# Elimination of Furocoumarins in Bergamot Peel Oil

## Composition, extraction methods and olfactory characteristics

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The operational conditions in order to obtain bergamot peel oil "bergapten free" have been studied. It has been found that the olfactory characteristics of processed bergamot peel oil remain almost unchanged.

The problems of photo toxicity of the bergamot peel oil, due to the presence of substances such as psoralens (also known as furocoumarins), has motivated the producers of perfumes and cosmetics to ask for the production of a bergamot oil deprived of such compounds. This product in the trade is known with the name of "defurocoumarined bergamot peel oil."

Examining the composition of the bergamot peel oil, three principal groups of compounds were distinguished: oxygenated compounds, which are primarily responsible for the olfactory properties of the oil; terpenic and sesquiterpenic hydrocarbons; and non-volatile ingredients that constitute the residue.

The non-volatile residue, which was contained in bergamot peel oil in the range of 4.5-6.5 percent, showed a very complex composition. It was mainly constituted of coumarins, psoralens, carotenoids, fatty acids and sterols, with coumarins and psoralens forming a large part (around 20 percent).

The oxygen heterocyclic compounds, with a coumarinic and psoralenic structure, that were present in the highest amounts were: bergamottin (5-geranyloxypsoralen), bergapten (5-methoxypsoralen), citropten (5,7-dimethoxycoumarin) and 5-geranyloxy-7-methoxycoumarin. The minor constituents were: oxypeucedanin, byakangelicol, 5-isopentenyloxy-7-methoxycoumarin, byakangelicin, oxypeucedanin and 5-isopentenyloxy-7-methoxypsoralene. The concentration of components having an oxygenated heterocyclic nucleus, determined by different authors, is reported in Table I (1-4).

The photo toxicity of bergamot peel oil is determined by the concentration of some furocoumarins, particularly bergapten. Considering that the average content of bergapten in bergamot peel oil equals 0.35 percent, the IFRA (International Fragrance Association) (5) recommends a maximum content of bergamot oil of 2 percent in compositions.

According to the literature (Table I), the bergapten content in the examined bergamot oil was about 0.25 percent, and consequently the quantity of bergamot peel oil in cosmetics and perfumery compositions could be increased to a 2.8 percent level.

In order to respect the IFRA recommendation, producers have begun to request bergamot peel oils deprived of bergapten ("bergapten free" oil).

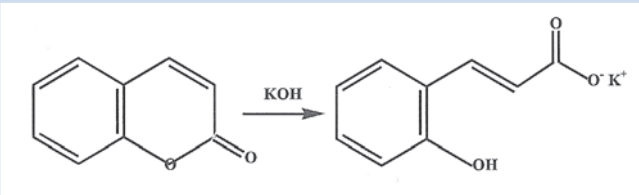
### Methods of Defurocoumarinization

In order to obtain a bergamot oil "bergapten free," different methods can be used:

1. Chemical or physical methods:
   - Chemical or "cold" process, which uses an alkaline aqueous solution
   - Physical "hot" process or rectification, which uses vacuum distillation (6)
   - Physical process, which uses supercritical carbonic dioxide (SC-$CO_2$) (7)
2. Genetic modification methods: bergamot peel oil is extracted by genetically modified fruits of bergamot that furnishes peel oil without bergapten or containing non-photoactive derivatives of bergapten (8)

The ideal technological process must realize the elimination of bergapten and still satisfy three fundamental conditions (9):

**In cold industrial process, a chemical property of the lactones is exploited in a way that, by treatment with alkaline hydrates, turns them into the sodium of the potassium salt of the corresponding oxiacids, following the opening of the lactonic ring** **F-1**




1. Olfactory properties must remain unchanged
2. Method must not eliminate all the coumarins and psoralenics com

pounds because some of them are useful to assure the preservation of the peel oil
3. Chemical and physical characteristics and the consequential indices obtained from the analysis must not be modified beyond reasonable terms due to the reduction of the bergapten concentration

## Chemical, or "Cold," Industrial Process for Defurocoumarinization of the Bergamot Oil

Chemical or cold industrial process is the most used method due to its inexpensiveness. Its operational simplicity doesn't involve sophisticated industrial equipment. In such a method, a chemical property of the lactones is exploited which, by treatment with alkaline hydrates, turns it into the sodium of the potassium salt of the corresponding oxiacids, following the opening of the lactonic ring (see F-1).

The treatment of bergamot peel oil with alkaline hydrates involves the destruction, for hydrolysis, of the lactonic ring present in the bergapten and citropten structure, while the lactonic ring of bergamottin and 5-geranyloxy-7-methoxycoumarin remains unchanged. This is accomplished through the steric protection of the geraniloxy group present in their molecule (10).

The experimental procedure can be described as follows: In a stainless steel reservoir, supplied with an agitator, a quantity of cold pressed bergamot oil, not more than 1/3 of the vessel capacity, is placed with an equal volume of aqueous solution of potassium idroxide, at a concentration ranging from 4-6 percent related to the initial bergapten content. This alkaline solution must be prepared the day before its use to allow for cooling and clarification of the solution. The speed of the agitator is then regulated to produce good contact of the two phases without provoking excessive turbulence that would determine oxidations owing to the air present in the container. The mixture is stopped after 6-7 hours, and the two phases (water/oil) are allowed to separate by decantation. The bergapten salt forms a semisolid floating phase on the oil phase, which is removed by mechanical separation, while the underlying emulsion (water/oil) is submitted to centrifugation in order to recover other oil which is added to the preceding one. In order to eliminate traces of the remained alkali from the defurocoumarinized oil, it is necessary to wash the oil with an equal amount of deionized water; an emulsion is formed that it is then submitted to centrifugation. The washing process is repeated until the washing water doesn't show alkaline reaction.

## Experimental

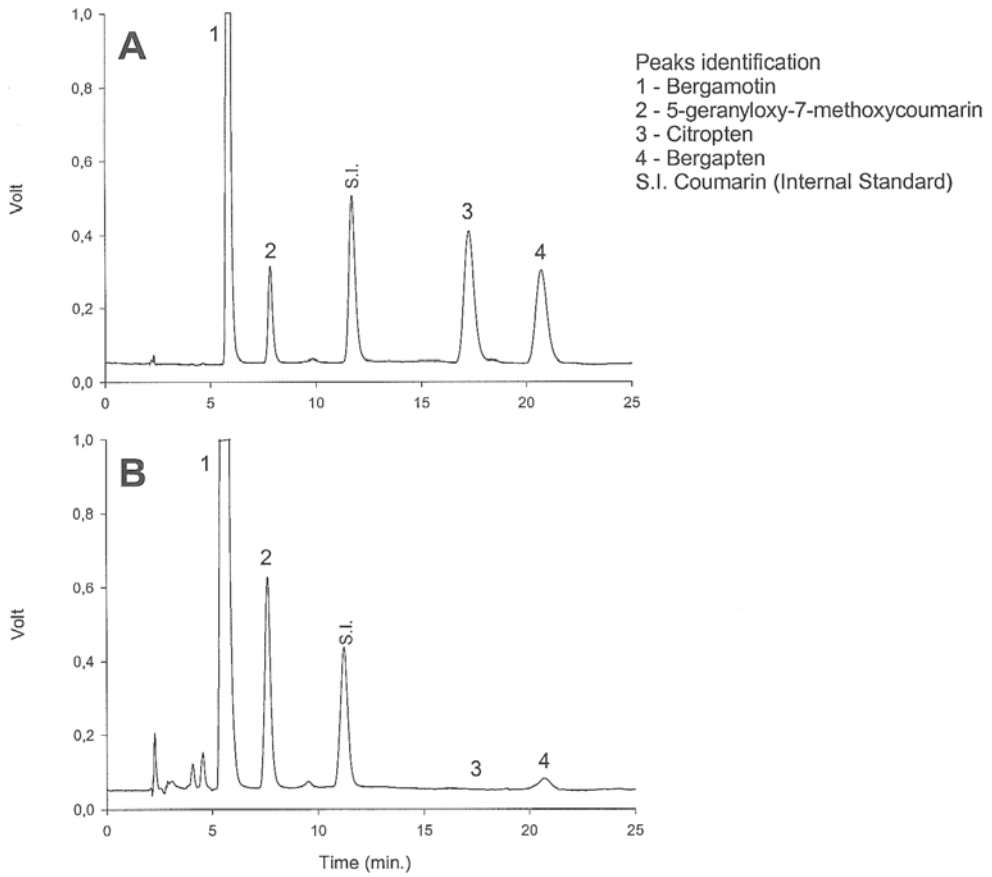
With the purpose of verifying the optimal conditions (concentration of the alkali and hours of agitation) to get a "bergapten free" oil having chemical and sensorial characteristics very similar to the natural oil, the described process for cold defurocoumarinization with alkali was reproduced in a laboratory scale. By using different concentrations of potassium hydroxyde solutions, we monitored over time the variation of bergapten content in the oil.

**Heterocyclic compounds containing oxygen in the bergamot peel oil "cold-pressed"** **T-1**

| | Calabrò G. e Currò P. (1975) | | Calvarano I. e coll. (1979) | | Mondello e coll. (1993) | | Gionfriddo e coll. (1997) | |
|---|---|---|---|---|---|---|---|---|
| | Range | Medium value | Range | Medium value | Range | Medium value | Range | Medium value |
| bergamottin % | 1.40-2.20 | 1.82 | 1.14-2.73 | 2.03 | 1.02-2.75 | 1.87 | 0.63-0.83 | 0.74 |
| bergapten % | 0.15-0.33 | 0.22 | 0.16-0.40 | 0.25 | 0.11-0.32 | 0.21 | 0.19-0.33 | 0.25 |
| citropten % | 0.14-0.24 | 0.18 | 0.16-0.30 | 0.23 | 0.14-0.35 | 0.22 | 0.12-0.32 | 0.20 |
| 5-geranyloxy-7-methoxycoumarin | 0.12-0.15 | 0.12 | - | - | 0.08-0.22 | 0.13 | 0.05-0.11 | 0.072 |

**The chromatographic profiles of the cold-pressed bergamot oil and corresponding defurocoumarinized oil***

**F-2**




*HPLC chromatogram of the furocoumarins in natural bergamot oil (A) and bergapten free oil (B)
Experimental setting:
HPLC Chromatograph Perkin Elmer LC 410; Luna Silica 2 (5µ) column; elution (isocratic) by exane + ethyl acetate (90:10) at constant flow 1.5 mL/min; sample: 20 µL diluted 1% (natural essential oil) or 20% (bergapten free) with eluent mixture; detector 315 nm.



A mass of industrial bergamot peel oil (4.5 kg), obtained by a special pelatrice, with an initial content of bergapten equal to 0.23 percent, was divided into three shares each of 1.5 kg and marked by the letters A, B and C.

Each of these shares was versed in a stainless steel vessel with the capacity of about 6 L and treated with a potassium aqueous hydroxyde solution, with a concentration of 4 percent in A, 5 percent in B and 6 percent in C. After 3, 6, 9, 12 and 18 h of agitation, using a palette of stainless steel operated by a rotating electric motor to the speed of around 120 rpm, we withdrew shares of 100 mL each of emulsion. After a complete separation of the phases by centrifugation, the oil deprived of furocoumarins (30-40 mL) was dehydrated with anhydrous sodium sulfate and analyzed by HPLC following Gionfriddo and coll. (4) for the bergapten content.

## Results and Discussion

The concentrations (mg/kg) of bergapten found after every treatment are reported in Table II. From experimental data it emerged that best results were achieved by using 6 percent potassium hydroxyde solution and 12 h of treatment. Nevertheless, in relationship to the product that is wanted, it is possible to use times and different concentrations considering that the yield and the quality of the oil decreased when increasing the time of agitation and the alkali concentration.

The oil treated for 18 h showed a marked dissolution in the odorous qualities. The same was true when the highest concentration of the alkali was used. Overall, the time of treatment had a greater affect on the olfactory quality of the oil compared to the alkali concentration.

In F-2, the chromatographic profiles (HPLC) of the cold pressed bergamot oil and of the corresponding defurocoumarinized oil are reported. It was verified (11) that the chemical cold process for the elimination of the bergapten resulted

**Bergapten concentration (mg/kg) of bergamot peel oil treated with potassium idroxide, at increased concentration and contact times** **T-2**

| Potassium idroxide concentration | Time (h) 0 | 3 | 6 | 9 | 12 | 18 |
|---|---|---|---|---|---|---|
| 4 percent | 2310 | 700 | 150 | 80 | 50 | 10 |
| 5 percent | 2310 | 650 | 112 | 43 | 22 | 10 |
| 6 percent | 2310 | 96 | 32 | 3 | t | t |

t = trace

in only little variation in the chemical and physical characteristics and a limited lowering of the specific weight, refraction index, non-volatile residue and of the spettrofotometric (CD and $E_{max}$) indices. These were all parameters that could be related to the non-volatile components of the residue. However, they didn't cause changes in the composition of the volatile fraction, and therefore didn't alter parameters such as gas chromatographic and the odorous notes.

## Conclusion

The suggested method to obtain bergamot oil deprived of bergapten is useful, since the oils obtained by the described procedure are qualitatively similar to the natural oils and can be used in perfumery and cosmetics.

The yield in defurocoumarinized oil varies between 88-90 percent with a loss of about 10 percent. The commercial quotation of the defurocoumarinized oil is about 10 percent more elevated in comparison to that of the natural oil. The annual quantities of this particular oil exported are hardly appraisable, because the defurocoumarinization process is often affected directly in the importing countries.

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