Black Perigord Truffle Aromatizers: Recent Developments

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Black Perigord truffles *(Tuber Melanosporum)* are underground mushrooms that grow in harmony with certain trees, especially oaks. They are found in several regions of Europe, particularly in Spain, Italy and France. The black truffle is highly valued by gourmets for its typical flavor, to the extent of being called "The Black Diamond of French Cuisine." The truffle grows underground, and is found traditionally by using pigs which can smell them.

Since the beginning of the century, the production of truffles has decreased in spite of efforts to increase this very valuable crop. Scientists have studied biology, ecology, and biochemistry of the black truffle. Its mode of development is now better known[1] and few innovations have appeared, e.g. specific fertilizer[2] or new cultural techniques. But nevertheless, the present production remains low and the black Perigord truffle is very expensive; consequently its use in cookery tends to decrease.

Usage In Food Industry

The food industry, particularly processed meat products, traditionally uses truffles to flavor its products. In France, the term "truffled" may only be used when black truffles, exclusively *Tuber Melanosporum,* are added to the meat in a range from three to five percent.[3] But because of its high cost, the truffle is used in only very small quantities (1%), and more often substituted by natural or artificial imitations.

Figure 1 shows the main imitations of black Perigord truffle. The major part of the artificial texture imitations are based on vegetable proteins and polysaccharides mixtures. Some of them, sun truffle for example, appear to be correct imitations of apertized truffles, but a microscopic analysis detects the absence of the characteristic spores of the black Perigord truffle. Nevertheless, in France, all these products are illegal and manufactured products obtained cannot be called truffled products.

In the case of aroma imitations based on mixtures of hydrolizates of proteins and pure chemical compounds, all products are disappointing. Taste tests measuring the similarity or dissimilarity to black truffle aroma, confirm the bad organoleptic quality of these products. Moreover, a chromatographic analysis showed easily that they are artificial aromatizers.[4] None of them is a reconstitution of the black truffle aroma. Recently, an alcoholic extract of truffles mixtured in vegetable oil was marketed. But, the taste tests conducted reported the low organoleptic quality of this product.

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Aroma Analysis

If the aroma impression was described as slightly sulfurous[5] and if some pure compounds were reported as reproducing the particular odor of black truffles[6-8] (see Table I) not many works were published concerning the aroma composition of the black Perigord truffle *(Tuber Melanosporum).* Only Ney and Freytag[9] identified some volatile compounds after steam distillation on a blend of black truffles *(Tuber Brumale and Tuber Melanosporum).* Also Claus, et al.[10] showed

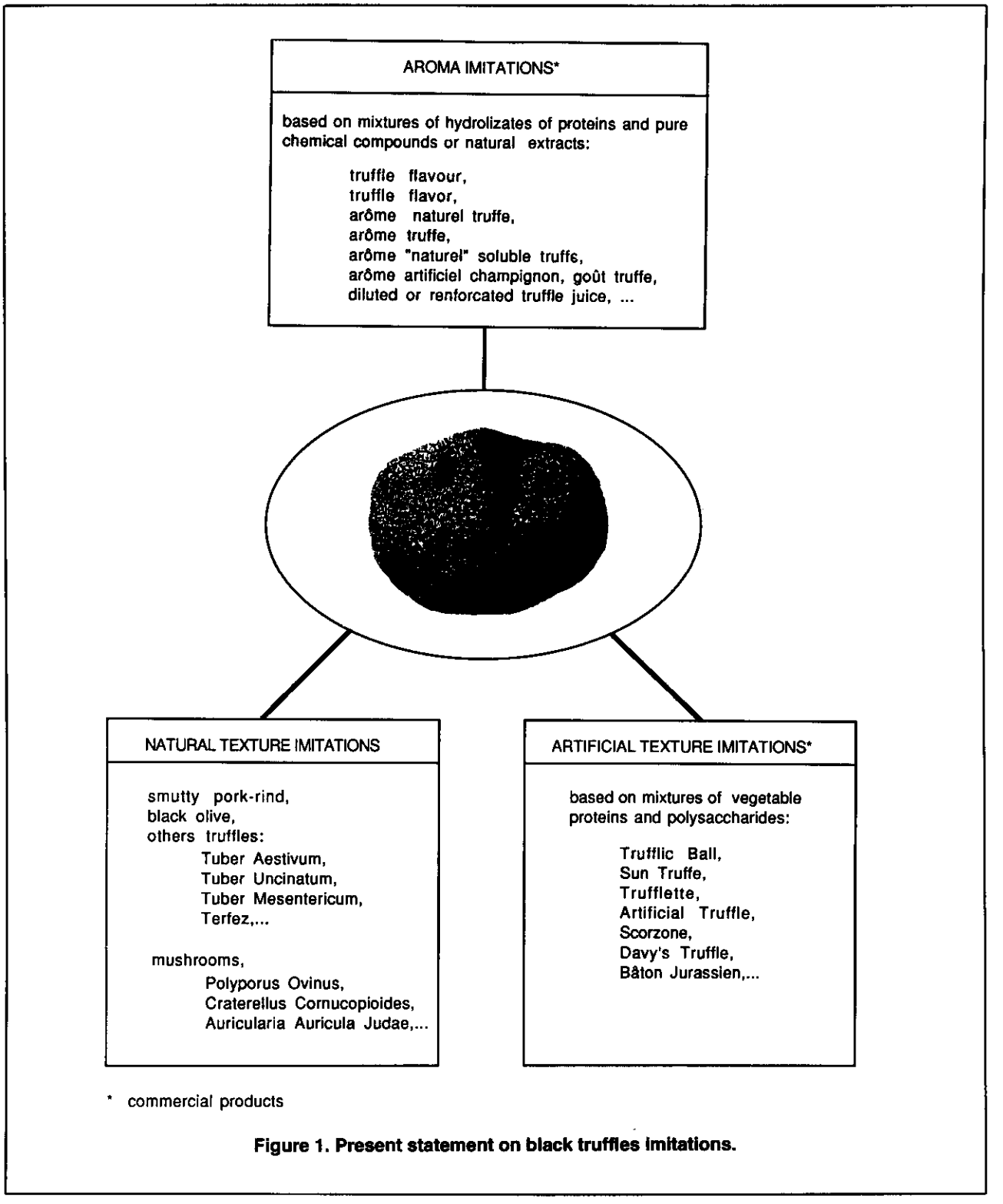
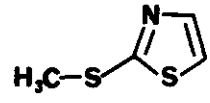
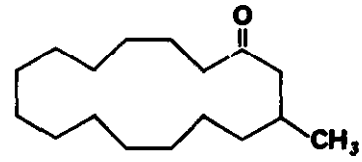


Figure 1. Present statement on black truffles imitations.

Table I. Chemical compounds reported as odorous-like black truffle.

Compound	Chemical Formula
diethyl sulfide	$H_3C-H_2C-S-CH_2-CH_3$
ethyl methyl sulfide	$H_3C-H_2C-S-CH_3$
2-thiomethyl 2- thiazoline	
Muscone	
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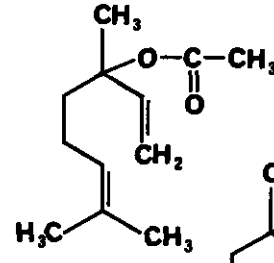
Lynalyl acetate	
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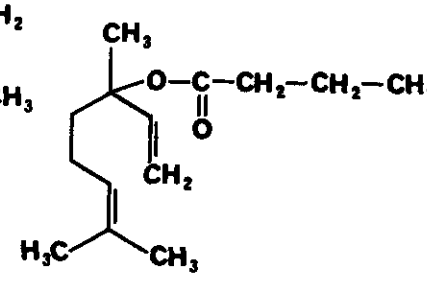
Lynalyl butyrate	

the presence of a steroidal pheromone—5-α androst-16-en-3α-ol (musk smell)—explaining, according to the authors, the ability for pigs to detect underground truffles.

Within the framework of The Truffle Project, i.e. a research program on the biochemistry of the black Perigord truffle during the maturation phase, we developed a Dynamic Headspace method for the analysis of aroma volatiles. Fresh truffles were collected directly from the field and analyzed the day after gathering. They were fully ripe and their organoleptic qualities were certified by a panel of experts.

The method used the new gas chromatographic device D.C.I. System (Desorption-Concentration-GC Introduction,[11] based on a cryogenic adsorption of volatiles on a Tenax GC trap following by a heat desorption. After optimization of the analytical conditions using Experimental Design,[12] and by combined D.C.I. System, Capillary Gas Chromatography, Mass Spectrometry and Data Bank, the major volatile compounds of the black Perigord truffle could be identified. Experiments were performed on truffle flesh[13,14] and

entire truffles.[15,16]

The analysis of truffles' aroma at their different stage of development, made it possible to set up correlations between aroma and metabolism.[17] Complementary experiments on atmosphere captures in cold storage for black truffles and on hapertized black truffles allowed us respectively to identify some minor compounds[18] and to study the aroma evolution during processing.[19,20] The results are summarized in Table II.

Table II. Volatile compounds identified in Black Truffle (Tuber Melanosporum) by Dynamic Headspace analysis.[a]

Compound[b]	Composition[c] (%)				
	fresh truffle		atmosphere	tinned truffle	
	flesh	entire	cold stored truffles	flesh	juice
acetaldehyde	4	4	5	2	3
dimethyl sulfide	4	7,5	7	5	6
propanal	-	tr	tr	tr	0,2
furan[d]	-	-	-	-	0,3
acetone	7,5	8	9	10	10
2-methylpropanal	6	5	6	7	6
2-methyl furan[d]	-	-	-	-	tr
isopropyl formate[d]	-	0,3	0,4	-	-
ethyl acetate	-	tr	tr	tr	0,2
2-butanone	2	2,5	3	6	5
2-methylbutanal	3,5	4	4	5	5
3-methylbutanal	tr	tr	tr	tr	tr
ethanol	35	27	29	34	30
1-methylpropyl formate[d]	-	0,6	0,7	-	-
2-pentanone[d]	-	-	-	-	tr
2-butanol	0,3	0,5	0,5	tr	0,4
1-propanol	1,5	1,8	1,7	tr	1
toluene[d]	-	-	tr	-	-
dimethyl disulfide[d]	-	-	tr	-	tr
2-methyl-2-butenal[d]	-	-	-	-	0,1
2-methyl-1-propanol	20	21	18	15	17
ethyl benzene[d]	-	-	tr	-	-
xylene[d]	-	-	tr	-	-
1-butanol[d]	-	-	-	-	tr
2-heptanone[d]	-	-	-	-	tr
2-methyl-1-butanol	16	17	15	15	15
3-methyl-1-butanol	tr	tr	tr	tr	tr
2-methyl pyrazine[d]	-	-	-	-	tr
1-pentanol[d]	-	-	-	-	tr
3-octanone[d]	-	-	-	-	tr
2-octanone[d]	-	-	-	-	tr
anisole	0,1	0,6	0,4	tr	0,5
1-hexanol[d]	-	-	-	-	tr
3-octanol[d]	-	-	-	-	tr
methyl anisole	-	-	-	tr	0,1
1-octen-3-ol[d]	-	-	-	-	tr
acetic acid	-	0,1	0,1	-	tr
2-ethyl-1-hexanol[d]	-	-	-	-	tr
benzaldehyde	-	-	0,1	tr	0,1
2-formyl thiophene[d]	-	-	tr	-	tr

a) After one cycle of Desorption.
b)The compounds are listed in the order of elution from the GC column.
c) Key: - = not detected; tr = < 0,1%.
d) Identified by mass spectral data alone.

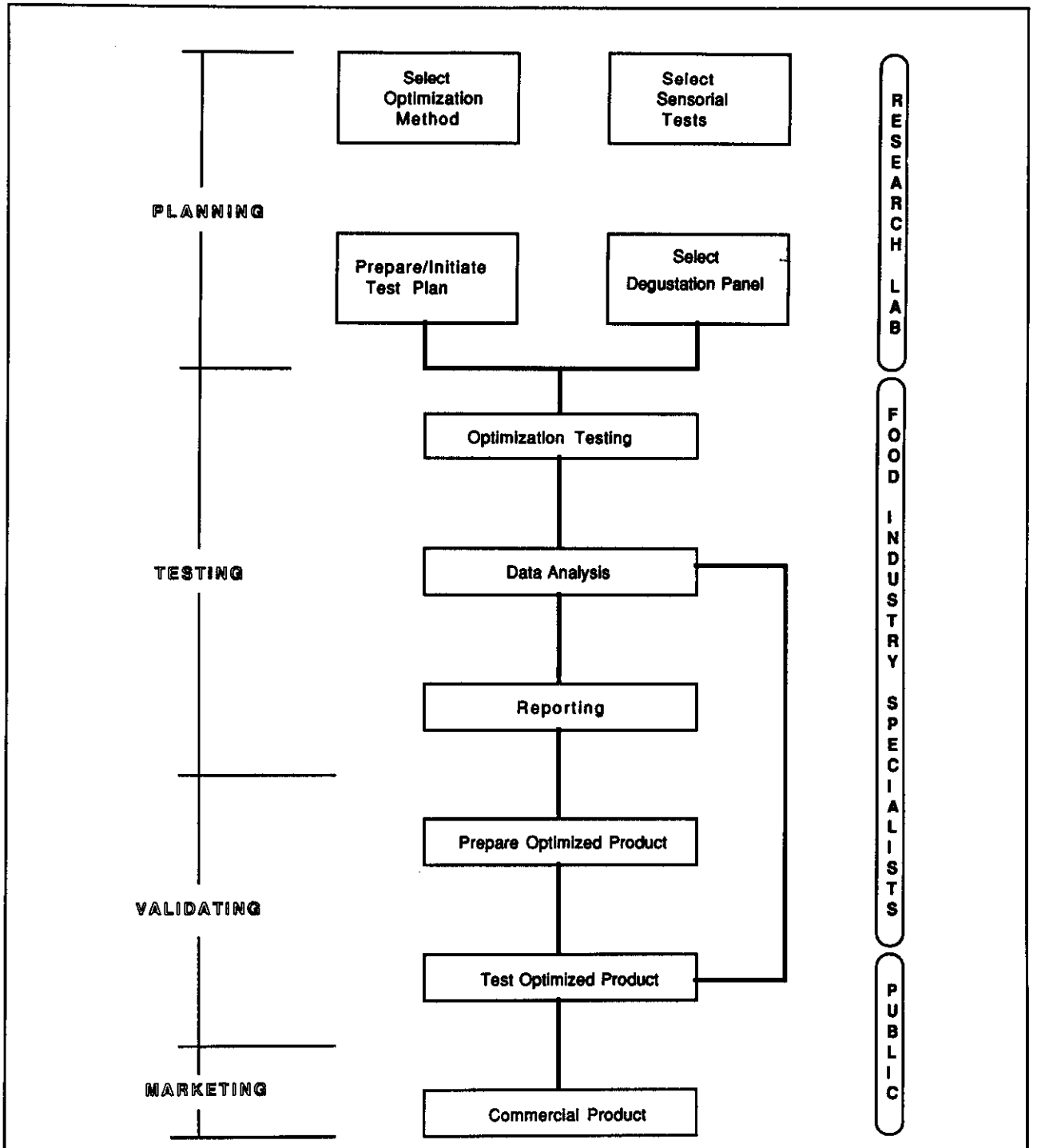


Figure 2. General steps in optimization of formulation method used.

Nature-Identical Aroma Formulation

In the same way that the analytical analysis of the white truffle *(Tuber Magnatum)* aroma[21] has lead to the realization of a synthetic white truffle aromatized edible oil,[22] our analytical study enabled us to elaborate the first nature-identical black Perigord truffle aromatizer.

A sensory analysis conducted by a panel of judges (trained in evaluation of truffles) reported that no single compound was uniquely responsi-

ble for black truffle aroma.[15] Preliminary tests showed that a mixture of nine compounds appeared to be sufficient to realize a correct black truffle aromatizer.[23] But the product has to be optimized in order to set up industrial production.

Some food technologists reported benefits of statistical methods which incorporate planned experimentation with special experimental design in order to optimize the relative proportion of ingredients in food systems.[24-29]

Figure 2 shows the general steps in the optimization method used. A fractional factorial design was arrayed for the nine factors representing each compound retained. The experimental domain was chosen from quantitative analytical analysis in order to realize a 1% aromatization. The sensory analysis was carried out by a panel of eight judges (food industry specialists) on the basis of a scoring evaluation.[30]

Samples of about 125 ml in an alimentary oil were prepared the day before the test. A notation scale was worked out from 5 (exceptionally good full truffle aroma) to 1 (aroma not different from solvent) for flavor imitation and powerfulness.

Twenty samples were presented at random to the judges at each of the two programmed sessions. In order to check the validity of the sensory analysis an unidentified natural parfumed oil was presented at each session.

The processing of the results[31] allowed us to determine the contribution of each compound in the final aroma impression by the quantification of the main effects and interaction effects (synosmic effect) of the nine factors. Also we were able to propose an orientation for an optimum formulation in the experimental range considered.[32]

Complementary experiments for these predicted optimum concentrations confirmed the predicted panel scores.[17] The optimized aromatizer obtained was patented[33] and tested for one year by well-known French cooks.

Black Perigord Truffle Aromatizers

European restaurants tested 2500 samples of 100ml perfumed oil. Culinary tests confirmed the good organoleptic quality of flavored products obtained for both cold and hot dishes. Tests presently carried out in the food industry, essentially in industrial cookery (vacuum packed prepared dishes) and in the salting industry (sausages, pies) were particularly promising.

The methodology of aromatization developed also was applied successfully to other alimentary supports, i.e. butter, duck and goose fats and vinegar. These products were presently under experimentation by cooks. On the other hand,

since truffles' extracts could be used by perfumers, we added the identified volatile compounds to traditional perfumery solvents, i.e. ethyl phthalate, di-propylene glycol or musk T, as well as a more volatile solvent, i.e. Forane 113. The results in perfume formulation were reported as being interesting.

Conclusion

There are many advantages of reconstituted black truffle aromatizers compared to present marketed products. They include:

- Better imitation. These nature-identical or reproduced aromas aromatizers more closely approached the fresh truffle aroma than the other imitations, even the alcoholic extract in which back notes were too strong. This was confirmed by the results of a taste test carried out by a panel of truffles' fanciers. Our aromatizers were reported as the only ones which duplicated the black truffle aroma.
- No off notes. During solvent extraction or heat treatment, e.g. apertization, some decompositions of vegetable matrix generated undesirable volatile and/or non-volatile compounds which participated at the flavor impression and modified it greatly.
- No solvent residues. Extracts prepared with organic solvents, i.e. alcohol, even diluted in vegetable oils, had an unpleasant taste and an important alcoholic note.
- More top notes. The reconstituted aroma had the typical top note of fresh truffle which is lost during heat or solvent extraction treatment and different in artificial products.

A detailed examination of the chromatographic profiles of the reconstituted and other aromatizers showed some or all of these four advantages.[34]

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