Application Note
No. 768/2021

The latest BUCHI innovations in the development process of a natural cosmetic ingredient

Manon Trinel[1], Hortense Plainfossé[1,2], Pauline Burger[2], Xavier Fernandez[1], Aurelie Demont[3], Claudia Blum[3]

[1] Université Côte d'Azur, CNRS, ICN, Parc Valrose, 06108 Nice cedex 2 (France)
[2] NissActive, Pépinière InnovaGrasse, Espace Jacques-Louis Lions, 4 traverse Dupont, 06130 Grasse (France)
[3] BUCHI Labortechnik AG, Meierseggstrasse 40, 9244 Flawil (Switzerland)

1. Introduction

The world's cosmetics market has been in constant evolution for more than ten years and France is a major player in this sector thanks to the brand image and quality it conveys [1]. Despite this marketing advantage, cosmetic companies must be innovative and adapt to the new expectations of consumers, turning towards more "natural" cosmetics [2]. The latter are increasingly concerned by the composition of the products they buy, and gradually reject synthetic ingredients, synonymous in their minds with danger to health and pollution. In order to meet the demands of these emerging trends, the global cosmetics industry is constantly looking for natural alternatives to the conventional ingredients, and to do so, favors the use of sustainable processes that are more respectful of the environment [3]. Technological advances are an undeniable asset in this race for "green optimisation". BUCHI has developed equipment allowing the automation and sustainable optimisation of several stages in the classic process of developing a natural cosmetic active ingredient (Figure 1).

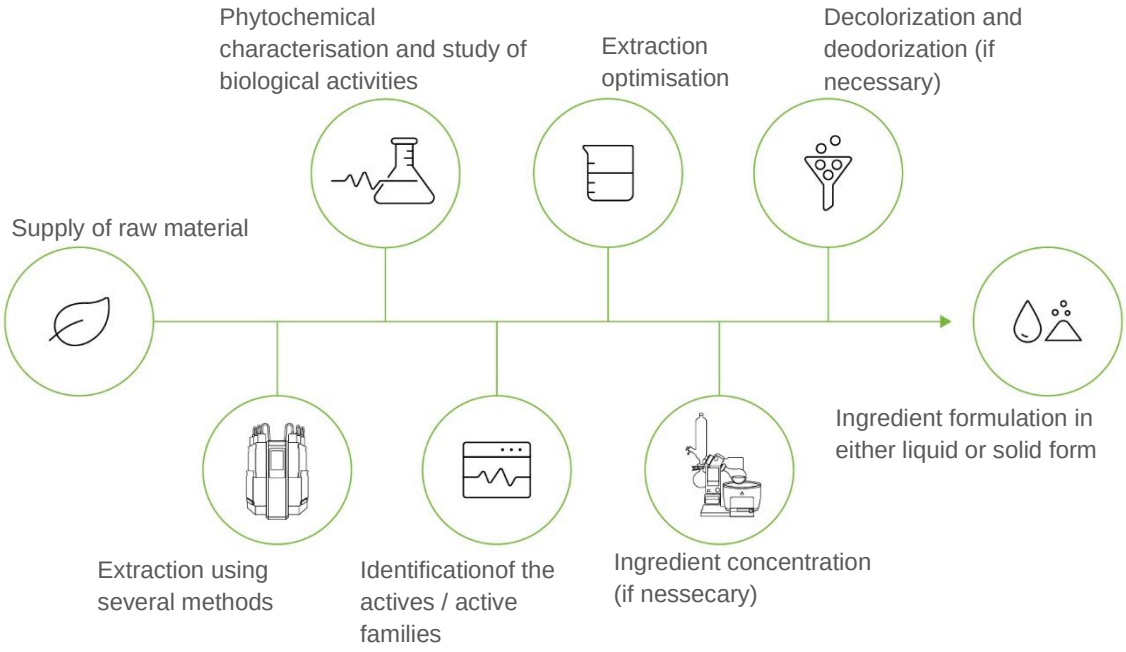

Figure 1: Development process of a cosmetic active ingredient of natural origin [4].

2. Development process of a cosmetic active ingredient

The development of a natural active ingredient is a long and delicate process that requires several stages.

2.1. Supply of raw materials

The first stage of the process consists in the search for potentially valorizable vegetable raw materials for the cosmetics industry. Traditionally, this stage is carried out using cultivated raw materials, mainly of plant origin, thus ensuring the sustainability of the supply chain and limiting the impact on biodiversity. Nevertheless, natural active ingredients can also come from other sources that produce molecules of interest such as insects like bees, snails, sponges, etc.

The pre-selection of the raw material is carried out according to several criteria: the innovative aspect (existence of cosmetic ingredients developed from this species or a related species, etc.), the legislation (protected species, NAGOYA protocol, etc.), the biological activities of the corresponding extract or the possibility of setting up a sustainable and profitable supply chain, are all parameters taken into account upstream of the R&D stages strictly speaking. A screening of numerous raw materials is necessary in order to select those which could be at the origin of the development of innovative cosmetic actives.

2.2. Extraction of the pre-selected raw-materials

The screening of a large number of pre-selected plants necessarily entails numerous extractions to be carried out. In addition, generally, several types of extractions with solvents of different polarities, thus influencing the nature and quantity of the extracted metabolites, are carried out from the same raw material in an attempt to extract the different families of metabolites of interest. The extraction technique most commonly used until now is cold or hot maceration. However, these techniques are very time-consuming, solvent-consuming and often have low yields for industrial transposition. Nor do they allow several extractions to be carried out at the same time with the same equipment. Soxhlet extractors are also widely used and have better yields since this technique allows the material to be exhausted in a few cycles, which would correspond to several successive macerations. Thus, the use of this system is more economical and environmentally friendly as it requires less solvent for a better yield.

However, once again, a Soxhlet extractor only allows one extraction at a time. It is therefore often necessary to use Soxhlet "ramps", where several Soxhlet are plugged in series. In order to optimize this step, Buchi Universal Extractor E-800 can be used (Figure 2). This technology based on the Soxhlet principle makes it possible to accelerate the extraction step by performing 6 parallel extractions under perfectly controlled and therefore repeatable temperature conditions. The various features of this system are the possibility of using solvents with a wide range of polarity and the ease of adapting the volume of the extraction chamber according to the volume of extract to be worked by adjusting the height of the level sensor. As the system is made of glass, it is possible to follow the extraction visually (Figure 3) and this facilitates the post-extraction cleaning stage. The precision of the control of the heating power, as well as the speed of the system to reach this set point, allows a great rigor in temperature control.

The efficiency of this system was evaluated by an experiment carried out in our laboratory by comparing the extraction of compounds contained in a co-product of the food industry, corresponding to blackcurrant skins (Ribes nigrum L.). We first carried out a hydroalcoholic extraction under the conditions presented in Table 1 and then an extraction of the same raw material using the Universal Extractor E-800 system using the parameters described in Table 2. As a result of this experiment, the average yield (yield of crude extract, i.e mass of dry extract/mass of plant dry matter x 100) with the Universal Extractor E-800 system was 21.0% compared to 15.3% with maceration. Thus, at equivalent temperature and solvent volume, we obtain an increase in yield of about 6% with the Universal Extractor E-800 system compared to conventional hot maceration. This equipment is therefore ideal for multiple extractions for screening natural raw materials. It allows several extractions to be carried out in parallel, with precise control of the extraction parameters.

Figure 2: BUCHI Universal Extractor E-800

Figure 3: Parallel extraction of 3 different raw materials

Table 1: Parameters used during the hot maceration of blackcurrant skins

Parameters	Hot maceration
Ratio raw material/ Solvent	1/10 (20g/200g)
Solvent	EtOH
Time (h)	2H
Temperature (°C) (Azéotrope binaire)	80-85 °C
Pressure (KPa)	atmospheric pressure

Table 2: Parameters used with the Universal Exctractor E-800

Parameters	Universal Extractor E-800
Ratio raw material/ Solvent	1/10 (20g/200g)
Method	soxhlet extraction
Solvent	EtOH
Number of extraction cycles	5
Heater level - extraction	10
Valve opening time	long
Rincing (min)	10
Heater level - rincing	10
Drying (min)	-
Heater level - drying	-

When the number of raw materials to be evaluated becomes large (several dozen), another interesting extraction system for screening is the pressurized solvent extraction system (PSE). This solid-liquid extraction technique is carried out at high temperatures and pressures, thus considerably reducing the extraction time. It is also automated and consumes much less solvent than traditional maceration methods or the Soxhlet system. Buchi offers this type of system with the Speed Extractor E-914 and Speed Extractor E-916.

Other extraction techniques exist such as supercritical fluid extractions (supercritical CO_2 in particular.) but they are not yet very widespread and above all are not suitable for screening a large number of samples. Nevertheless, it is obvious that the single or multiple PSE and Soxhlet methods can only be used in the context of the R&D of a cosmetic active ingredient, and greatly facilitate them as has been shown, but are not applicable on an industrial scale. At present, maceration remains the most widespread extraction technique, however, from an environment-friendliness point of view, greener solvent choices should be preferred, solvent reduction in water/solvent ratios or times and temperatures optimised and lowered as much as possible.

Once the extractions have been carried out, the removal of the solvent or solvent mixture is a necessary and often neglected step. The most commonly used technique is vacuum distillation of the solvent using a rotary evaporator, although a drying option is available on the Extractor E-800. However, this technique presents application difficulties, especially for hydroalcoholic mixtures. Indeed, with this type of mixture the boiling temperature is high, which results either in the need for excessive heating of the water bath, which could damage certain heat-sensitive compounds, as well as the risk of reflux if the boiling temperature is exceeded, or in excessively long drying times.

In order to optimise distillation speeds, it is recommended to increase the temperature difference between the bath and the condenser or between the vapours and the condenser. As temperatures cannot usually be increased, the speed can be modulated by lowering the condenser pressure and temperature while keeping the bath temperature constant. In order to avoid overloading the condenser, this optimisation can only be achieved with a condenser that has a larger surface area than the standard V-condenser. This is necessary to ensure total condensation of the solvent and to protect the vacuum pump.

The larger surface area available on the high-performance condenser allows more steam to be condensed at the same time and therefore a faster distillation speed can be achieved with optimal distillation parameters. The following results (Table 4) were obtained when drying a hydroalcoholic extract of myrrh on a Rotavapor R-300 equipped with a B-305 bath, F-314 chiller, I-300 pro interface and V-300 vacuum pump and anti-foam probe.

Table 3 : Evaporation parameters of a myrrh extract

	Standard condenser	High performance condenser
Surface [cm2]	1500	3000
Bath temperature [°C]	45	
Chiller temperature [°C]	5	
Pressure [mbar]	10	
Rotation [rpm]	250	
Total volume [mL]	50	
Evaporation time [min]	47.58	32.28
Evaporation speed [mL/h]	63	93

A significant time saving is observed for the evaporation of a volume of 50mL of myrrh extract when the high performance condenser is used with optimal temperature conditions for the myrrh. As the temperature parameters cannot be altered to dry this extract more quickly, this change of glassware offers an interesting solution.
The options, such as the high-performance condenser, the large bath capacity (5L) or the anti-foam probe present on the BUCHI Rotavapor® R-300 (Figure 4) make the researcher's work easier and allow to get around the difficulties encountered when evaporating hydroalcoholic mixtures. The digital interface for temperature and speed control is very easy to use. The probe equipped with the foam sensor to avoid "flush" effects is also undeniably practical (Figure 5). It prevents material loss, eliminates cleaning and contamination problems, and allows unattended operation of the evaporator. The ability to program an evaporation for a given solvent or solvent mixture and to repeat this program is particularly appreciated, especially during multiple extractions. The system is self-contained and automated and offers ease of use to the operator, who can perform other tasks in parallel with this extract concentration step.

Figure 4: Rotavapor® R-300

Figure 5: Antifoam sensor on a Rotavapor® R-300

2.3. Phytochemical characterisation and study of biological activities

Once the extract has been concentrated using the rotary evaporator, a step to characterise its phytochemical composition is often carried out. This enables the main families of metabolites characterising the extract to be identified. This characterisation is made possible using analytical techniques such as High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) or Thin Layer Chromatography (TLC, HPTLC) coupled with different detectors such as UV detectors, light scattering evaporative detectors, mass detectors, etc. The phytochemical profiles of the different extracts obtained from the same raw material according to the extraction conditions are compared in order to determine which protocol allows the extraction of the most compounds of interest (when they are known).
On the other hand, the biological activities of these extracts are evaluated by tests, most often in vitro, selected according to the type of activities sought. The most common tests in cosmetology are enzymatic or chemical reduction tests that measure the antioxidant (Ferric reducing-antioxidant power test, ORAC [Oxygen Radical Absorbance Capacity] antioxidant index, etc.), whitening (L-DOPA), anti-inflammatory, anti-hyaluronidase or anti-collagenase activities of the extracts. There are many tests, some of them very specific, which can also be carried out on so-called ex vivo or in vivo models, but their cost and difficulty of carrying them out excludes them in the case of the study of many raw materials.
Linking the biological activities of the extracts with their phytochemical composition makes it possible to select plant materials of interest for the development of cosmetic ingredients.

2.4. Identification of actives molecules/family of actives molecules

Once the species of interest have been selected, the next step is the characterisation of the molecules/families of active molecules. To do this, fractionation steps of the extract with an often complex phytochemical composition are necessary.
For a long time, the purifications of extracts have been carried out using low-resolution, time-consuming and solvent-consuming techniques such as liquid-liquid partitioning, acid-base washes or chromatography on solid supports such as Medium Pressure Liquid Chromatography (MPLC) or Solid Phase Extraction (SPE). Currently, research is being carried out to optimise purification methods that minimise the use of organic solvents, which are automated, faster and more decisive, in short, more efficient. Among the new methods developed are flash and preparative chromatography, which have become widespread. They allow, through a wide choice of solvents, cartridges or columns, the separation of all types of compounds. Indeed, when choosing the column or cartridge, the diameter and height of the column playing on the quantity of stationary phase and therefore on the quantity of extract mass that it will be possible to inject into the system is the first influential parameter. The nature of the stationary phase (silica, grafted silica, alumina, etc.) must also be adapted to the polarity of the plant extract. Indeed, purifying an extract composed mainly of lipophilic molecules will be easier with a classic silica, whereas a rather polar extract will adapt better to a C18 grafted silica column for example. Then, the granulometry of the silica is an equally important parameter and depends on the fineness of separation that one wishes to obtain or on the complexity of the extract to be separated.
In addition, as the system is under pressure, it is possible to work with a small grain size, thus further improving the separation resolution. Finally, the choice of the eluent(s) and the flow rate

makes it possible to envisage as many separation processes as there are combinations, i.e. an almost infinite possibility. As the number of separation tests in a complex matrix can be numerous, the automation of the systems is an ideal technological advance.
To meet these expectations, BUCHI has developed a fully functional system with a dual-use capability (flash or prep), the C-850 flash-prep system (Figure 6), which stands out for the ease of use of the software interface. The integration of the UV and LED detectors within the system is also a considerable advantage for controlling the separation of compounds. The very compact geometry of the C-850 flash-prep system and the fact that the fraction collector is a closed system saves space in the laboratory and can be used without specific benchtop adaptations. The ease of maintenance of the various parts of the system is also a major advantage of this unit.

Figure 6: PURE Flash-prep C-850

The fractions thus separated can be characterised from a phytochemical point of view and their biological activities can be evaluated. It is then possible to identify the compounds or families of compounds responsible for the activity.

2.5. Extraction optimisation

The identification of families of active molecules is essential to optimise the plant's extraction conditions in order to maximise the concentration of these molecules in the objective extract.
The optimal extraction process must allow the extraction of the compounds of interest while being economically viable and having little impact from an environmental point of view. Various extraction parameters such as the plant matter/solvent mass ratio, the nature of the solvent (green solvents are currently increasingly preferred), the maceration/extraction time if this method is chosen or the extraction temperature are tested. As the Universal Extractor E-800 system offers the possibility of simultaneously testing different extraction methods including different solvents, it is once again a support of choice for the development of a cosmetic ingredient by facilitating this stage of extraction optimisation. Subsequently, the phytochemical profiles and yields of the different extracts obtained by concentration with the Rotavapor® R-300 allow the selection of optimal conditions for this extraction.

2.6. Discolouration and/or deodorisation of the extract

Depending on the raw material, the solvent and/or the extraction conditions used, the extract obtained may have a colour and/or odor that may be disturbing when formulated into the finished product. Indeed, the incorporation of such an ingredient may induce a more or less intense colouring or a more or less marked odour of the finished cosmetic product, which will depend in particular on the percentage of ingredient formulated. A stage of decolourisation and/or deodorisation of the extract may then prove necessary in order to meet the expectations of formulators, and ultimately consumers, who generally prefer a cosmetic with little or no colouring, and not too marked or unpleasant odour [3]. Different techniques such as molecular distillation or adsorption on activated carbon are used for these stages of decolourisation (Figure 3) and deodorisation [5]. Activated carbon adsorption is a simple method which consists of incorporating

a given quantity of activated carbon in relation to the mass of extract to be treated in solution in a suitable solvent, leaving the extract and the carbon in contact for a given time and then filtering to remove the carbon. This technique is easy to implement, does not require heating and is therefore more environmentally friendly. However, being non-specific, coal can adsorb active molecules and cause the extract to lose activity. It is therefore a question of optimising the decolourisation/deodorisation conditions in order to find the best compromise between loss of colour/odour and preservation of the claimed activities of the active ingredient. Numerous parameters can influence such adsorption: quantity of activated carbon in relation to the mass of extract, the contact time between activated carbon and extract, the temperature at which adsorption is carried out, etc. In order to illustrate the different yields that can be obtained by varying these parameters, an experiment was carried out in the laboratory on an ethanolic extract of Prunus domestica L. The results of this experiment are presented in Table 4. Although it requires specific equipment and energy consumption, compared to absorption on activated carbon, molecular distillation is a commonly used alternative for the decolourisation/deodorisation of extracts in cosmetics. This technique consists of a short-path distillation under high vacuum and at a high temperature. However, the contact time is very short in order to limit the denaturation of heat-sensitive molecules. The advantage of this technique is that there is therefore no risk that active molecules in the extract will be eliminated during the decolourisation

Table 4: Parameters applied during the various discolouration tests on Prunus domestica L. by classical absorption

	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Activated carbon content	1%	3%	5%	10%	10%	10%
Contact time	15 min	15 min	15 min	4 h	15 min	15 min
Temperature	TA	TA	TA	TA	TA	70 °C
Yield	87%	85%	79%	92%	84%	87%

If the developed active ingredient has no particular colour or odour, it is valued as it is, thus avoiding any loss of business.

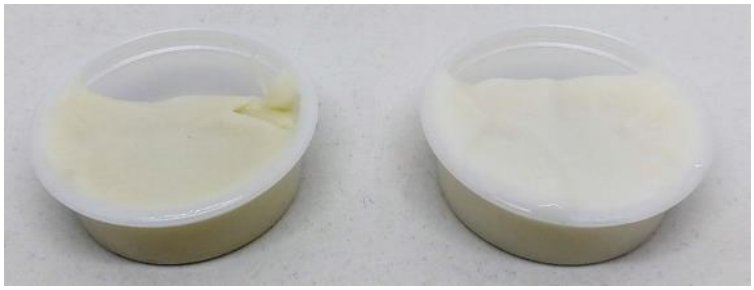

Figure 7. Result of an optimized activated carbon decolourisation on an extract of Quercus pubescens without decolourisation (left) and decolourised (right) formulated at 3%

2.7. Ingredient formulation

Vegetable extracts are often obtained in the form of pastes that are impossible to formulate, so it is necessary to process them. At the request of formulators and to facilitate the incorporation of ingredients into cosmetic formulas, cosmetic ingredients are available in liquid and solid forms. Some ingredients are only available on the market in one form or another, but this is most often dependent on the demand of the formulators and not on the raw material or the choice of the producer. On the contrary, it is even appreciated by a producer that both forms are offered.
The liquid ingredient is obtained by direct maceration of the plant material of interest in a solvent compatible with cosmetic formulations such as glycols or vegetable oils or by dilution of the active extract in an appropriate liquid carrier. After objectification of the extraction conditions, in order to extract the majority of the compounds of interest and possibly decolorization and/or deodorisation and dilution steps, the ingredients in liquid form do not require any additional processing step.
Conversely, to offer an active ingredient in solid form, a stage of putting the extract on a support is necessary, by drying or atomisation for example. Spraying enables the extract to be deposited on a carrier material such as maltodextrin in order to obtain a homogeneous, stable powder that can be easily incorporated into cosmetic formulas. We have mentioned maltodextrin as an

example of a carrier but other polysaccharides (starch, sucrose, gum arabic, etc.), proteins (gelatin, casein, etc.) or synthetic polymers (acrylic polymer, etc.) can also be used.
This step and the optimisation of these conditions can be easily carried out using the Mini Spray Dryer B-290 (Figure 7). Its ease of use and the numerous application notes offered by BUCHI for this type of equipment and scientific publications allow a quick introduction to the system. The optimisation of the atomisation conditions enables yields of around 70% to be achieved with the B-290 Mini Spray dryer, which is very encouraging for industrial transposition. Moreover, the work of optimising the conditions is facilitated by the efficiency of the drying system (of the order of 1 L/h). Also, as the unit's module is made of glass, it is possible to visually control the spry process and facilitates the cleaning of the system.

Figure 8: BUCHI Mini Spray Dryer B-290

3. Conclusion

The traditional methodology for the development of a cosmetic active ingredient of natural origin is constantly being questioned in order to optimise it and to ensure that it fits in with the current sustainable development policy. The stages of development must be adapted to the raw materials used, while favouring "green" eco-extraction and objectification techniques for the development of ingredients with proven activities. These stages of selecting the raw materials of interest and optimising the extraction conditions prove to be time-consuming, and the technology and automated techniques offer significant time and efficiency savings. In this search for optimisation, BUCHI has proved to be a player of choice since it offers machines that allow better repeatability and greater precision, while making the user's task easier and reducing the environmental impact of the processes (energy savings, reduction in solvent volumes, etc.).

4. References

[1] Business France, Fiche secteur Cosmétiques 2017, 2017.
[2] A. Beyer, Benchmarking Co. 2009.
[3] Agrobiobase 2011.
[4] H. Plainfossé, P. Burger, S. Azoulay, A. Landreau, G. Verger-Dubois, X. Fernandez, Development of a Natural Anti-Age Ingredient Based on Quercus pubescens Willd. Leaves Extract A Case Study. Cosmetics. 2018, 5,15.
[5] A. Kerdudo, P. Burger, F. Merck, A. Dingas, Y. Rolland, T. Michel, X. Fernandez, Comptes Rendus Chim. 2016, 19, 1077.
[6] H. Plainfossé, P. Burger, G. Verger-Dubois, X. Fernandez, Eco-conception of an anti-age ingredient based on Quercus pubescens Willd. Leaves extract. H&PC Today. 2018, 13(4).