Product differentiation and quality approach in the French market for oysters and mussels.

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Abstract

France is one of the world top producers of oysters and mussels by aquaculture with 140 000 tonnes of oysters, 60 000 tonnes of mussels and an ex-farm turnover accounting to 350 millions US$ in 1996. The French market is by far the largest one in Europe for oyster and is supplied entirely by domestic production, with very little external trade. On the contrary, the French market for mussel is a very competitive one, widely open to imports. Given the evolution of market conditions due to the development of supermarkets, to the competition of new value added products and to the modification of consumer's habits, the French oyster and mussel producers have started to implement various strategies of product differentiation and of quality approach. These numerous strategies which are built on geographical origin, on process of production or on marketing promotion may induce a confusion in the consumer's mind. A typology of these strategies has been drawn in order to clarify the situation and to assess their sustainability. Since any quality approach has a counterpart in term of production costs, an enquiry has been carried out at the level of supermarkets in order to assess the price the buyers are ready to pay for labelled oysters and mussels. The results of this study show important variations according to the localisation of the supermarkets, especially due to the distance to the coast and to the size of the town. Contractual relationships between producers and supermarkets as well as better production organisation prove to be important perquisites to make successful product differentiation and quality approach.

key words: aquaculture, oyster, quality, product differentiation, seafood market study

OYSTER AND MUSSEL SECTOR IN FRANCE: PRESENT STATE OF SUPPLY AND DEMAND

Bivalves farming remains the first aquacultural activity in France. It accounts for 75% of the total volume and 60% of the total value of the French aquaculture. Cup oyster (*Crassostrea gigas*) and mussel (*Mytilus edulis* and *Mytilus galloprovincialis*) culture have been increasing regularly since 1985, but flat oyster (*Ostrea edulis*) production remains marginal because of the presence of the parasite called Bonamia which does not make it possible to grow this species at a large scale. Most of the oyster production is realised in bags on tables on the intertidal zone, or in deep water on cultivated beds. In the Mediterranean lagoons, the oysters are hanged on ropes under tables. Most of the juveniles come from natural collecting in two places of the French coast (Arcachon lagoon and estuary of the Seudre river, both on the Atlantic coast), but spat from hatchery begins to be used (not more than 10%). Most of the mussels are reared on wooden poles called "bouchots" on intertidal areas, but new developments have taken place especially on the Mediterranean coast and also on the Atlantic coast using long-lines offshore. All the juveniles of mussels come from the wild. After a big depletion between 1985 and 1990, natural stocks of mussels have been dredged again. Nevertheless, these landings of mussels from the wild are very fluctuating and have been dramatically low since 1995.

It is thanks to this activity that France is the second largest aquaculture producer in Europe, behind Norway. This sector is all the more important as it gives employment to 10 000 persons at full time, and to 12 000 others at part time only during the pick season for oysters around Christmas (Ifremer, 1997). In some coastal regions, it is the only activity all year round. So it participates widely in the coastal and land management. Nevertheless, its situation is often fragile because of the still increasing tourist pressure and of environmental concern. Though an extensive production since no artificial food is distributed, bivalves farming has an impact on the environment because of the high density of animals and because it requires space along the coastline where other potential users are numerous.

Specificities of the oyster sector

The oyster production is assessed around 140 000 tonnes per year, but it is very difficult to have reliable

data of production. Indeed, there is a poor knowledge of the enterprises as economic units of production. The only official source of information is the administrative register of leases, but there is a disconnection between the way these leases are distributed and the way the enterprises are organised. For instance, people who are registered may be totally out of the business while their lease is used by a relative or a neighbour who has better to hide this fact for fiscal reason. Moreover, there is a deep intricacy between production and trade inside most of the enterprises which makes the production enquiries difficult to realise. Some enterprises only do the production phase while other enterprises do both production and trade, not only for their own production but also for the other producers or even for imported products. In particular, a production site plays a major role in the French oyster sector. It is the basin of Marennes Oléron which is specialised in trade. Almost half the marketed production of this basin comes from other regions of production and is just fattened in Marennes Oléron, most often in special earth ponds so called « claires ».

Production sites are scattered all along the coast, including sites in the Mediterranean area in the Thau lagoon and in Corsica, with different environmental conditions. So, in spite of the fact that almost all the production relies on one species only, i.e. *Crassostrea gigas*, there are heterogeneous products depending on the production sites. For instance, oysters from the Mediterranean are quite different from oysters from Normandy, as well for the shell aspect as for the meat content. Moreover, the environmental conditions have a great impact on the characteristics of the product with great variations all along the year. In particular, the maturation process makes it difficult to sell oysters in summer.

The French market for oysters is characterised by the fact that all the production is sold alive and that there is almost no international trade. The only significant flows are exports to Italy and imports from Ireland. Indeed, the consumption of raw cup oysters in the half shell is almost limited to France since the Spanish market has not substituted the cup oyster to the flat one. From the production point of view, the lack of farming know-how and the absence of natural reproduction of Crassostrea gigas otherwise than along the Atlantic French coast may explain the disinterest from other European countries. In Ireland, the production has been supported by French-Irish joint ventures but does not know large expansion since the French production meets the demand on the French market.

With some delay in comparison to other fresh agricultural products, the market share of super/hypermarkets has recently increased. It represented 50% of the sales in 1997. As for the price, a decrease has been observed three years ago at the ex-farm level, but not at the retail level (Table 1). The seasonnality of the consumption is still very high, with 55% of the household consumption in one month only between mid-December and mid-January (FIOM, 1997). The oyster consumers are older than the average and belong mostly to the higher income bracket (Girard, 1995).

Specificities of the mussel sector

The production from aquaculture is around 60 000 tonnes per year, and is easier to estimate than the oyster production since the enterprises are usually larger and most of them market only their own production. Landings from fisheries have to be added, but they are variable and are not so well taken into account in the statistics (Table 2). As for oysters, production sites are scattered all along the French coasts, which makes it possible to have mussels good for consumption, i.e. with a sufficient meat content, a large part of the year. Moreover, two species are cultivated, *Mytilus edulis* on the Atlantic coast and *Mytilus galloprovincialis* mainly on the Mediterranean coast but also in some sites on the Atlantic coast. As these two species have different biological schedules, it enlarges the marketing period of mussels.

There is an active trade of mussels inside the European Union and imports from other parts of the world are increasing in particular from New Zealand. The French market is quite attractive since it accounts for 25% of the European apparent consumption of mussels while the French production does not exceed 15% of the European production. French imports of mussels are for different purposes (Paquotte, 1996). First, it may be a question of seasonal complementarity from February to April when imports from U.K., Ireland and the Netherlands supply the market while the French production is very low. Second, even in period of full French production, there are imports of specific products like extra large mussels from Spain or ready to cook pre-packed mussels from the Netherlands. It may be also a question of price competitiveness with imports of cheap mussels from Spain (small size) or from Ireland It has to be noticed also the development of imports of value added products especially from Spain, Denmark and the Netherlands (frozen, canned, vacuum packed or in prepared dishes).

The market share of super/hypermarkets is still higher for mussels than for oysters: it reached 60% in 1997. After a decrease in the early 90's, the price has remained stable but it is variable according to the sites and the processes of production. The so called « moules de bouchot » from Brittany get a more and more significant price premium while mussels from the Mediterranean have difficulties to maintain their price.

Table 1. Evolution of quantities and price of oysters (Crassostrea gigas) on the French market

	1993	1994	1995	1996	1997
Production (tonnes)	146 347	144 328	149 629	147 150	n.a.
Imports (tonnes)	2 612	1 441	1 470	1 525	1 700
Exports (tonnes)	4 357	4 270	4 284	4 718	4 100
Ex-farm price* (US$/kg)	1.96	1.94	1.47	1.58	n.a
Retail price* (US$/dozen)	3.22	3.07	3.06	3.19	3.23

* Current price, on the basis of 1 US$ = 6 French Francs
Source: FIOM

Table 2. Evolution of quantities and price for mussels on the French market

	1993	1994	1995	1996	1997
Aquaculture production (tonnes)	64 413	64 194	61 962	63 350	n.a.
Fisheries production (tonnes)	27 366	32 698	10 796	9 658	n.a.
Imports of fresh product (tonnes)	23 233	21 186	24 225	34 495	35 955
Imports of processed product (tonnes)	4 127	4 617	3 899	4 664	4 500
Imports of canned mussels (tonnes)	n.a.	10 841	9 965	9 404	8 950
Exports of fresh product (tonnes)	2 590	1 778	1 622	1 727	1 696
Ex-farm price* (US$/kg)	1.14	1.20	1.20	1.33	n.a.
Retail price* (US$/litre)	1.77	1.73	1.74	1.80	1.85

* Current price, on the basis of 1 US$ = 6 French Francs
Source : FIOM

QUALITY APPROACH AND STRATEGIES OF PRODUCT IDENTIFICATION AND DIFFERENTIATION

The context of quality approach in the case of the French oyster and mussel sector

Still more than for other agrofood sectors, the public policy about shellfish has focused till now on the sanitary aspects. It has resulted in the implementation of numerous regulations. The other main concern of the public policy towards agriculture and food industry has been to support these activities in response to the degradation of the quality of the products due to the industrialisation of the production processes. Among others, poultry and veal industries are good examples of quality policy following such a phenomenon of industrialisation. On the contrary, this last issue is not relevant in the case of the oyster and mussel sector since there has been very few industrialisation of the processes (Paquotte, 1995).

Even in the case of oysters which have not to face an international competition, it has to be taken into account now that molluscs enter a market where the products are evaluated by the consumers not only according to the price but also to different aspects like the convenience or the image. The competition comes from more and more numerous meat and seafood products which will be compared to oysters and mussels. In particular, the demand for oyster may be considered as optional and variable since it is not a main component of the meals and it is rather expensive. This fragility has been underlined by the dramatic decrease in price of possible substitutes like smoked salmon or « foie-gras ». In the case of mussels, the increasing imports of processed products proves also that the competition is not any more based only on price.

This awareness of the quality issue is also a consequence of the economic difficulties of the sector, with decreasing ex-farm prices and steady quantities while production costs increase. Indeed, productivity gains are difficult to obtain in this sector where labour is intensively used in a context of small scale farms with little investment capacity. In a situation of increasing financial costs due to investment in order to comply to the European sanitary regulations, the price decrease has induced a reduction of the average profit margin in the French shellfish sector, with recently some cases of bankruptcy. In the mussel sector, the development of off-shore plants has facilitated merges and new investments which have made it possible to take advantage of economies of scale. But the oyster activity is still very traditional and labour represents 35% of the production costs. Moreover, there is an increasing competition among the different regions of production which pulls prices down but also stimulates the attempts to differentiate the products and to build quality policies.

Given the variety of production sites, the variability of the quality of the products along the year and from one year to another, the main objectives are first a better identification of the products, then a

differentiation of the products and also an increase of their quality. In a context of reduction of the profit margin and of increasing competition with other seafood and meat products, the overall objective is to ensure better profitability of the activity and a higher remuneration of the labour.

A typology of the quality approaches in the French oyster and mussel sector

Quality approaches and product differentiation have been an issue in the French oyster and mussel sector for about ten years, but most of the realisations are less than five years old. Three main types of quality approach may be distinguished, which are respectively based on advertisement, specificity and top grade quality (Charles, 1997).

The actions of the first type do not refer to quality approach, but to promotion and communication policy. The actions of the second type point out the specific characteristics of a particular product but do not aim explicitly at any superior quality. This specificity may be due to geographical reasons or to production process reasons. The third type of action is used to promote products the quality of which is considered as superior. This top grade quality may also be justified by the location of the production or by a special process (Figure 1).

In every case, such a quality approach may be supported or not by a certification. The certification procedure may be just the registration of a brand or may result from a more complex procedure associating producers, an approved certifying institution and the agreement of the administration. In particular, this agreement is required for the use of official French or European quality marks.

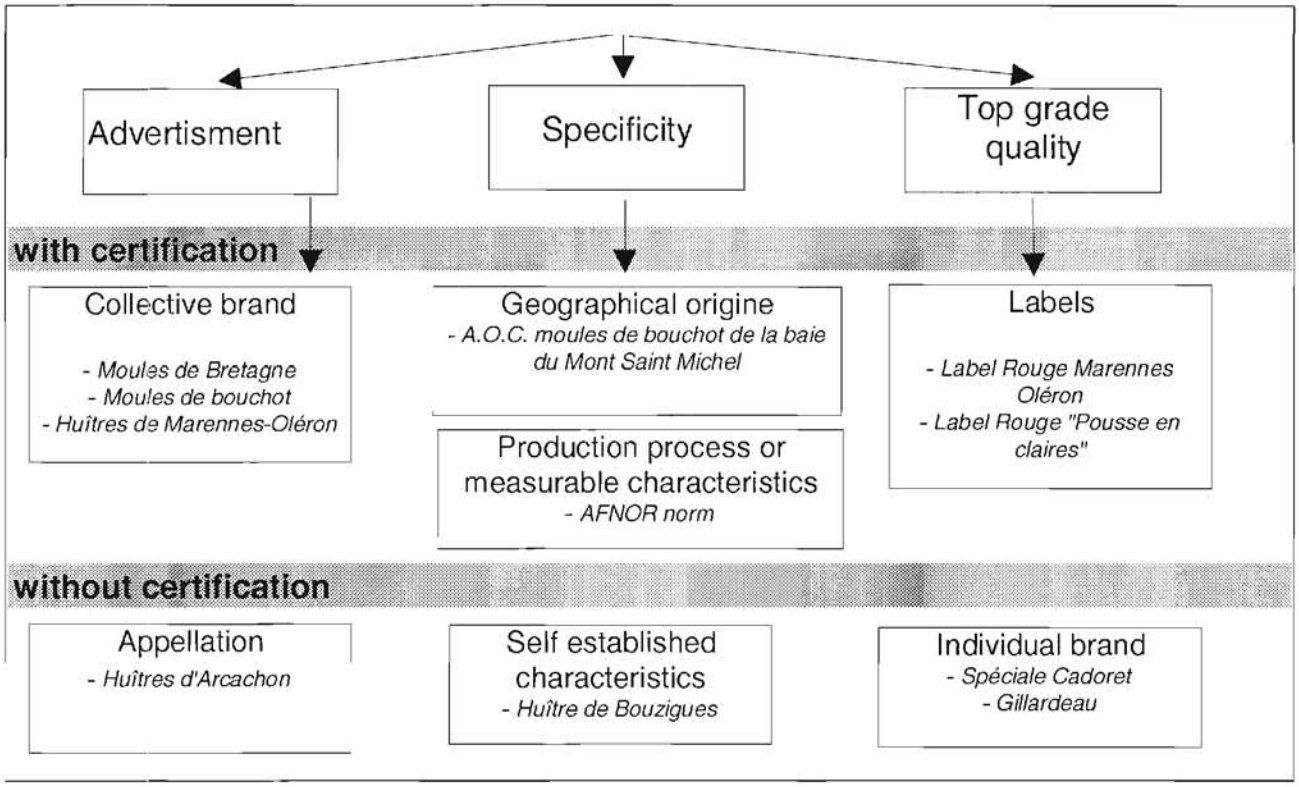

Figure 1. Typology of the different quality approaches for oysters and mussels in France

Among all the quality approaches, it is possible to quote some appellations which aim only at communicating and at advertising the product without any certification procedure: « Huîtres d'Arcachon » for oysters produced in the Arcachon basin, « Huîtres de pleine mer » for oysters farmed in offshore conditions and « Moules de Charron » for mussels coming from the coast close to the village of Charron. Most often, the appellations used for promotion and communication purpose are registered as collective brands. That is the case of the « Moules de bouchot » which are farmed on poles or « Moules de pleine mer » which are farmed on long-lines by opposition to bottom farmed mussels and to dredged mussels. The best known registered collective brand is « Marennes Oléron » for oysters. To be allowed to be sold under that appellation, the oysters have to stay a minimum time in the Marennes Oléron basin, wherever they come from.

The certification of specificity has been until now mostly applied to the production process with the

appellations « spéciale » and « fine » which are based on the duration of the farming cycle in the earth ponds called « claires ». Some measurable characteristics like the meat content and the overall weight are the support of official national norms (AFNOR). As for specificity due to the geographical origin, it has been developed either without any certification by Mediterranean producers of the Thau lagoon with the « Huître de Bouzigues » or with a heavy certification procedure in the case of the « Moules de bouchot de la Baie du Mont Saint Michel ». In that case, a procedure of « Appellation d'Origine Contrôlée » has been carried out, at the image of what has been commonly developed for wine and cheese in France.

Following the example of the poultry industry, a group of producers of the Marennes Oléron basin tried to promote top grade quality products with the help of the « Label Rouge » which is a French official quality mark widely used for agricultural products. The attribution of this label is linked with some constraints in the production process and with measurable characteristics of the final product. Given the poor results of this attempt in terms of volume of production, some producers have decided to go further with a much more severe set of criteria. This « Label rouge pousse en claire » is given to oysters which have spent at least 4 months in « claires » at a density below 5 oysters per m2, which have been farmed with special traditional processes and which comply to higher standards of shape and of meat content. On the other side, some individual producers have specialised in top grade quality products without any certification process. That is the case of producer's brands like « Gillardeau » or « Papin » for oysters from Marennes Oléron, or like « Cadoret » for oysters from Brittany.

Present results of these quality approaches

In spite of that multiplication of quality approaches, the French market is still dominated by two major appellations which are « Marennes Oléron » for the oysters and « Bouchot » for the mussels. Indeed, almost half of the production of oysters is sold as « Marennes Oléron » and 65% of the mussel production comes as « Bouchot ». In both cases, they get a premium price on the market.

The « Label rouge » is still marginal for oysters because producers have difficulties to get a price premium. This failure may be explained by a lack of real differentiation from other oysters. Indeed, given the variability of oysters quality, it is possible to find on the market oysters providing the same quality as the labelled ones, but at a lower price. On the contrary, the top grade quality oysters which are sold by producers under their own name got a real price premium, up to 100% more in the traditional outlets.

The appellations based on the geographical origin are numerous but they compete with each others and so are self-limited. In most cases, these regional approaches are supported by local authorities and by local economic development organisations without any coordination at a national level. This situation does not make possible a clear identification of the products and is not compatible with the constraints of super/hyper markets which can not afford to propose as many geographical appellations as there are French regions. There is already a large number of appellations which induce confusion in the consumer's mind. A survey carried out in the Metropolitan Paris area between December 24 and December 31 1996 in six hypermarkets has shown that 21 different appellations were in use for oysters. Among them, only 5 could be found in more than one hypermarket while 16 were exclusive to one hypermarket. In this sample, three quarters of the appellations referred to the geographical origin and only one quarter had a brand name not related to the geographical origin (Garnaud, pers. comm.).

As for the attempt to launch an A.O.C. (« Appellation d'Origine Contrôlée ») in the Baie du Mont Saint Michel, it is still in stand-by after five years of common reflection with the producers and the public authorities. The analysis of the set of criteria which has been drawn up shows that two different types of quality approach has been mingled inducing confusion among the producers. Indeed, not only this project of A.O.C. refers to a special site of production which gives specific characteristics to the product in terms of colour and taste, but it is aiming too at defining a top grade quality product through parameters like shell size and meat content. The problem is that if all the producers of the Baie du Mont Saint Michel can easily comply with the specificity of the product since it is due to general characteristics of the site, not all of them can comply all year long with the superior quality criteria. So, they are afraid to lose the price premium they are used to get with the usual appellation « Moules de bouchot de la Baie du Mont Saint Michel » if the A.O.C. is implemented.

ATTITUDE OF CONSUMERS AND DISTRIBUTORS OF OYSTERS AND MUSSELS TOWARD QUALITY

Attitude of consumers

In order to assess the attitude of consumers towards the quality of shellfish, a study has been carried out in 1996 about oysters (Ifremer-Isara, 1997). This study was based on a panel of 1 000 super/hypermarkets customers in 5 cities: two cities close to production centres (Montpellier and La Roche sur Yon), two inland cities (Lyon and Limoges) and Metropolitan Paris.

At a general level, all cities mixed, the main features of French consumers' attitude towards oysters are the following:
- The notoriety of Marennes-Oléron oysters is very high, far beyond other regions (Bretagne, then Bouzigues and Arcachon);
- More than half the population of the panel is unable to differentiate oysters from various origins; This proportion is lower in the coastal cities.
- Even in cities close to the production centres, the production processes are poorly known, especially by women; The fattening process in « claires » is better known by elder people and by Parisians;
- Oysters are usually thought as natural products; The consumers do not pay attention to the external aspect and to the shape of the shell;
- Around two thirds of the panel prefer oysters with high meat content and half of the panel prefer oysters with a lot of water;
- The price of oysters is not well known since the coefficient of variation (standard deviation / average estimated price) is above 50% in general, but only 40% in the coastal cities;
- 75% of the panel are in favour of certified regional appellations (A.O.C), but this proportion is much lower among elder people;

The main differences in consumer attitude towards oysters come from the age and the geographical position. Elder people, who are big consumers, rely on their own knowledge of the product and do not wish the implementation of official labels or appellations which they would not trust. In the coastal cities, the purchase frequency is higher and there are more regular consumers while in other cities the consumption is limited to the end of the year. People in the coastal cities praise the products of their region and do not really long for labels or other quality marks. The preferences in terms of meat content and water quantity are also different since people in the coastal cities look for higher meat content and less water. In Paris and Lyon, the appellation Marennes Oléron is particularly known and receives a price premium but it is not linked to any knowledge of the specific characteristics of the product.

Attitude of distributors

In order to assess how the distributors consider these quality approaches, a study has been carried out with 31 super/hypermarkets in Brittany, a region which is on the Atlantic ocean but which has also a large hinterland (Charles, 1997). Although more than half of the panel claim to give more importance to the quality of the product than to the price, very few among them are able to quote a quality approach for oysters or mussels: only 30% know the collective brand « Huîtres de Bretagne », 26% the « Label Rouge Marennes Oléron » and 38% local brands. Most of them rely on their personal knowledge of the producer and would prefer to promote products from nearby production sites.

Nevertheless, 75% of the distributors are in favour of the implementation of quality approaches and half of them would even consider it as a major criteria in the purchase decision. They would be ready to pay a premium for such a label, as long as it stays inside the range 5%-15%. But most of them give priority to quality marks linked to the geographical origin rather than to quality marks linked to production process. From the distributors' point of view, the consumers pay attention to the external aspect of the product (87%), to the price (81%) and to the origin (68%) but not to a certification of quality (3%). They even think that the consumers would be confused by two many quality marks.

This study has also revealed different attitudes between distributors in cities directly on the seaside and distributors in cities more than 25km away from the seaside. So, if 87% of the inland distributors would rely on a certified quality approach, only 36% of the seaside distributors would do it. These last ones give much more importance to the personal relationships with producers and consider that their customers are connoisseurs at 66% (but only 33% inland) and would definitely prefer local products to any other certified product. In particular, the seaside distributors are not eager to pay any quality certification more than 5% above the regular price, while inland distributors would be ready to go till 25%. In the case of seaside distribution, it does not seem likely that any quality approach could be profitable with such a small premium.

ARE THE LEGAL SIGNS OF QUALITY SUITED TO OYSTERS AND MUSSELS ?

In spite of the various and numerous attempts which have been being undertaken for several years to identify and to differentiate oysters and mussels in France, the results are quite disappointing as well in terms of market share of labelled products as in terms of consumers' and distributors' attitude. These results seem all the weaker as the same approaches have given great results in other agrofood sectors like wine, cheese and poultry.

Indeed, oyster and mussel farming sectors are characterised but a « domestic mode of coordination », according to the classification given by Eymard-Duvernay (1992). This means that a great importance is given to the personal relationships between actors all along the production and distribution chain. Since there is very little industrialisation of the production processes, most of the products being sold fresh, this mode of coordination is still relevant in oyster and mussel farming while it has disappeared in most other food

sectors. Nevertheless, this type of relations has not been pursued by the super/hypermarkets which have recently become the major distributors for these products and have put a lot of pressure on price. As there is little industrial processes on which to base certified quality marks and develop an « industrial mode of coordination », the price has become the major criterion in the purchase act, either from distributors or consumers. So, a new mode of coordination has appeared, mostly based on the price which becomes the only criterion to arrange the different products. In this kind of « commercial mode of coordination » which results mainly from the balance between supply and demand, the producers are in weak position in front of the distribution and become price takers.

Actually, supply and demand should not be the only parameters to be taken into account in the price formation since the lack of industrial process and the high dependence of oysters and mussels on natural conditions are at the origin of a high variability in the characteristics of the products. There is a large range of qualities, but it is difficult to establish objective links between the geographical origin or the production processes and the final characteristics of the products. This is certainly the main reason why so many attempts have failed, by lack of objectivation of the parameters making the products different. Given the large variation of the product characteristics around a standard, the specifications for a top grade quality product have to be put very high in order to be sure it will be always superior to the ordinary products and deserve its price premium in consumer's mind. Moreover, oyster and mussel farming is characterised by transfers of animals from one site to another during the rearing cycle. These transfers, which are often necessary to give the best products at the lowest cost, make more difficult the definition of a quality mark based on the geographical origin.

In order to get a clear definition of what is the quality for products like oysters and mussels and facilitate the use of agricultural quality marks, research programmes about significant quality criteria and quality assessment methods have to be carried out in cooperation with the professional organisations. Following the example of other agricultural sectors, the improvement of husbandry practices and the reduction of the dependence on the medium thanks to zootechnical progress seems to be the key to the quality control issue. In order to implement quality approaches based on the certification of production methods and processes, a better zootechnical control should be required. But it has to face the reluctance of the producers who are afraid to lose the image of « natural product » they are very proud of. For instance, the French organisation of oysters and mussels producers has not yet accepted to transfer the recent advances in genetics and to use triploïdic oysters which do not mature in summer and would produce a regular product all year long. So, there is a contradiction between the aim at differentiating these products with the help of the legal agrofood quality marks and the willingness to keep the farming process totally depending on the environmental conditions for a question of image.

DISCUSSION

The oyster and mussel sector in France is far behind other agrofood sectors in terms of:
- knowledge of consumer's preferences,
- objectivation of links between origin or production processes and final characteristics of the products,
- quality control all along the year and from one year to another.

Like for other agricultural products, there is a possibility to enlarge the market for oysters and mussels and to get a better valorisation with on the one hand standardised products (with a better quality control) and on the other hand specific products with quality marks (« green » products, top grade quality labels, certification of origin etc.).

The analysis of the recent evolution of the sector and of the consumers' attitude shows that it is necessary to improve the transmission of information all along the distribution chain and to have a better control of the production processes. In particular, three issues have to be taken into account:
- the definition of an identical set of criteria to define the quality and to differentiate the products from the producer to the consumer
- the improvement of scientific knowledge, particularly in physiology and genetics, to alleviate the present zootechnical constraints to an efficient product differentiation and to quality control,
- the coordination of the different local and regional approaches in order to reduce the number of appellations, to give more credibility to the most relevant ones and to avoid confusion in the consumer's mind.

Producers have also the possibility to develop a partnership with the super/hypermarkets chains, as it has been done for meat or some seafood products. These partnerships are based on contracts which guarantee the origin and the production processes according to a set of criteria established by the distributor. It is a way to ensure the producer its quality approach will be worthwhile thanks to the market power of the supermarket, and to guarantee to the distributor a good quality of the products in order to establish customer loyalty. Until now, no distributor has given its own brand to oysters and mussels yet, but it could be done soon in the framework of such a partnership.

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Conclusion

Although this review is incomplete and has not dealt, for instance, with the use of organic materials such as dicoumarin as a rodenticide, or 2-heptadecylglyoxalidine as a fungicide, the same rules regarding their use as hygiene aids should be applied as for those materials discussed in detail. Although there may be many difficulties, analytical and toxicological, in the use of processing aids and hygiene aids, it seems wrong that one should be unduly biased by these difficulties and argue that chemical aids in food processing should be banned. This is the negation of chemical progress. These difficulties should be a challenge to the food chemist to surmount them. If a real functional value (Coppock, 1951) can be demonstrated for a new processing aid or hygiene aid, then its use should be carefully considered on its merits for the purpose intended, and only if the proposed use involves a potential hazard to health should it be discarded. It is unfortunate that this problem is not being approached here as actively as in America and we are accumulating toxicological and analytical data less rapidly. Also, should not the food scientist do more to inform the public of the steps taken to safeguard it from the use of substances that might endanger its health ? A little wise publicity could remove many misconceptions which are becoming a danger to the progress of food science and which can only be met by telling the public the facts in a simple understandable manner.

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MANUFACTURE OF ALGAL CHEMICALS. IV.*—Laboratory-Scale Isolation of Fucoidin from Brown Marine Algae

By W. A. P. BLACK, E. T. DEWAR and F. N. WOODWARD

Methods for the extraction and isolation of fucoidin from the common brown algae indigenous to Great Britain have been worked out on the laboratory scale, with a view to the ultimate development of a process suitable for large-scale production. Extraction involves treatment of the weed with hydrochloric acid at 70° for one hour at pH 2·0–2·5. The crude fucoidin is isolated by fractional precipitation with alcohol, and purified by treatment with formaldehyde.

Introduction

Fucoidin, a polyfucose ethereal sulphate occurring in the Phaeophyceae, was first described

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and named by Kylin (1913), who prepared (Kylin, 1915) it from *Laminaria digitata* and showed that it contained the methylpentose, L-fucose, by isolating the phenylhydrazone on hydrolysis. He claimed that pentoses were also present in the hydrolysate. Bird & Haas (1931) obtained from *L. digitata* fronds a product containing ash, 30·9%, and sulphate on hydrolysis, 30·3%, and considered fucoidin to be a carbohydrate ethereal sulphate since the total sulphate on hydrolysis was approximately double that found in the ash. Lunde, Heen & Öy (1937) prepared fucoidin by precipitating the droplets exuded from fresh *L. digitata* fronds in alcohol. Their product contained ash, 26–30; sulphate in ash, 17–19; total sulphate, 35·5–37·7; and methylpentose (by distillation with hydrochloric acid), 33–37%. The ash consisted chiefly of sodium sulphate, with small quantities of potassium, calcium and magnesium sulphates. Since only about 80% of the molecule could be accounted for by this analysis, Lunde *et al.* (1937) proposed the formula $(R{\cdot}R^1{\cdot}O{\cdot}SO_2{\cdot}OM)_n$ for fucoidin, where R is fucose (as $C_6H_{10}O_4$), R^1 is unknown, and M may be Na, K, $Ca_{0·5}$ or $Mg_{0·5}$.

Another water-soluble polysaccharide closely related to, if not identical with, fucoidin was isolated from *Macrocystis pyrifera* by Hoagland & Lieb (1915) and shown to contain L-fucose and a high proportion of calcium and sulphate. Nelson & Cretcher (1931) showed the presence of an ethereal sulphate grouping and confirmed that fucose was the only sugar obtained after hydrolysis. Their product, however, had a uronic acid content of 2·6%, which was considered to be due to contamination with alginate.

Percival & Ross (1950) prepared fucoidin from *Fucus vesiculosus, F. spiralis, Himanthalia lorea* and *L. cloustoni*, by extraction with boiling water for 24 hours, removal of alginates and proteins with lead acetate, and precipitation of fucoidin as a lead hydroxide complex by addition of barium hydroxide. The resulting complex was decomposed with dilute sulphuric acid, and the fucoidin was isolated after prolonged dialysis and several treatments with Filter Cel. The various specimens gave similar analytical data, but the purest (from *H. lorea*) contained: fucose on hydrolysis (method of Cameron, Ross & Percival, 1948), 43·9, (chromatographic method of Flood, Hirst & Jones, 1948), 48·4; total sulphate, 32·4; metals, 6·9; ash, 22·6%; $[\alpha]_D$—140° in water. The ash was mainly calcium sulphate. When allowances were made for adsorbed water and ethanol, which were found to be still retained by the polysaccharide after drying at 40°/0·1 mm. for 18 hours, the following figures were obtained: fucose on hydrolysis (chromatographic method), 56·7; total sulphate, 38·3; metals, 8·2%. Small quantities of uronic acid (3·3%), galactose (4·1%) and xylose (1·5%) were also detected. These workers believe that the principal constituent of fucoidin is a polyfucose monosulphate and that the other constituents arise from adventitious impurities. A calcium polyfucose monosulphate, $(C_6H_9O_3{\cdot}SO_4{\cdot}Ca_{0·5})_n$, would give on hydrolysis: fucose (as $C_6H_{12}O_5$), 66·9; total sulphate, 39·2; calcium 8·2%.

Conchie & Percival (1950) methylated fucoidin from *F. vesiculosus*, and on hydrolysis the carbohydrate portion was found to contain L-fucose, 3-methyl fucose and 2 : 3-dimethyl fucose, roughly in the proportions 1 : 3 : 1. The main residue in fucoidin was therefore believed to be a 1 : 2-α-fucopyranose unit carrying a sulphate group on C_4. They advanced two possible theories of accounting for the free fucose and 2 : 3-dimethyl fucose residues in methylated fucoidin: (1) Some fucose residues might carry two sulphate groups, whereas others (linked 1 : 4) are unsubstituted by sulphate groups:

$$\begin{array}{l} \qquad\qquad\qquad\quad SO_4^- \\ \qquad\qquad\qquad\quad \overset{3}{|} \\ \text{—1F2—1F2—1F4—1F2—1F2—} \\ \quad \overset{4}{SO_4^-}\ \overset{4}{SO_4^-} \qquad\quad \overset{4}{SO_4^-}\ \overset{4}{SO_4^-} \end{array} \quad (\text{F} = \text{L-fucopyranose})$$

(2) Another possible explanation is that the free fucose originates from branching points at C_3 carrying terminal groups having free hydroxyls on C_2 and C_3:

$$\begin{array}{l} \qquad\quad F_4SO_4^- \\ \qquad\quad | \\ \qquad\quad \overset{3}{|} \\ \text{—1F2—1F2—1F2—1F2—} \\ \quad \overset{4}{SO_4^-}\ \overset{4}{SO_4^-}\ \overset{4}{SO_4^-}\ \overset{4}{SO_4^-} \end{array}$$

These workers state, however, that a great deal of work must yet be carried out before the constitution of fucoidin is settled.

Fucoidin was considered by Kylin (1915) to be a cell-wall mucilage of the brown algae, particularly rich in the laminarias. It is now known, however, that fucoidin is present in all

common brown seaweeds, but to a much greater extent in the Fucaceae than the Laminariaceae, and undergoes a seasonal variation similar to alginic acid and cellulose, the other constituents of the cell-wall (Black, paper in preparation).

Apart from any industrial use which may be found for fucoidin itself when available in quantity, the polysaccharide should prove a much more suitable source of L-fucose than the present rather tedious process from the whole weed (Hockett, Phelps & Hudson, 1939). Although the Phaeophyceae are the best source of this sugar, L-fucose is also present in gum tragacanth (James & Smith, 1945), sea-urchin eggs (Vasseur, 1948), blood-group substances (Bray, Henry & Stacey, 1946) and frog-spawn mucin (Folkes, Grant & Jones, 1950).

The samples of fucoidin prepared in this investigation were dried either in a vacuum over phosphorus pentoxide or in an oven at 100–105° overnight, and therefore must still contain the bound water and ethanol revealed by Percival & Ross (1950). No correction has been made for these adsorbed solvents.

Species examined

The species used in this investigation, together with details of their composition, are recorded in Table I. After collection, the samples were dried on a rack at 25–30° for 48 hours and ground in a Christy and Norris mill, fitted with a 64-mesh screen. Fucose (as $C_6H_{12}O_5$) was estimated throughout by the improved colorimetric method (Black, Cornhill, Dewar, Percival & Ross, 1950), and the other constituents by the methods previously employed by one of the authors (Black, 1948). Because of the uncertainty of the exact fucose content of fucoidin, it is not yet possible to convert combined fucose figures into fucoidin. Ashing was carried out at 450° for 4–6 hours in a Wild Barfield furnace.

Table I

Composition of species examined

Species	Habitat	Date collected	% Chemical composition (dry basis)					
			Fucose (as $C_6H_{12}O_5$)	Ash	Mannitol	Laminarin	Alginic acid	Organic nitrogen
1. *Pelvetia canaliculata*	Atlantic Bridge	Mar. 1949	11·2	24·1	8·5	2·48		1·55
2. *Ascophyllum nodosum*	Cullipool	June 1945	9·0	17·3	9·0	4·27	24·4	1·33
3. *F. vesiculosus*	Port Appin	Nov. 1946	9·7	20·5	13·7	4·85	15·2	1·18
4. *F. vesiculosus*	Port Appia	Dec. 1945	10·1	26·0	10·6	2·6	15·0	1·20
5. *L. cloustoni frond*	Cullipool	Oct. 1948	3·14	20·3	15·4	29·2	10·0	1·70

Determination of optimum conditions of extraction

Several extractions were carried out to determine the effect of pH, temperature, extraction time and the ratio of extracting liquid to weed. The results obtained are recorded in Table II. An impeller type of stirrer was used in Expt. 1–7, and the rate was kept constant at approximately 500 r.p.m.

The dried milled weed was treated for the required time with the extracting liquid as shown in Table II at the pH and temperature indicated. After extraction, the weed residue was centrifuged, washed with water, air-dried at 65°, weighed and analysed. In Expt. 7, the weed was stirred with 0·17N-hydrochloric acid (200 ml.) at 70° for 1 hour, centrifuged and washed. The weed residue was then treated with water (150 ml.) to give the same volume as in the first extraction, the pH was reduced to 2·3 with 10N-hydrochloric acid (1·0 ml.), and the mixture was stirred at 70° for 1 hour. After centrifuging and washing, the weed residue was again treated with water (150 ml.), 10N-hydrochloric acid (0·5 ml.) added to pH 1·9, stirred at 70° for 1 hour, and the weed residue centrifuged and washed. Expt. 12 was carried out in the same way, except that water only was used in each extraction.

Extraction in the cold, even at the low pH of 1·5 (Expt. 2), removes very little fucoidin, and treatment with alkali at 75° is little better (Expt. 1). Stirring with hydrochloric acid at 70° for 1 hour at pH near to 2·5 extracts about 50% of the total fucoidin (Expt. 3, 4 and 5), whereas three acid extractions remove more than 80% (Expt. 7). The importance of controlling pH is shown in Expt. 6, where conditions identical to those in Expt. 5 existed except for pH, and the fucoidin extracted was only 22% as compared with 51% in Expt. 5.

As found in the preparation of laminarin (Black, Cornhill, Dewar & Woodward, 1951), the

Table II

Effect of pH, temperature, extraction time and ratio of extraction liquid to weed on the extraction of fucoidin from P. canaliculata *and* F. vesiculosus

Expt. No.	Species	Wt. of weed, g.	Extracting liquid and conditions	pH of mixture	Vol. of water washings, ml.	Wt. of weed residue as % of weed	Analysis of weed residue				Fucose extracted as % of total fucose
							Ash, %	Ash as % of total ash	Fucose, %	Fucose as % of total fucose	
1	*F. vesiculosus* Table I (3)	20·71	Stirred with 0·1N-NaOH (200 ml.) at 75° for 1 hr.	9·1	2 × 80	55·5	20·9	56·5	12·3	70·4	29·6
2	*P. canaliculata* Table I (1)	248·0	Stirred with 0·16N-HCl (2500 ml.) at 15° for 6 hr.	1·5	2 × 500	64·6	10·9	34·7	13·9	80·2	19·8
3	*P. canaliculata* Table I (1)	24·92	Stirred with 0·1N-HCl (250 ml.) at 70° for 1 hr.	2·7	2 × 50	59·4	12·6	31·1	9·5	50·4	49·6
4	*F. vesiculosus* Table I (3)	24·18	Stirred with 0·1N-HCl (250 ml.) at 75° for 1 hr.	2·5	2 × 50	61·2	8·1	24·3	6·9	43·5	56·5
5	*F. vesiculosus* Table I (4)	250·1	Stirred with 0·17N-HCl (2500 ml.) at 70° for 1 hr.	2·3	2 × 750	57·2	10·1	22·2	8·6	48·7	51·3
6	*F. vesiculosus* Table I (4)	252·0	Stirred with 0·1N-HCl (2500 ml.) at 70° for 1 hr.	4·5	2 × 750	61·4	16·2	38·2	12·8	77·8	22·2
7	*F. vesiculosus* Table I (4)	20·46	Stirred 3 times with HCl (200 ml.) at 70° for 1 hr.	1·9–2·5	1 × 80 after each extraction	44·0	3·5	5·9	4·23	18·4	81·6
8	*P. canaliculata* Table I (1)	25·23	Heated with water (250 ml.) at 100° for 3 hr.	5·7	2 × 60	55·9	18·9	43·8	9·0	44·9	55·1
9	*F. vesiculosus* Table I (3)	20·11	Heated with water (200 ml.) at 100° for 7½ hr.	5·8	2 × 80	48·0	13·9	32·5	8·0	39·6	60·4
10	*F. vesiculosus* Table I (3)	20·32	Heated with water (400 ml.) at 100° for 7½ hr.		2 × 80	42·8	14·5	30·3	7·3	32·2	67·8
11	*F. vesiculosus*, Table I (3)	10·21	Heated with water (200 ml.) at 100° for 15 hr.		2 × 40	41·0	13·2	26·4	5·3	22·4	77·6
12	*F. vesiculosus*, Table I (4)	20·39	Heated 3 times with water (400 ml.) at 100° for 7½ hr.		2 × 80 after each extraction	46·3	20·0	35·6	6·82	31·3	68·7

Table III

Fraction	Wt. as % of weed	Ash, %	Fucose, %	Fucose as % of total fucose	Fucose as % of fucose extracted
Precipitate B	6·60	22·2	11·1	7·6	12·5
Fucoidin C	13·6	26·4	31·3	43·9	72·7

chief disadvantage of aqueous extraction is the large volume of liquid retained by the weed residue, which renders the separation of the residue from the solution difficult. With water, however, there is less chance of degrading the fucoidin by partial hydrolysis than when acid at 70° is used. Aqueous extraction at 100° for 3 to 7½ hours removes 55 to 60% of the fucoidin (Expt. 8 and 9), although more efficient extractions can be obtained by increasing the water : weed ratio (Expt. 10), the extraction time (Expt. 11), or the number of extractions (Expt. 12).

From these experiments, hydrochloric acid extraction at 70° for 1 hour at or near pH 2·5 appeared to be the most satisfactory, three such treatments being necessary to remove 80% of the fucoidin.

Isolation of crude fucoidin from *F. vesiculosus*

The centrifugate and washings from Expt. 9 (Table II) were evaporated at 50°/20 mm. to dryness, and the dark-brown glass (11·15 g.) redissolved in water (100 ml.). The solution was treated with alcohol (25 ml.) to 20% (v/v) concentration, and the precipitate B was centrifuged, washed with alcohol (ethanol) and ether, and dried to a brown powder. This precipitate contains most of the soluble alginate. The alcoholic centrifugate was then treated with alcohol (125 ml.) to 60% (v/v) concentration, and the crude fucoidin C was centrifuged and isolated as above as a brown powder. The small quantity of laminarin present is not precipitated at this concentration of alcohol. The analysis of these fractions is given in Table III.

Effect of adding formaldehyde to seaweed extracts

When the combined centrifugates and washings from Expt. 12 (Table II), to which 40% formaldehyde (1·0 ml.) had been added as preservative, were evaporated *in vacuo* at 50° to dryness, the resulting glass was found to be only partially soluble in boiling water (100 ml.). The dark-brown residue A was centrifuged, washed with hot water (2 × 35 ml.), alcohol and ether, and dried. The centrifugate and water washings (130 ml.), which were now almost colourless, were treated with alcohol (56 ml.) to 30% (v/v) concentration, and precipitate B was isolated as a white powder. The alcoholic centrifugate was then treated with alcohol (139 ml.) to 60% (v/v) concentration, and fucoidin C isolated as a white powder. The analysis of these fractions is recorded in Table IV.

Table IV

Fraction	Wt. as % of weed	Ash, %	Fucose, %	Fucose as % of total fucose	Fucose as % of fucose extracted	Total sulphate	$[\alpha]_D$ *c*, 1·053 in water
Residue A ..	.. 23·9	16·8	11·4	27·0	39·3		
Precipitate B	.. 4·5	30·1	10·9	4·9	7·1		
Fucoidin C ..	.. 6·4	29·5	42·5	26·9	39·2	26·2	— 121°

The formation of this insoluble residue A has led to a serious loss (27%) of fucose, although the resulting fucoidin C is purer than that recorded in Table III and almost completely free from colour. This resin-like compound A was found to be insoluble in organic solvents (benzene, carbon tetrachloride, acetic anhydride, acetone and ethyl acetate), and only slightly soluble in boiling concentrated hydrochloric acid and 40% sodium hydroxide. It contained Kjeldahl-N, 1·17, and alginic acid, 1·6% [standard precipitation method of Cameron, Ross & Percival (1948)].

When the combined centrifugates and washings from Expt. 7 (Table II), to which 40% formaldehyde (1·0 ml.) had also been added, were neutralized with sodium hydroxide, evaporated *in vacuo* to dryness, and treated as described for Expt. 12, similar results (shown in Table V) were obtained.

Table V

Fraction	Wt. as % of weed	Ash, %	Fucose, %	Fucose as % of total fucose	Fucose as % of fucose extracted
Residue A ..	.. 18·6	16·4	12·7	23·4	28·7
Precipitate B	.. 1·6	35·9	—	—	—
Fucoidin C ..	.. 12·1	37·4	32·2	38·5	47·2

That the formation of this insoluble residue was due to some complex with formaldehyde was shown as follows : Dried milled *F. vesiculosus* [Table I (4) ; 20·48 g.] was stirred with 0·17N-hydrochloric acid (200 ml.) at 70° for 1 hour, and the weed residue was centrifuged and washed with water (2 × 40 ml.). The centrifugate and washings (236 ml.) were divided into

two. One portion (118 ml.) was neutralized with sodium hydroxide, evaporated *in vacuo* at 50° to dryness (5·25 g.) and taken up in boiling water (100 ml.). When this dark-brown solution was centrifuged, only a very small residue was isolated (0·18% of the weed). The second portion (118 ml.) was stirred again at 70° for 1 hour, neutralized with sodium hydroxide and treated in the same way as the first portion. The insoluble residue amounted to 0·78% of the weed, thereby indicating that very little insoluble material is formed after 1 and 2 hours heating in the absence of formaldehyde.

When, however, the dark-brown centrifugates from these two experiments were combined, 40% formaldehyde (0·5 ml.) added, and the liquid was evaporated to dryness and taken up again in boiling water (100 ml.), an insoluble residue amounting to 12·8% of the weed was obtained (Found: ash, 13·0%). The solution after the separation of this residue was almost colourless.

Preparation of crude fucoidin from different species

Fucoidin was isolated from *P. canaliculata* [Table I (1)], *F. vesiculosus* [Table I (4)] and *A. nodosum* [Table I (2)], by the following general procedure: The dried milled weed (20 g.) was extracted three times with hydrochloric acid (200 ml.) at 70° for 1 hour at pH 2·0–2·5, as described for Expt. 7 (Table II), in the absence of formaldehyde. The normality of hydrochloric acid required to bring the pH within the range 2·0–2·5 at the first extraction varied slightly with different species, but generally lay between 0·10 and 0·17N. The combined centrifugates and washings were neutralized with sodium hydroxide, evaporated *in vacuo* at 50° to dryness, redissolved in water (125 ml.), treated with alcohol (54 ml.) to 30% (v/v) concentration, and precipitate B isolated as a brown powder. The centrifugate was then treated with alcohol (134 ml.) to 60% (v/v) concentration, and the crude fucoidin C isolated as a sandy-coloured solid. The analysis of the various fractions is shown in Table VI.

With *L. cloustoni* [Table I (5)], the frond (50·86 g.) was first stirred in the cold for 10 minutes with 0·09N-hydrochloric acid (500 ml.) to remove the bulk of the laminarin, centrifuged, and washed with water (2 × 100 ml.). This treatment was found to remove 31·7% of the total fucoidin. The frond residue was then treated with water to 400 ml., the pH reduced to 2·3 with 10N-hydrochloric acid (2·0 ml.), and the mixture stirred at 70° for 1 hour to extract the fucoidin. After two further extractions at 70° for 1 hour at pH 2·2, the combined centrifugates and washings from the 70° extractions were neutralized, evaporated to dryness, and treated exactly as described for the Fucaceae.

Table VI

Fraction	Species	Wt. as % of weed	Ash, %	Fucose, %	Fucose as % of total fucose	$[\alpha]_D$ in water
Weed Residue A	*P. canaliculata*	42·2	4·1	4·32	16·3	
	F. vesiculosus	42·9	2·6	4·81	20·4	
	A. nodosum	43·5	2·3	3·18	15·4	
	L. cloustoni frond	27·5	4·8	3·43	30·0	
Precipitate B	*P. canaliculata*	10·1	26·5	10·2	9·2	
	F. vesiculosus	6·5	32·7	11·8	7·6	
	A. nodosum	13·3	28·9	13·1	19·4	
	L. cloustoni frond	2·0	27·3	12·2	7·6	
Fucoidin C	*P. canaliculata*	25·5	30·1	33·3	75·9	− 110° (*c*, 0·908)
	F. vesiculosus	17·2	30·0	36·4	61·9	− 98° (*c*, 0·44)
	A. nodosum	15·7	28·7	30·7	53·5	− 115° (*c*, 0·910)
	L. cloustoni frond	1·9	31·5	33·3	20·2	− 106° (*c*, 0·452)

The results show that crude fucoidin, containing more than 30% fucose, can readily be prepared by fractional precipitation, with yields for the Fucaceae exceeding 50%. The poor yield (20·2%) in the case of *L. cloustoni* frond is due partly to the low initial fucose contents of the Laminarias, and partly to the loss (31·7%) of fucoidin in the initial laminarin extraction.

Purification of crude fucoidin

(1) *Reprecipitation with alcohol.*—Crude fucoidin (0·886 g.; fucose, 33·3; ash, 30·1%) was dissolved in water (9 ml.), alcohol (3·9 ml.) was added to 30% (v/v) concentration, but no precipitate formed. Alcohol (17·1 ml.) was then added to 70% (v/v), when the fucoidin appeared as a semi-colloid. This was coagulated immediately on adding sodium chloride (0·1 g. in 1 ml. water). The sticky precipitate was centrifuged, washed with alcohol and ether, and dried to a sandy-coloured powder (0·818 g.). (Found: ash, 29·8; fucose, 38·7%.)

Although the fucose content has been increased, reprecipitation does not remove the colour.

(2) *Precipitation with lead acetate and barium hydroxide.*—This method was used by Percival & Ross (1950) in their preparation of fucoidin. Crude fucoidin (1·803 g.; fucose, 33·3; ash, 30·1%) was dissolved in water (20 ml.), and lead acetate solution (2·0 g. $PbAc_2,3H_2O$ in 10 ml. water) added, but no precipitate formed. Cold saturated barium hydroxide (30 ml.) was then added until the solution was just pink to phenolphthalein. The lead hydroxide–fucoidin complex was centrifuged, washed with water (2 × 20 ml.), and decomposed by suspending in water (50 ml.) containing 4N-sulphuric acid (10 ml.), stirring for 3 hours, and leaving overnight. The lead sulphate was centrifuged, washed with hot water (2 × 20 ml.), and the centrifugate and washings were dialysed against tap-water until free from acid (3 days). The solution was then evaporated *in vacuo* at 50° to 20 ml., a small precipitate centrifuged, the solution treated with sodium chloride (0·1 g.) and alcohol to 70% (v/v) concentration, and the fucoidin was isolated as a brown powder (1·251 g.). (Found: ash, 24·6; fucose, 35·5%; i.e. 74·0% of the fucose in the crude product.)

This treatment, although it effects a slight purification, leads to considerable loss of fucoidin.

(3) *Treatment with formaldehyde.*—Crude fucoidin (1·460 g.; fucose, 36·4; ash, 30·0%; Table VI) was dissolved in water (50 ml.), 40% formaldehyde (0·50 ml.) added, and the solution evaporated at 50°/20 mm. to dryness. The dark-brown glass was extracted with hot water (20 ml.), and the insoluble residue A was centrifuged, washed with hot water (2 × 10 ml.) alcohol and ether, and dried to a brown powder. The light-brown centrifugate and water washings were treated with sodium chloride (0·1 g.) and alcohol to 70% (v/v) concentration, and fucoidin B isolated as an almost white powder. The analysis of A and B is recorded in Table VII.

Table VII

Fraction	Wt. as % of crude fucoidin	Ash, %	Fucose, %	Fucose as % of total fucose in crude fucoidin	Total sulphate	$[\alpha]_D$ in water (*c*, 1·06)
Residue A	.. 29·2	20·3	26·8	21·5		
Fucoidin B	.. 62·7	31·1	44·1	76·0	26·3	− 123°

The formation of this insoluble residue has therefore effected considerable purification, although it also leads to a 21·5% loss of fucoidin. This fucoidin B compares favourably in fucose content with the purest sample isolated by Percival & Ross (1950). The ash, however, is higher than that reported by these workers, although the total sulphate is lower. Expressed as a percentage of total fucose in the original *F. vesiculosus*, the yield of fucoidin B is 47·0%.

Large-scale preparation of fucoidin from *P. canaliculata*

The methods previously worked out on the small scale for preparing crude fucoidin and purifying the crude product have been used for the preparation of a quantity of fucoidin from *P. canaliculata* [Table I (1)]. The weed (200·4 g.) was extracted three times with hydrochloric acid (2 l.) at 60–70° for 1 hour at pH 2·1–2·2, exactly as described for Expt. 7 (Table II), except that the washing with water after each extraction was omitted. The combined centrifugates were neutralized with sodium hydroxide, evaporated *in vacuo* at 50°, and the extract dried for several hours at 50°/10 mm. Toluene was used as preservative, when it was necessary to leave the solution overnight during evaporation. The extract was dissolved in water (1250 ml.), treated with alcohol (540 ml.) to 30% (v/v) concentration, and precipitate B isolated as a brown powder. The centrifugate was then treated with alcohol (1340 ml.) to 60% (v/v), and crude fucoidin C isolated as a sandy-coloured solid (51·1 g.).

The crude product C (50·25 g.) was redissolved in water (500 ml.), 40% formaldehyde (17·5 ml.) added, the solution evaporated *in vacuo* at 50°, and the glass dried for several hours at 55°/10 mm. The glass was extracted with hot water (700 ml.), the insoluble residue D centrifuged, washed with hot water (300 ml.), alcohol and ether, and dried to a chocolate-coloured solid (13·54 g.). The centrifugate, with the water and alcohol washings, was treated with sodium chloride (3·0 g.) and further alcohol added to 70% (v/v) concentration. The fucoidin E, which was precipitated as a toffee-like solid, was centrifuged, washed with alcohol and ether, dried and ground to a light-brown powder (33·96 g.). The analysis of these fractions is given in Table VIII.

When fucoidin E (1·499 g.) was dissolved in water, and treated a second time with 40% formaldehyde (0·50 ml.) as described above, no insoluble residue was obtained, and the fucoidin was recovered unchanged in 95·9% yield on precipitation with alcohol.

Table VIII

Fraction	Wt. as % of weed	Ash, %	Fucose, %	Fucose as % of total fucose in weed
Precipitate B	6·25	25·6	10·4	5·8
Crude fucoidin C ..	25·5	30·7	35·7	81·3
Residue D	6·9	24·1	26·2	16·1
Fucoidin E	17·2	32·0	41·1	63·2

Summary

Methods for the extraction and isolation of fucoidin from brown marine algae have been worked out on the laboratory scale.

Optimum conditions for the extraction of fucoidin consist of stirring for one hour at 70° one part (by weight) of the dried milled weed (all passing 64 mesh) with ten parts (by volume) of hydrochloric acid at pH 2·0–2·5. This treatment removes about 50% of the fucoidin, whereas three acid extractions remove more than 80%. Fucoidin can also be extracted by heating one part of the dried milled weed with ten parts of water at 100° for 3 to 7½ hours. This removes 55–60% of the fucoidin, but more efficient extractions can be obtained by increasing the water : weed ratio, the extraction time or the number of extractions. Aqueous extractions, however, are not recommended because of the difficulty of separating the weed residue from the solution.

Crude fucoidin is isolated from the acid extracts by neutralization and evaporation to dryness, solution in water, and fractional precipitation with alcohol at 30 and 60% (v/v) concentration. The 60% fraction is crude fucoidin containing 30–36% fucose (as $C_6H_{12}O_5$). This crude product has been isolated in 76% yield from *P. canaliculata*, 62% from *F. vesiculosus*, 53% from *A. nodosum* and 20% from *L. cloustoni* frond. Fucoidin, containing more than 40% fucose, can be prepared from the crude product by treating with formaldehyde, and separating the insoluble compound formed. In this way fucoidin, containing fucose (as $C_6H_{12}O_5$), 44·1; ash, 31·1; total sulphate, 26·3%; and $[\alpha]_D$—123° in water, has been isolated from *F. vesiculosus* in 47% yield.

In a larger scale experiment fucoidin, containing 41·1% fucose, has been prepared from 200 g. of *P. canaliculata* in 63% yield.

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