RIVO-Netherlands Institute for Fisheries Research

P.O. Box 68
NL 1970 AB Ymuiden
The Netherlands
Phone: +31 255 564646
Fax: +31 255 564644
E-mail: fisheriesresearch.asg@wur.nl
Internet: www.rivo.wageningen-ur.nl

Centre for Shellfish Research
P.O. Box 77
NL 4400 AB Yerseke
The Netherlands
Phone: +31 113 672300
Fax: +31 113 573477

Report

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Comments of RIVO to the report

"Bromophenols: cause off-flavour in marinated herring and other foods"

A.A.M. Schelvis-Smit and M.J.J. Kotterman

Commissioned by: GAB Robins
Mr. Niall Pritchard
Infinity House
South County Business Park
Leopardstown Dublin 18
IRELAND

Contract number: 04.071

Approved by: H. van der Mheen
Head dpt. Aquaculture

Signature: _____

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Summary

In commission of GAB Robins Scully Tyrrell Ltd RIVO has evaluated the report of Jan W. Henfling. Identified in the documentation with nr: 1740-1747 + 1749-1756 + 1758-1759 RIVO has dealt mainly with those topics and opinions that are plainly not correct, not justifiable or misleading. Topics or opinions raised by Jan W. Henfling, which are not discussed in this RIVO report, do not necessarily reflect the opinion of RIVO. In this evaluation, the report will be discussed page by page.

Note: Page 8 (1748), 14, 16, 19 (1757) are missing in our version of the final report.

1. Comments to the report

Page 2 “Introduction”

“The taint arose gradually following processing…..”

This argument is repeated within the report several times, but this is not according to the reaction mechanisms of bromophenol formation that allegedly took place in the herring of Errigal fish. This will be explained later.

“In all cases phenol contamination of acetic acid has been proven or has been identified as the most…”

It has never been scientifically proven that the presence of phenol in acetic acid in those cases was the cause of tainting. Accordingly, no evidence of this claim has been presented in the Henfling-report.

“Phenol was only identified in the acetic acid used for the tainted product, not in other batches of acetic acid or batches obtained from other processors”.

Batches of tainted herring were detected where the production charts suggests (as far as we know, 14-01-05) these batches of marinated herring were made with other acetic acid (so, with no phenol)! So, either other batches of acetic acid contained phenol also, or phenol was not the cause of the tainting.
That tainting occurred also in other batches is denied in a next statement; “..or by Errigal just prior to the incident with another batch of acetic acid….”

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“The phenomenon as it occurs…..have been described and explained fully”

Again, in literature no evidence has been presented [1]. Some literature even suggests that the marinate cannot be the cause of bromophenol formation as analysed in the tainted herring [2].

Page 4 “Bromophenols as constituents or contaminants of food”

Bremelmans et al [1] pointed to bromophenols as the cause of tainting, but they stated that the herring was not contaminated after harvest, that is shipping, storage. Marinating at this point was also excluded. In fact, when they inspected batches, prepared over a two-month period, of

suspected herring, some batches were tainted whilst others were not. This could be due to the use of clean or phenol contaminated marinates. But, the intensity of the tainting varied for different fish within one batch. If this taint was produced solely by the marinate, all fish would have approximately the same tainting. This suggests that the (chemical composition of the) fish itself also influenced the tainting process. In agreement with this statement is the development of bromophenols in shrimps during storage without any chemical treatment [3] .

Page 5 “Physical-chemical properties of bromophenols”

Physical-chemical properties of compounds are often described in terms of polar (hydrophilic) or non-polar (hydrophobic or lipophilic). Known examples of polar compounds are sugar and ethanol (dissolve readily in water). Examples of non-polar compounds are vitamine D and the infamous DDT, both dissolve only in fat.

In environmental research, the level of the distribution of a certain compound between a non-polar phase (octanol) and a polar phase (water) is often described by Log P. Compounds with high Log P values (6-10) like DDT and dioxines are extremely hydrophobic and will dissolve in octanol (lipophilic phase, like fat) 10^6 till 10^{10} times better than in water. Fish contains lipids and lipid-like compounds, so these compounds will prefer to be solubilised in the fish (fat) rather than in the water surrounding the fish. This can result in bioconcentration (concentration in organism is higher than in the surrounding environment) or even bioaccumulation (compounds will not be excreted from the organisms, even when in a very clean environment. DDT is an infamous example).

In this case, it is interesting to know how Bromophenols behave. The Henfling report suggests that Bromophenols are not likely to be accumulated by herring from the environment, so the source of the Bromophenols must be the suspected marinate.
However, the explanation in the Henfling-report is not accurate. “ Compounds with Log P values well below 3 will be solubilized mostly in the aqueous phase” Log P 3 means that a compound has a distribution over water and octanol of 1 to 1000. In other words, 99.9 % of the compound is solubilized in the octanol, whereas only 0.1 % is solubilized in the water. In databases the bioconcentration factors of other compounds in fish have been described ranging from 490 fold at Log P 2.7 to 4910 at Log P of 2.9 [4]. Only compounds with Log P lower than 0 will be solubilized more in the water than in octanol.

In the Henfling-report rather old (1979), and low Log P values are used. Log P values are 1.69, 2.37, 3.00 and 3.74 for 2-bromophenol, 2,6 dibromophenol, 2,4 dibromophenol and 2,4,6 tribromophenol respectively[2].
However, more recent Log P values are higher. Data from the Environmental Protection Agency (EPA) show Log P values for 2-bromophenol and 2-6 dibromophenol, the most important compounds with respect to tainting, of 2.40 (calculated) and 2.35 (experimental data) for 2-bromophenol; 2,6 dibromophenol has Log P of 3.29 (calculated) and 3.36(experimental data). (www.epa.gov/oppt/p2framework/docs/epiwin.htm).
Also for 2,4,6 tribromophenol significantly higher Log P values have been documented; 4.18 at www.gerstelus.com/appnotes/ new/Gerstel%202001/an-2001-09.pdf and 4.23 at www.epa.gov/epaoswer/hazwaste/id/organobr/acommrev.txt - 84k. With these more recent, higher Log P values it is safe to say that these bromo-phenols will solubilize preferably in a non-polar environment, eg the fat of fish.
Bemelmans and Braber [1] also suggested that fish can accumulate bromophenols fish from the surrounding water. In the communication of Errigal it is clearly stated that washing of the tainted product had no effect, what underlines the fact that bromophenols prefer to solubilize in the fatty fish rather than in water.
It must be noted that not only Log P determines the distribution of these types of compounds between fish and the surroundig water. In life fish, depuration can take place (active transport of these compounds out of the fish) so that accumulation of the compounds is decreased.

Page 8 misses in our copy of the final Henfling report!

We have commented the page 8 ”3. Sources of Bromophenols” from the version of 14-02-01(1660).

A rapid loss of bromophenols in fish when transferred to clean water can take place when the fish is capable to actively excrete these compounds. If the fish is not capable of doing so, bromophenols will still be slowly “washed out”. The driving force is the chemical equilibrium of bromophenols between water and fish. When the clean water contains no bromophenols there is no equilibrium and the bromophenols will move slowly into the water. The higher the Log P of the compound the slower this process becomes. The period required for depurination of salmon, stated by Henfling as “within days”, can not be found in the original article [2]

We do not support the statement that “herring will never contain bromophenols from an internal source”. As stated previously, prawns were shown to develop bromophenols during storage without any chemical treatment [3].

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The herring, as stated in paragraph 3, passively acquired the environmental levels of bromophenols in the surrounding sea. Detection levels of bromophenols in water are approximately 100 times lower than in fatty fish. So, if the fish would acquire the same levels of the surrounding water and would show a strong off-flavour, the water would have a 100 times stronger smell. Clearly, this was not the case. If the herring did acquire the bromophenol from the water, levels of bromophenols could be (according to the Log P values of the bromophenols) 100 to 1000 times higher than in the water, resulting in a strong tainting of the herring (stronger than the water) .

Bromophenols are not analysed for the OSPARCOM. Therefore : “the presence of bromophenols has not been perceived as a problem” is not informative.

3

Post harvest production of bromophenols has been observed in cooked and frozen shrimps. This shows that these off-flavours can be produced without chemical treatments, meaning that artificially added phenol from acetic acid is not an absolute requirement for the production of BP in fishery products [3]**.**

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The statement of 3.3 about the absence of bromide in cleaning products does not have any bearing on this case since bromide is ample present in the marinate.

3.4

Again it is suggested that the fishing waters are constantly monitored for bromophenols, which is not the case. As bromophenols will accumulate 100-1000 times in the herring while eating, low unobserved concentrations of bromophenols in the water could have large effects on the tainting of herring.

page 11. “Bromophenol formation in brine: the chemical process”

Reaction mechanisms

Please note that step one, the oxidation of Br- (bromide, a rather normal ingredient of salt in low concentrations) by hydrogen peroxide to HOBr is the rate-limiting step (the slowest step in the chain of reactions, leading towards bromophenols). This is crucial for the formation of bromophenols, without this slow step no bromophenols will be formed.
The step where actually bromophenols are formed however is rapid, minutes to hours. This information has been deleted from the final version of the report, in stead, the phrase “ the process may gradually continue over a prolonged period of time expressed in days rather than hours” has been inserted. This statement is not according to the reaction mechanism.

To understand the formation process of bromophenols, it is vital to have more insight in the use of hydrogen peroxide in the marinating process. This strong oxidiser is used to oxidise pigments from the herring (“bleaching”), to give it a nice colour. As such this compound is well known for bleaching human hair.
To be able to bleach the pigment in the fish, this compound will have to react strongly with these compounds (a strong oxidiser, or better yet, a strong bleaching agent). As result, it will also react rapidly with other compounds that can be oxidised (again, look at the instructions for using hydrogen peroxide to bleach hair). This makes this compound rather unstable; in fact, this compound will already degrade rapidly in pure water in the presence of light.

As soon as the marinate is added to the herring, the hydrogen peroxide will start to react with many of the organic compounds of the herring, especially the pigments. This results in two important observations;
A the hydrogen peroxide will be depleted rapidly
B bromide will have to compete with all the other compounds in the mixture to react with hydrogen peroxide (and, as stated before, the reaction rate of bromide with hydrogen peroxide is already low).
As soon as all the hydrogen peroxide is depleted, no HOBr or Br_2 will be formed and the formation of bromophenols will stop accordingly.

The study of Adams et al, [5] cited in the report, was performed in pure water or in water with some organic acid. They did not use hydrogen peroxide to produce the reactive bromine species (HOBr and Br2) from bromide. They added the active bromine in high concentrations directly to phenol, which only shows that bromine can react with phenol. No new information useful for this case can be subtracted from this article. However, it shows that under optimal conditions (no other compounds than phenol present for the active bromine to react with) the reaction yields are already low (1-2 %). Also, short storage times of the reaction mixture without phenol decreased the amount of bromophenols formed, again showing that the reactive bromine species are instable and decompose rapidly. Bromine, being a reactive compound, will likely react with other organic compounds in the herring marinate.

Types of bromophenols formed

In the article of Boyle et al [2] the presence of 2-bromophenol in brine-cured herring is seen as an indication that natural sources are at least partly responsible, since the above described reaction mechanism is not likely to produce 2-bromophenols, more likely are 4-bromophenol and 2, 4, 6-tribromophenol. This is supported by the article of Steeg et al[6]. In a brine with off-flavour, many bromophenols and chlorophenols were detected, however, no 2-bromophenol! This is nicely illustrated by the reaction mechanism in the Henfling-report; first 4-bromophenol is formed, than adition of bromide on the ortho-positions takes place (resulting in 2, 4-

dibromophenol and 2,4,6-tribromophenol). According to dr. Fransen (bio-organical, biochemical expert Wageningen University, personal communication) a mixture of 4 and 2-bromophenol can be expected in a releative amount of 4:1 as result of the reaction of bromine with phenol. ***Analysis of all the bromophenols and their distribution would give more information of the origin of these bromophenols. Unfortunately, Leatherhead has not analysed 4-bromophenol, the key intermediate in the formation of bromophenols. If the bromophenols in the tainted herring of Errigal were due to reaction of bromide with phenol, the reaction maechanismes predict a high percentage of 4-bromophenol, and much lower 2-bromophenol***

What can be concluded from the solid facts about reaction mechanisms

Bromophenols (mono, di and tri) can be formed in an acidic solution, containing hydrogen peroxide, bromide (or only bromine) and phenol. These conditions do resemble the brine (if phenol is present) to marinate the herring. However, brine is far from "optimal for the formation of bromophenols".

Addition of other (organic) compounds hydrogen peroxide can react with (herring), will lower the yield of active bromine formation (thus the yield of bromophenols) and will deplete the hydrogen peroxide sooner.

It is clear that the production of bromophenols will proceed better and with higher yield in the brine without herring added. After addition to the herring, the production rate of bromophenols will drop rapidly. As the brine is prepared at forehand, and stored overnight, the major part of bromophenol production, if any, has occurred before the brine is added to the herring.

Since bromophenols will be adsorbed by the (fatty) herring, and thus the threshold for detection will be raised, it is curious that the bromophenols are detected after shipment only.
NOTE, if the herring is shipped with FRESH shipment marinade with peroxide, production of bromophenols can occur during shipment. However incomplete, the production charts so far available suggest that even after the problem of tainted herring was discovered (and a suspected batch of acetic acid was identified and removed) batches of tainted herring were shipped.

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The taste threshold for 2,6 dibromophenol in prawn meat is 0.06 ug per kg. This threshold value differs some between different authors. Curiously, in the same article of Whitfield [3], in which the threshold of 0.06 ug per kg was determined in prawns, an iodine off-flavour in other prawns, supposingly caused by 2,6 dibromophenol could only be detected at concentrations of over 32 ug per kg! This new threshold is higher than any of the observed concentrations in figure 2. Clearly, thresholds cannot easily be used within different products, not even within the same (prawns).

2. Conclusions

Again, it has never been scientifically proven that phenol in acetic acid was the cause of off-flavour in fishery products.

The mixture of ingredients used to produce brine is not a perfect environment for the production of bromophenols when phenol is present. However, some formation of bromophenols can occur.

The claim that contamination of acetic acid with phenol is highly probable is not sustained by any evidence.

The production charts of Errigal itself suggest that off-taint has occurred in herring, produced by another batch of acetic acid free of phenol!

Thresholds of bromophenols in herring are not likely to be the same as in prawns. As the fat content of herring is higher than that of prawns, a higher rather then a lower threshold can be expected. Even so, thresholds can vary within prawns already 500-fold.

To the opinion of RIVO the final conclusion in the Henfling-report is not stated by any solid evidence.

3. Literature cited

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