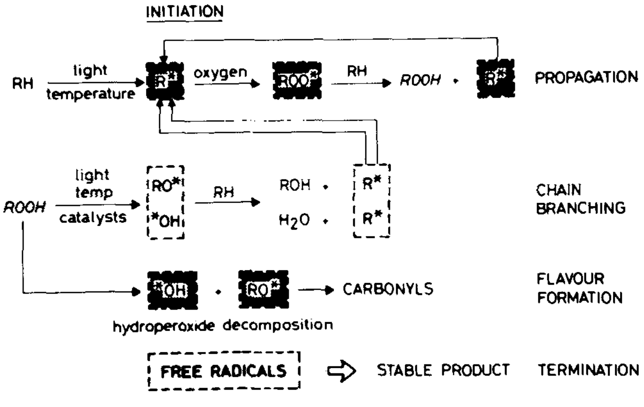


FIG. 1. Autoxidation of fats.

The proceedings of the SIOL conferences mention metal catalysis as the factor involved, but in normal practice iron, nickel, copper, magnesium, cobalt, etc., are not present, and if they are, their concentration is very low. Such very low concentrations can be detected by sophisticated atomic absorption techniques so that refining can be done in such a way that the most stringent demands can be met. We may wonder whether a particular type of oxygen, for instance singlet oxygen, might be responsible for the first attack on the fat molecule. Rawls (16) and Clements (17) have investigated this possibility. Although the systems used by them are no doubt valuable from a theoretical viewpoint, later investigations have shown that the highly reactive singlet oxygen is not present in edible oils.

Morita and Fujimaki (10) postulated that the hydroperoxides could not be responsible for the ultimate formation of the volatile aldehydes. However, for their experiments they used methyl linoleate oxidized to a high degree; the peroxide value was 50, which means that the substance contained around 1% hydroperoxides. Irrespective of the fact that at a peroxide value of 0.1 or lower strong off-flavors are formed, there are objections in regard to the chromatographic techniques they used, for the hydroperoxide fractions contained also aldehydes, which is not so surprising considering the high degree of oxidation. On the basis of their findings, only hydroperoxides with double bonds at the 9- and 13-position may be present. However, also Privett (19) thinks of undetected initial substances. In this connection I would like to quote Horikx (unpublished communication, 1959):

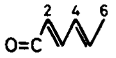
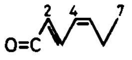
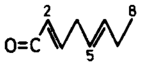
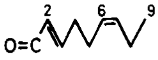
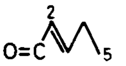
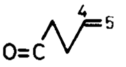
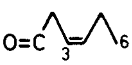
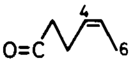
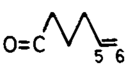
> The aldehydic flavor components of natural oils are formed by the action of atmospheric oxygen; freshly deodorized oils, which contain no volatile aldehydes, are without flavor. But in deodorized oils in which the peroxides have been destroyed almost completely, reaction with oxygen at room temperature for only a few hours is sufficient for the appearance of detectable amounts of flavor components. This level is reached at an oxygen absorption of 1 mmol/kg = 22 ml O_2/kg. This amount of oxygen is comparable with the oxygen solubility in fat that is in contact with air of 1 atmosphere; chemical reaction of such an amount cannot readily be detected. If the flavor threshold is considerably lower than 1 mg/kg (the linseed hardening flavor, caused by 6-trans-nonenal is perceptible at levels of 1 mg per 10^9 g), even less oxidation is necessary for flavor development.
>
> It is thus reasonably certain that the initiating decomposition of the hydroperoxides is the source of the reversion and rancidity off-flavors. The difference between both types is one of perception level: reversion flavors are caused by carbonyls which are perceptible in sometimes very low concentrations; rancidity flavors are composed of aldehydes having a relatively high perception level.

It may also be useful to mention the conclusions Grosch (14) arrived at in 1975. In his review he stated that the numerical values of carbonyls or their derivatives are insufficient to predict reversion. According to Marcuse, the so-called TBA value is not of much use either, as 2-heptenal reacts, for instance, different from 2-octenal. For rapeseed oil stored for 3 mo, Naudet (20) did not find a good correlation with the TBA or anisidine values either. If a reaction is carried out on aldehydes in the oils, the results are confusing, for also keto acids, dicarbonyls, etc., react.

It would, therefore, be necessary to determine what

TABLE II

Threshold Values of a Number of Aldehydes

Aldehyde	Flavour description	Mean threshold (mg/kg)	
		Odour	Taste
	green, banana	0.3	0.04
	rancid hazelnuts	10	0.5
	rotten apples	4	0.055
	nutty	1	0.15
	starch glue fried fat	-	-
	chicken fat	2	0.25
	tangerines	-	-
	cucumber-like	2.5	0.35
	tallowy, green	0.25	0.02
	cucumber-like	0.01	0.002
	apple	2.5	0.35
	creamy, burned milk methional-like	0.025	0.01
	green beans, tomato	0.15	0.15
	green, creamy, methional-like	0.002	0.00055
	green, potato peel like	0.025	0.02
	orange	34	5.5
	licorice root	-	-
	melon-like	5	1
	cucumber-like	1.55	0.35

aldehydes contribute to the off-flavors and to what extent. This requires a detailed and rapid analysis of the off-flavors. From the analytical results, a selective carbonylogram can then be derived.

According to Meijboom (21), the interaction between the aldehydes also plays a part, which may decrease or increase the flavor strength. To elucidate this, we synthesized a number of aldehydes (Table II) in order to establish if there is a correlation between flavor and molecular structure and here we enter the domain of perception. It was difficult to establish a regularity and this was one of the reasons why we had a number of aldehydes tested by panels to find out whether there is a certain pattern of flavor strength increase. The following principles were found in a majority of cases:

(a) The chain should contain at least nine carbon atoms;
(b) a CH_3-CH_2-CH=CH- group should be present;
(c) mono- and diunsaturation enhances the flavor strength;
(d) in a chain with more than twelve carbon atoms three or more double bonds are needed;
(e) an isolated double bond is required to give more strength of flavor.

The final conclusions were, however, not very encouraging and no strict regularity in a type of smell of specific intensity could be established.

In Europe it is still a problem how soybean oil should be processed to avoid deterioration in taste and to obtain a product – a table oil or a spread – which meets the highest demands. It was thought that the beans from the fields of Illinois might be damaged during transport to New Orleans and that this damage to the cells would be sufficient to cause the reversion. But experiments we carried out with beans harvested in the pod and treated most carefully and worked up under carbon dioxide, showed that the oil developed the well-known aldehydic off-flavors within a few hours, as predicted by Horikx.

When we realize that a fully purified soybean oil or a soybean oil synthesized from pure fatty acids can reach a peroxide value of 3 to 4 without any taste deviations occurring, the conclusion must be drawn that the undesired aldehydes are formed from the linolenate peroxides, but we do not know what extra pro-oxidants are really involved right from the start. Because of their non-volatility, the hydroperoxides themselves are no flavor carriers and it has also been shown that they have no taste, which was confirmed by Prevot in 1971 (22).

Natural antioxidants, the tocopherols, are present in table oils, margarines, and spreads in sufficient quantities and a normal refining process leaves ample amounts of these important substances intact. Extra addition of antioxidants is, therefore, not necessary; as a matter of fact, extra amounts cannot counteract the reversion.

The volatile aldehydes are also known to play an important part in vegetables, fruits, and meat. In our laboratory at Duiven, The Netherlands, extensive research on meat flavor and the precursors was performed by Peer et al. (23) and Schutte and Koenders (24). Also in this case, the balance between desired and undesired is sometimes a question of the amount of aldehyde and of the way the mixture is assessed by the consumer.

I may also mention the carbonyls isolated from cooked chicken by Harkes and Begemann (25). Again, there is a clear relationship between the highly unsaturated fatty acids and the aldehydes found. It is known that highly unsaturated chains play a certain part and that too large amounts of these substances affect the flavor adversely.

The aldehydes isolated from vegetables over the past ten years by different researchers have been compiled by Varoquaux and Avisse (26). Dubois (27) states that in fact the polyunsaturated fatty acids present in minor amounts in the cell walls of fruits and vetetables are responsible via enzymatic attacks, first by lipase, later by lipoxygenases, for the formation of a host of aldehydes. They are first flavors that are wanted but on standing of the vegetable or on processing, too much of them is formed and within a short period unwanted mixtures are obtained, described as malodorants. Of course, other classes like sulphur-containing volatiles develop and here the interaction is even more subtle.

Figure 2 shows the correlation between the amount of hexanal and the sensorial evaluation of green peas after some hours' standing at 20 C. As to the sensorial evaluation, we may ask ourselves whether it is possible to correlate objective and subjective methods.

The workhorse hexanal is found in fairly large amounts in vegetables, fruits, butter, fats, meat, and it can be measured directly or by means of headspace analysis.

Apart from the oxidative influences, microbial deterioration also gives rise to the formation of off-flavors. This does not take place in waterfree oils, but it occurs, however, in storage tanks which are not properly cleaned so that a deposit may be formed. In butter and other dairy products, bacteria may cause enzymatic β-oxidation of fatty acids. In this way, a number of methyl ketones may be formed (28). Keenan (29) mentions the objection of too many acids being formed and the formation of bacterial metabolites such as acetaldehyde, diacetyl, dimethylsulphide, and methional, which smell unpleasant when present in large amounts. Diacetyl may even be converted into the much less flavor-carrying butane diol.

Conversion takes place via acetoin, which has also a low flavor potency. As a result, there is a loss of the diacetyl desired under the influence of the diacetyl reductase present in a number of *Lactobacillus* species and *S. diacetilactis.* Bitter substances may be formed by microbial action and these are very unpleasant in even small quantities. Too high concentrations of acetaldehyde sometimes cause a so-called green flavor, to be distinguished from the aldehyde flavors which are sometimes also referred to as green. Too much ethanol with formation of ethyl esters is the cause of an atypical aroma.

FUTURE DEVELOPMENT OF FLAVOR RESEARCH

When we read recent reviews on flavor and food it becomes abundantly clear that industrially prepared foodstuffs are under pressure through consumerism. We see that legislation has to follow the general public in setting very strict lines in all countries. Additives are in low esteem with the general public. As coloring matters can easily be observed, the main pressure is in this area. The list of coloring matters permitted has been reduced in the EEC countries in the past thirty years from approximately 20-30 to only a few. Within two years not more than six will be left over. Neither in the EEC nor in Sweden, the difference between natural and synthetic colors will be legally admitted. In this latter country even, coloring food with palm oil concentrates containing the original carotenes is not allowed.

When colors fade away because their use is not permitted, the impression to the consumer of such grey or discolored foods will not make them attractive. It is known that noncolored or miscolored food tastes repulsive.

It leaves little doubt that flavor addition, which for many products is already restricted or totally prohibited in some countries, will be under severe scrutiny in the years to come. We know that the trend will be towards natural and bland, and in many European countries less salty. But even so, natural flavors need to be tested thoroughly, because natural does not necessarily mean harmless. That's why Unilever spends annually more than $3,000,000 on safety testing in order to be sure that all their products are safe from a toxicological point of view.

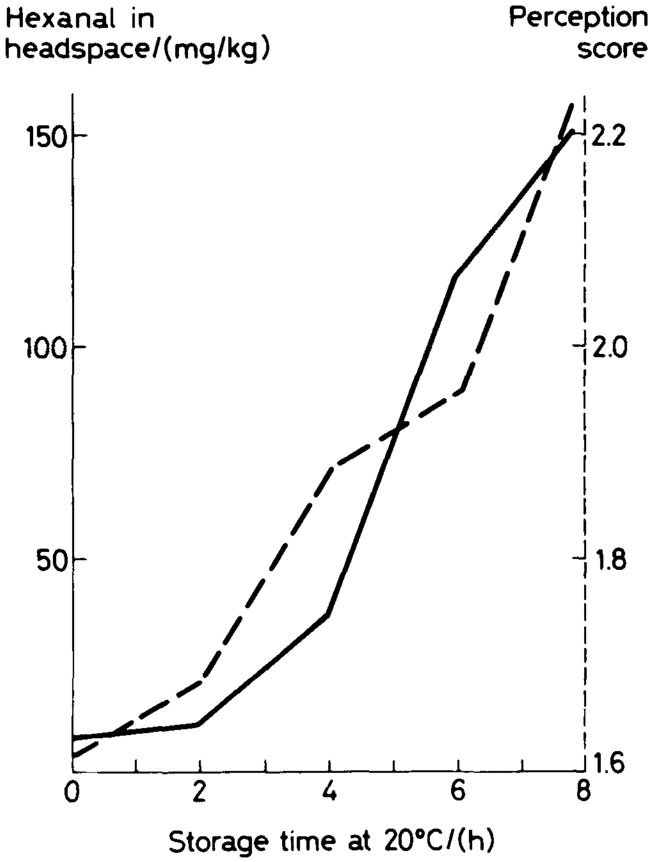


FIG. 2. Hexanal production and perception of degradation of peas (according to Bengtsson and Bosund, 1967); *Rev. Fr. Corps Gras* 22:373 (1975).

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