2ND PERIODIC ACTIVITY REPORT

FINAL REPORT

FP & Contract No. COOP-CT-2005-513216

AMAY

"Aloe vera- Maximising yield of effective compounds and development of natural cosmetic products with standardised content of these compounds"

Instrument: Cooperative Research (CRAFT)

D 5.8 Final Report (month 24)

Reporting period: 15 April 05 – 14 April 07

AMAY start date: 15th April 05 **Duration: 24 months**

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PUBLISHABLE EXECUTIVE SUMMARY

Natural substances and plant extracts are increasingly important components in the cosmetic and pharmaceutical industry as well as in the wellness sector thanks to its inherent characteristics and their high acceptance among the consumers. Therefore, the market share of natural products has been growing very fast, and for instance plant based drugs represent today a market share of approx. 30 % of pharmaceuticals.

Aloe Vera is benefiting from this demand on natural ingredients thanks to its unique healing and nutritional characteristics: The Aloe Vera leaf contains over 75 nutrients and 200 active compounds, including 20 minerals, 18 amino acids, and 12 vitamins and works effectively against sunburn and UV irradiation. However, up to now no objective method for quality demonstration and assessment of Aloe Vera has been established, which affects the competitiveness of European producers since they can only compete against imported raw material through offering a higher quality product.

The AMAY project aims at obtaining:

1. Exchange of best practises for plantations (maximising yield) regarding soil quality, cultivation methods, plant cultivar
2. Isolation and Quantification of Aloverose by NMR (irregularly acetylated polymer of Mannose)
3. Development and testing of new products with standardised content of effective compound

Strategic and industrial objectives of the project are:
The AMAY project has two main strategic objectives. On the one hand to investigate the dependence of Aloe Vera cultivation parameters and the content on active compounds leading to the growth of plants allowing for a maximum yield of these active components. On the other hand, AMAY aims to develop an analysis method, which allows the determination of active components in Aloe Vera and to derive therefore natural cosmetic products with a standardised and certifiable Aloverose content. In a more detailed way the objectives can also be listed as follows:

Primary industrial objectives

- Exchange of best practises for plantations (maximising yield) regarding soil quality, cultivation methods and plant cultivar.
- Development and integration of a feasible procedure to isolate and quantify the Aloverose by NMR (irregularly acetylated polymer of Mannose)
- Establishing a new standard on Aloe Vera detection
- Development of new products based on Aloe Vera with standardised content of effective compounds

Primary social objectives:

- High quality natural cosmetics containing Aloe Vera which meet expectations of EU consumers, in terms of wound healing, anti-inflammation
- Greater consumer confidence through avoiding adulterationof the Aloe Vera products and compounds, respectively.
- Employment creation by maintaining competitiveness of European natural cosmetic sector

The R&D programme for the AMAY project is structured as follows:

1. Definition of requirements: sampling, collection of end-users requirements
2. Comparison of the Aloe vera cultivation: analysis of soil, comparison of cultivation methods and climate and assessment of results
3. Purification and standardisation of active compounds, chemical characterisation and development of an alternative-test development
4. Product development of cosmetics and dermatological evaluation
5. Project management, pre-exploitation and dissemination measures

For more information about the project, visit our website: www.insidealoe.de

To achieve the AMAY project objectives, a strong and complementary project consortium was selected including industrial SME end-users.

SME-PARTNERS OF THE PROJECT:

PLANTSCIENCE- Plant Science Services GmbH (Germany), ALFAVERDE- Alfaverde Productos Naturales S.L. (Spain), POULIMENOS- Poulimenos Cacti (Greece), FARO- Piante Faro (Italy), TELA- TELA GmbH (Germany), GRADIENS- Gradiens Termékfejlesztó Kft. (Hungary), TOPORIINA- Toporiina Oy, QUIVERA- Quivera Cosmeticos S.L. (Spain)

RTD PARTNERS OF THE PROJECT:

TTZ - TTZ-Bremerhaven (Germany), SPECTRAL- Spectral Service GmbH (Germany), STATNI ZDRAVOTNI USTAV (Czech Republic)

WITH THE SUPPORT OF THE EUROPEAN UNION

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Section 1 – Project Objectives and Major Achievements during the Reporting Period

The **Strategic and industrial objectives of the project are**:

AMAY project has two main strategic objectives. On the one hand to investigate the dependence of Aloe Vera cultivation parameters and the content on active compounds leading to the growth of plants allowing for a maximum yield of these active components. On the other hand, AMAY aims to develop an analysis method, which allows the determination of active components in Aloe Vera and to derive therefore natural cosmetic products with a standardised and certifiable *Aloverose* content.

Primary industrial objectives

- Exchange of best practises for plantations (maximising yield) regarding soil quality, cultivation methods and plant cultivar.
- Development and integration of a feasible procedure to isolate and quantify the Aloverose by NMR (irregularly acetylated polymer of Mannose)
- Establishing a new standard on Aloe Vera detection
- Development of new products based on Aloe Vera with standardised content of effective compounds

Primary social objectives:

- High quality natural cosmetics containing Aloe Vera which meet expectations of EU consumers, in terms of wound healing, anti-inflammation
- Greater consumer confidence through avoiding adulteration of the Aloe Vera products
- Employment creation by maintaining competitiveness of European natural cosmetic sector

For achieving the project objectives, the AMAY work is organised in seven main actions, which are to be performed in the different R&D Work Packages all through the project long. These are named as follows:

- WP 1: *Definition of requirements* for the Aloe vera cosmetic production and for the technical and definition of a protocol for the technical and experimental specifications for Aloe vera sampling.
- WP 2: *Comparison of the Aloe vera cultivation*, by means of analysing macronutrients in the soil of the plantations and comparing cultivation methods and climate conditions.
- WP 3: *Purification and standardisation of active compounds* for a further structural analysis with NMR. Furthermore, the development of an alternative test in order to

compare the results obtained with the NMR, with a cheaper but reliable technology..

- WP 4: *Product development* of cosmetics containing standardised amount of active compounds. Product testing will comprise safety and efficacy tests.
- WP 5: *Project management, dissemination and pre-exploitation measures*.

Objectives for the reporting period

In the covered reporting period of the AMAY project, which comprises from the beginning of the project (15th of April 2005) until the end of the project (14th April 2007), the objectives were to comply all objectives set for the whole project duration:

First, to define and compile all necessary requirements for the good execution of the project, laying down the specifications. The corresponding SMEs (cosmetic companies as well as growers) together with the research partners set their requirements, which should guarantee that the final achievements correspond to the needs of the end-users. This also stabilised the base to co-ordinate the technical development.

First technical steps for the soil analysis, comparison of cultivation methods, purification of the active compounds, were developed during the first 6 months, being optimised during the second 6-months period.

By the date of the mid-term meeting, the assessment of results of the soil analysis and the comparison of the cultivation methods had been done and could be presented and discussed with the Aloe vera farmers.

The purification of the active compounds continued and was optimised the second year project. NMR was applied and validated for the quantification of Aloverose. HPLC method development and optimisation continued during the second year. The development of both methods was concluded by the end of the project and presented at the final meeting.

Before month 18, samples of the Aloe vera clones distributed at the beginning of the project for their plantation in the 4 farm locations were collected and analysed by means of NMR for Aloverose quantification. The results of this action extend the conclusions obtained from the tasks performed in WP2.

The development of the cosmetic products containing specific amounts of the active compounds and the dermatological and consumer testing of the products took place mainly in the 2nd project year and lasted until the end of the project.

The launching of the project webpage and the design of the project flyer were carried out during the first period of the project. Extensive dissemination has been performed at various fairs, conferences, exhibitions and among partners' contacts and through the project web site, own partners website and the distribution of flyers (See Section 4: Dissemination and Use).

According to the *Description of Work*, the specific objectives and achievements/deliverables for the mentioned period, which are extensively described in this report, are summarised as follows:

Table 1: List of work packages, as stated in the *Description of Work*

WP No	Work package title	Partner Short Name	Person-months	Start month	End month	Deliverable No
WP 1	Definition of requirements	SANTAVERDE	15,25	0	2	**D 1.1** **D 1.2**
WP 2	Comparison of the Aloe Vera cultivation	TTZ	23,5	2	14	**D 2.1** **D 2.2**
WP 3	Purification and standardisation of active compound	SPECTRAL	19,5	5	17	**D 3.1** **D 3.2** **D 3.3** **D 3.4**
WP 4	Product development and evaluation	GRADIENS	25	16	24	**D 4.1** **D 4.2** **D 4.3**
WP 5	Project management, dissemination and pre-exploitation measures	SANTAVERDE	13,5	0	24	**D 5.1** **D 5.2** **D 5.3** **D 5.4**
	TOTAL		95,75			

All deliverables in the previous table are due to the end of the project. The AMAY partners have achieved the planned tasks in a timely manner, although some minor rearrangements of the time structure and work organisation were necessary to perform the work (detailed in the next paragraphs and further on in this report).

Detailed progress of the work performed by the partners in the corresponding WPs as well as its resulting deliverables and milestones is described later in section 2.

Problems and corrective actions during the reporting period

At the beginning of the project, the consortium performed some rearrangements to the initial Work Plan in order to optimise the effectiveness of the work. The main modification, which is mainly related to the WPs timetable, was, performed during the first 6 month of the project

and already contemplated in previous reports (first Progress report, mid-term report and 18 months progress report. Such modification supposed the change of the scheduled timetable for WP2 and WP3. The partners agreed during the kick-off meeting that the related tasks could be brought forward since the test materials (Aloe vera leaves) could be available for testing soon and having the result of their analyse in an early stage would facilitate the performance of other tasks in both WP (see kick-off meeting minutes, Annex I)

Additionally, WP4, scheduled from month 17, started in month 6. The partners involved in the development and the testing of the cosmetic products foreseen in AMAY agreed on starting the task as soon as the material produced in WP3, ingredient for the cosmetic product, was available (see mid-term meeting minutes in *Annex XVIII*), and taking into account the existing technical risk, so they could take corrective actions on time if needed. The dermatological test of the products started subsequently right after the product production, also a bit sooner than foreseen in the workplan.

Section 2 – Work Package Progress

The following table shows an overview of the work plan progress until the end of the project.

WP	Work Package Title	WP Leader	Total Length (Months)	Elapsed Time (Months)	Left Time (Months)
1	1. Definition of Requirements	*SANTAVERDE*	2	2	Finished
2	2. Comparison of Aloe vera cultivation	*TTZ*	12	12	Finished
3	3. Purification and standardisation of active compounds	*SPECTRAL*	13	13	Finished
4	4.Product development and evaluation	*GRADIENS*	8	8	Finished
5	5. Project management, pre-exploitation and dissemination	*SANTAVERDE*	24	24	Finished

Table 2: Work package progress

A detailed description of each Work Package and its tasks can be found in the following pages.

2.1 Work Package 1: "*Definition of requirements*"

WP leader: **SANTAVERDE**

This WP aimed to define a protocol for the technical and experimental specifications for Aloe Vera sampling (dates and quantities of plants and soils), and develop a set of requirements for the Aloe Vera cosmetic production, laying down the specifications to guarantee that the final developments correspond to the needs of the end-users.

Tasks	Partners Involved
1.1 *Sampling schedule and protocol* (months 1-2)	SANTAVERDE, TTZ, PLANTSCIENCE
1.2 *Collection of end-users requirements* (months 1-2)	SANTAVERDE, TTZ

Task 1.1 Sampling schedule and protocol

TTZ together with SANTAVERDE and with support of PLANTSCIENCE developed a sampling protocol "Deliverable 1.1" (*Annex IV*) and distributed it to the Aloe vera growers (ALFAVERDE, QUIVERA, POULIMENOS, FARO) during the kick-off meeting. The content of the protocol was discussed and agreed by all partners.

Task 1.2 End-user requirements

TTZ together with SANTAVERDE and with support of PLANTSCIENCE prepared 2 questionnaires which were distributed to the cultivators (ALFAVERDE, QUIVERA, POULIMENOS, FARO) and to the cosmetic companies (SANTAVERDE, GRADIENS, TOPORIINA) during the kick-off meeting. The content of the questionnaires was discussed and approved. The questionnaire addressed to the growers aimed to get a detailed description of their Aloe vera plantation in order to study the influence of the biological and environmental conditions on the yield of effective compounds, while the one for the cosmetic companies summarised their expectations regarding sensorial properties, physical-chemical characteristic, purity, biological activity, cost of the Aloe vera ingredient and the requirements of the final products. Their own expectations as well as their costumers' expectations were taken into account to set the end-users requirements.

The questionnaires can be found in *Annex II* and *Annex III*.

The content of the filled questionnaires was summarised by SANTAVERDE and constitutes the Deliverable 1.2 "*End-user requirements*" (*Annex V*)

Additionally, in order to update the assessment of the state of the art which was carried out during the project proposal stage (see DoW) and be aware of any new development of importance for envisaged tasks, partners SANTAVERDE, TTZ, TELA, SPECTRAL and PLANTSCIENCE carried out and extensive literature search. TTZ compiled the input of the other partners and summarized it in a survey that can be found in Annex VI This survey has been actualised during the project with new references of the interests of the AMAY project and its objectives.

Achievements

	Due Date	Actual Submission date	Lead Contractor
D 1.1 Sampling protocol	May 2005	May 2005	SANTAVERDE
D 1.2 End-user requirements	May 2005	May 2005	SANTAVERDE

Deviations/Remarks

No deviations occurred

2.2 Work Package 2: "Comparison of Aloe vera cultivation"

WP leader: **TTZ**

The objective of WP2 was to determine whether the content of effective compounds in the plants varies because of the outer conditions by means of analysing the macronutrients in the soil of the Aloe Vera plantations and comparing the cultivation methods and the climatic conditions.

Aloe Vera is a subtropical plant coming originally from Middle and North Africa although currently main production areas are Southeast Asia and the USA. Compared to this production the amount of Aloe Vera grown so far in Europe can be neglected specially because only some regions in Spain, Italy or Greece are climatic suitable. The plant needs a humid-arid climate and temperatures that rarely fall under 10ºC besides particular rainfalls. It has also been proved that at least 60% sand clayey soil with little organics is required. Therefore, climate and soil, growth rate, watering methods, harvesting periods or cultivation methods can play a very important role in the active compounds production from the Aloe Vera, which has been studied during this project.

Cultivation methods have either been conducted in the laboratory or under climatic conditions, which differ greatly from those in Europe. Therefore their validity for the AMAY project is limited. Yin-Tung Wand and Kimberly J. Strong from the texas A&M University carried out a two-year study monitoring several physical and chemical properties of field-grown Aloe Vera leaves and concluded that the some parameters found in weekly testing of fresh Aloe Vera leaves did show seasonal fluctuations.

As told before, Aloe Vera grows in arid climates but it may be cultivated outdoor in warm climates and needs only a moderate amount of water. The plant takes approximately four years to reach maturity and has a lifespan of about 12 years. They can be harvested every 6-8 weeks by removing 3 to 4 leaves per plant.

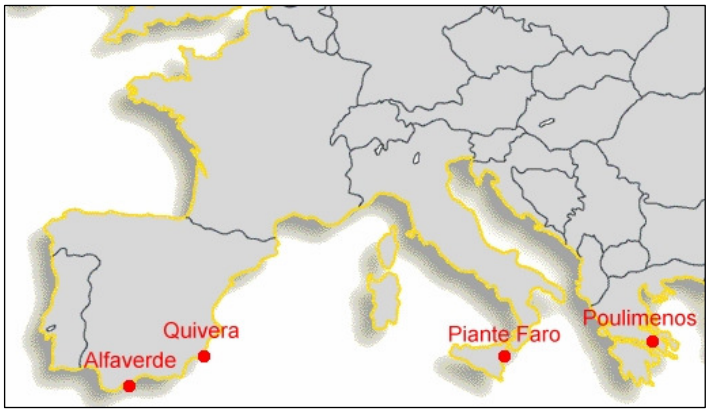


Figure 1 illustrates the location of the 4 plantations

Work Package 2 is conformed as follows:

Tasks	**Partners Involved**
2.1 *Soil Analysis* (months 3-6)	TTZ (leader) ALFAVERDE,QUIVERA,FARO,POULIMENOS
2.2 *Cultivation method, climate and plant clone (*months 3-14)	TTZ,PLANTSCIENCE ALFAVERDE,QUIVERA,FARO,POULIMENOS
2.3 *Assessment of results* (months 13-14)	TTZ, PLANTSCIENCE ALFAVERDE,QUIVERA,FARO,POULIMENOS

Task 2.1 Soil analysis

In accordance with the Description of Work, the Aloe vera growers (ALFAVERDE, QUIVERA, FARO, POULIMENOS) provided TTZ with the soil samples taken at their plantations as described in the sampling protocol (D 1.1, *Annex IV*). They also provided the figures obtained after applying the quick-test distributed and explained during the kick-off meeting to determine the content of soluble N and P on-site.

TTZ initiated then analysing the soil samples provided by the growers. 3 samples from each plantation were analysed in terms of acidity, organic matter content, maximal water holding capacity, portions of clay, silt and sand, and the content of macronutrients N, Ca, Mg, K and Ammonium was also determined.

The laboratory results of the soil analysis constitute the Deliverable 2.1 (*Annex VIII*). An extensive description of the material and methods employed for the soil characterisation can be found in the same document. Some relevant results are shown in the following pages.

Task 2.2 Cultivation methods, climate and plant clone

TTZ compiled the data provided by the growers in the questionnaires developed in WP1 (*Annex III*) about their plantations characteristics regarding varieties, fertilisation, watering, harvesting and clime data from the last 3-5 years, pictures of the plants and plantation, etc. It was also taken into account if the plants were grown in the ground or in pots as well as if they were place in shade or sun.

The list of the different cultivation methods of the 4 Aloe vera growers (Deliverable 2.2) can be found in *Annex IX.*

Additionally, PLANTSCIENCE provided the growers (ALFAVERDE, QUIVERA, FARO, POULIMENOS) with five Aloe vera plants (Sweet Aloe). The plants belong to the cultivar "sweet", which is a cultivar of *Aloe vera Barbadensis Miller* containing a very low amount of bitter substances (Aloin). Aloin is an anthrol derivative contained in the yellow bitter fluid

(resin) that seeps out from in between the outer skin of the leaves and the inner transparent water-like gel. The plants were planted under different conditions, e.g. outside and inside a greenhouse, etc.

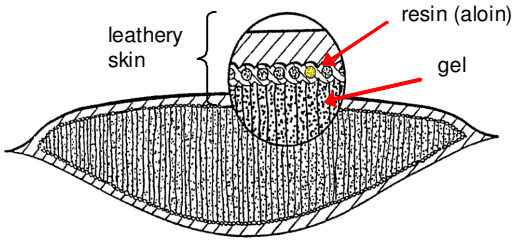


Figure 2: Internal structure of the Aloe

PLANTSCIENCE delivered in person the plants to grower ALFAVERDE during a technical meeting in Spain in May 2005 where TTZ and SANTAVERDE were also present. Later PLANTSCIENCE met the grower QUIVERA, also in Spain. PLANTSCIENCE made sure that the plants were properly placed and gave the growers indications for the care of the plants.

TTZ met the grower POULIMENOS in Greece in May 2005 and provided them with the plants as well as with the necessary instructions.

The sweet Aloe was meanwhile sent to the Italian grower FARO by post with the corresponding indications.

During the mid-term meeting, the growers were requested to provide the RTD SPECTRAL with leave samples of the sweet aloe plants, in order to quantify by means of NMR the content of active compounds (WP3) and this way estimate the influence of soil conditions, cultivation methods and climate on the yield of effective compounds. It was not possible to analyse the clone planted at FARO's plantation since the 5 plants did not grow properly and died before any samples could be sent to SPECTRAL, probably due to a bad shipping. Although the size of the leaves was still not adequate for analyse (too small), the growers selected the biggest leaves as samples to be sent for analyse.

The pictures of the clone plants after 1 year plantation and the samples there of for analysis provided by the growers can be found in Annex XXI (M2.2 Samples of plants form the different Aloe vera cultivators to be analysed).

Figure 3: Sweet Aloe plants 1 year after plantations (parter QUIVERA)

The following two tables summarize the figures obtained of the NMR analysis of the sweet aloe plant leaves inner leaf (IL) as well as whole leaf (WL) performed by partner SPECTRAL. The sample AMY 19472 Spectral corresponds to a plant that grew at SPECTRAL´s facilities and was also subject of analysis.

IL	AMY17826	AMY17853	AMY17926	AMY19472
	Alfa Verde	Poulimenos	Quivera	Spectral
Aloverose	14,8	10,2	9,1	
Glucose	17,8	14,9	10,5	
Malic Acid	13,8	21,1	21,2	
Dry Matter	2,7	1,3	1,3	

WL	AMY17826	AMY17853	AMY17926	AMY19472
	Alfa Verde	Poulimenos	Quivera	Spectral
Aloverose	5,3	5,2	5,3	4,2
Glucose	8,7	8,7	5,0	5,9
Malic Acid	4,1	13,3	10,4	15,5
Ciotric Acid	0,8	1,8	5,1	1,4
WLM	4,5	16,8	18,8	12,5
Dry Matter	7,4	3,9	3,5	2,4
Aloin*	1119,0	18,8	4,6	26,3

Table 3: Results of the NMR analysis of plant clone leaf samples (Inner and whole leaf)

Compared to the rest of leave samples, the content of Aloin (1.119 ppm) detected in the sample provided by ALFAVERDE is very high.

Task 2.3 Assessment of results

The first laboratory results from the soil analysis were discussed during a technical meeting between SANTAVERDE, TTZ and PLANTSCIENCE in Hamburg (Germany). Later on, when the soil from all plantations had been analysed, the 6 months technical meeting took place in Bremerhaven (Germany) in August 2005. The results were discussed and compared with the data available from the cultivation methods in the different plantation sites gathered by TTZ and the analysis of the leaves conducted by SPECTRAL, especially regarding Aloverose content (WP 3). TTZ, SANTAVERDE, TELA, SPECTRAL and BIOZOON were present at this technical meeting.

TTZ summarized the laboratory results carried out in task 2.1 (Deliverable 2.1, *Annex VIII*) and compared them with the cultivation methods in the different plantations (task 2.2, Deliverable 2.2, *Annex IX*) and with the Aloverose content in the leaves analysed by SPECTRAL (WP3). The report resulting from this assessment constitute a milestone of the AMAY project (Milestone 2.1, *Annex XI*)

PLANTSCIENCE contributed to this task with their knowledge through a fluent communication and meetings with the responsible partner of the task (TTZ).

The following figures summarize the most relevant outcomes of this assessment, which

constitutes a milestone of the AMAY project. The final results of the task were presented by the task leader (TTZ) during the project mid-term meeting. The results of the analysis of leave samples from sweet aloe clones regarding their Aloverose content, completes this task and was discussed during the final meeting and included in the relevant deliverable report (Annex X).

The figure below schematically represents the characterisation of the different soils regarding the particle size. All lines have a similar progression, which shows that all soils analyses were loamy sand. The only difference can be seen in the sample QS3 from partner QUIVERA. Unlike the other 2 soil samples, this one is loam.

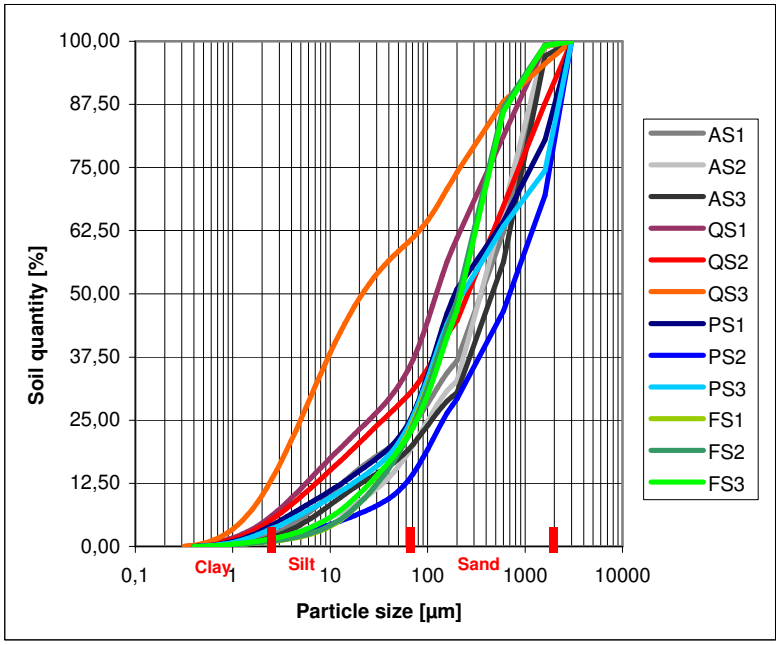


Figure 4: Particle Size Distribution of the different soils analysed.

The following diagram shows the maximal water holding capacity and the loss of ignition measured in the soils. Both parameters are related to the organic content: the max water holding capacity gives an indication of the contained organic matter, which is water storing. The loss on ignition indicates the content of organic matter, which is the one which glows. We can see from the diagram that the soil from the grower POULIMENOS shows the highest maximal water holding capacity, although not the highest loss of ignition which should correspond. This is due to the fact that the soil contained a substrate which stores a high amount of water but does not glow since it is inorganic.

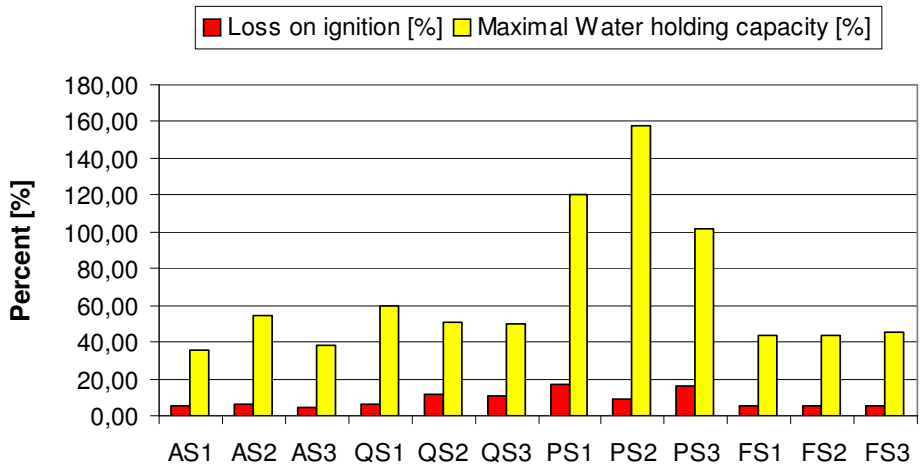


Figure 5: Maximal Water holding capacity & Loss on Ignition

LUFA[1] is an agricultural examination and research center of Münster which sets the recommended minimal and maximal concentrations for the nutrients by plants (not particullary for Aloe vera but for plants in general). This diagram represents in a logarithmical scale the concentrations of the 5 most indicative nutrients found in the 4 types of soils and compares it with the recommendations of LUFA

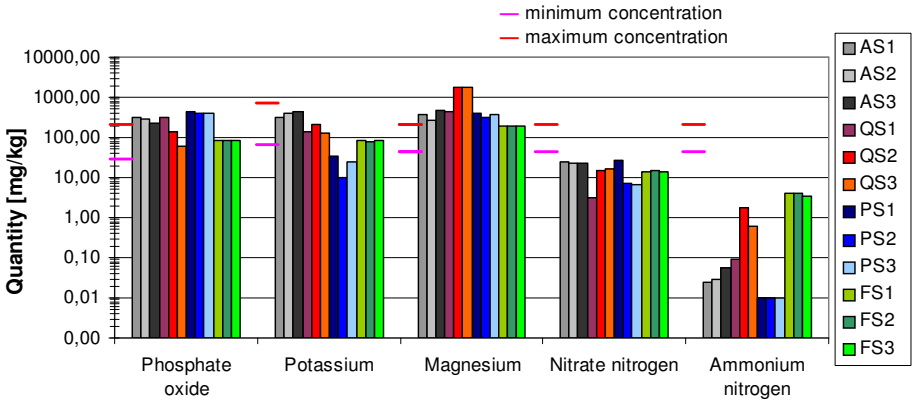


Figure 6: Nutrient concentrations measured in the different soils

As seen in the diagram, generally the soils comply with the recommendations with the exception of Nitrogen, which concentration seems to be too low in most cases. Additionally, only in the case of POULIMENOS, the concentration of Potassium in soil seems to be low.

[1] Landwirtschaftliche Untersuchungs- und Forschungsanstalt Nordrhein-Westfalen - LUFA NRW – is the acredited institute for agrarian and environmental analytic of the chamber or agriculture in the Nordrhein-Westfalen region in Germany

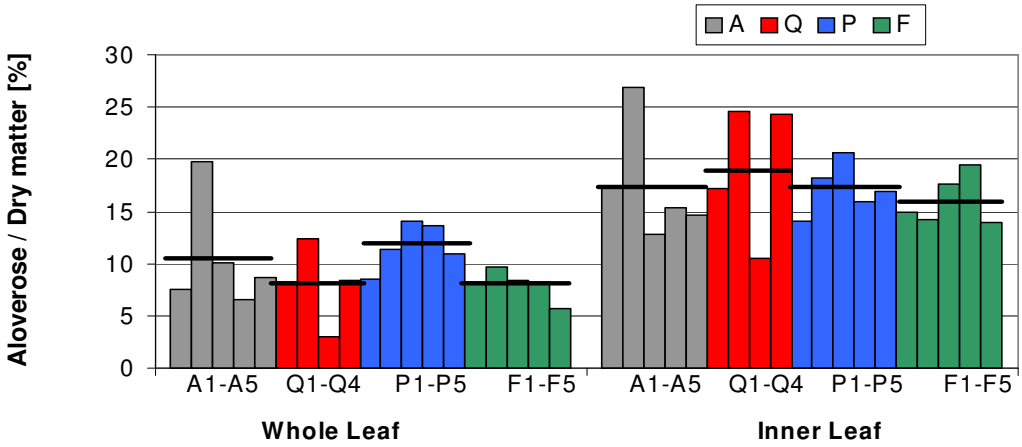


Figure 7: Content of the polysaccharide Aloverose

Figure 6 shows the content of Aloverose measured by means of NMR by SPECTRAL in the Aloe vera leaves provided by the growers. The content of Aloverose was measured in the whole leaf as well as only in the inner leaf (the inner gel without the outer rind). The content in plants from ALFAVERDE and QUIVERA varies strongly, while we find small difference in plants from POULIMENOS and FARO. The plants from QUIVERA show the lowest value on Aloverose in Whole Leaf, whereas in the gel they show the highest content.

The content of Aloverose in the samples coming from ALFAVERDE and QUIVERA varies a lot, while in the case of leaf samples from FARO and POULIMENOS, the content in Aloverose is quite regular.

The black lines in the diagram represent the mean value. A low content on Aloverose in the whole leaf does not mean a low content in the inner gel, as can be seen in the case of the leaves from QUIVERA

The four studied growers from the Mediterranean area produce *Aloe vera* plants, their leaves carrying a relatively high content of Aloverose. They all employ different methods and procedures of cultivation but none of them seemed to be wrong with regards to the Aloverose production. The *Aloe vera* plants showed to be very resistant to different climatic conditions. Irrigation and other cultivation methods do not seem to have badly influenced their growth and the production of Aloverose. However, the results of this study have shown that the Aloverose production was higher on coarse soils than on fine soils.

Achievements

The tasks have been accomplished. Preliminary laboratory results from soil analyses were ready in August 2005 and were discussed during the 6 months technical meeting. Some missing data were completed afterwards and some information from the growers had to be

checked. The final laboratory results of the soil analyse were available in October 2005. Further comparison with cultivation methods and leave analyse results for the assessment of results was performed until January 2006 and could be discussed with the growers and the rest of the AMAY partners at the mid-term project meeting on the 20th April 2006. In the second year project, the sweet aloe clones planted at the beginning of the project by the different partners were analysed and the task was completed with the outcomes of these analyses (WP3).

The outcome of WP2 constitutes a milestone in the investigation of how to assure a high production of the polysaccharide Aloverose in aloe vera plants taking into account the two influencing factors cultivation methods and climate. As stated in the Annex 1 of the contract, it was the aim of the WP2 to analyse the macronutrients of the soil of the different Aloe vera plantations and to compare the cultivation methods and the climatic conditions in the four sites. The work conducted by the AMAY partners has been in accordance with the objectives stated in the DoW and the personal and economic resources allocated for the tasks. The partners involved in WP2 are planning further collaboration in this field, since there are other aspects involved in the production of the Aloverose which could be investigated. Unfortunately, this could not be performed in the frame of the AMAY project since the partners did not count with the necessary resources. Further agronomic work is necessary to get to know more about the production of the polysaccharide Aloverose in the plant, where for instance genetic pathways and of the synthesis of compounds play and important role. A study about the aloverose productivity per hectare would make sense in order to assess how many leaves could be harvested in a year from a single Aloe vera plant and how much aloverose can be produced in an Aloe vera plantation. Also interesting for further studies would be the investigation of the best harvest time regarding aloverose production. The comparison of aloverose yields in Aloe vera plantations in other parts of the world would be also of great interest. Taking into account these aspects, at the time this report is being written, some of the AMAY partners involved in WP2 are setting up a European project to be submitted in the frame of the FP7.

List of deliverables:

	Due Date	**Actual Submission date**	**Lead Contractor**
D 2.1 Soil analysis results	September 2005	September 2005 (Revised: October 2005)	TTZ
D 2.2 List of different cultivation methods	June 2006	May 2006	TTZ

List of Milestones:

	Due Date	Actual Submission date	Lead Contractor
M 2.1 Assessment report	June 2006	May 2006	TTZ
M2.2 Samples of plants from the different Aloe vera cultivators to be analysed	June 2006	June 2006	-

Deviations/Remarks

During the kick-off meeting, it was agreed to bring forward WP 2 (Comparison of the Aloe Vera cultivation) and WP 3 (Purification and standardisation of active compounds) to start already in parallel to WP1 (Definition of requirements) at month 1. It was possible then to start the soil and leave analyses as soon as the responsible partners (TTZ and SPECTRAL) received the samples. This allowed TTZ to begin establishing a comparison of the soil analysis results and cultivation methods (WP1) with the analysed content of Aloverose in the leaf samples (WP3) from an earlier stage.

2.3 Work Package 3: "Purification and Standardisation of active compounds"

WP leader**: SPECTRAL**

The objective of this WP is the isolation and purification of the active compound of Aloe vera for a further structural analysis by NMR. Furthermore, it is foreseen to develop an alternative-test in order to compare the results obtained with the NMR, with a cheaper but reliable methodology.

Work Package 3 is divided as followed:

Tasks	**Partners Involved**
3.1 *Purification of Aloverose* (months 5-10)	SPECTRAL, TTZ, TELA
3.2 *Structural analysis of Aloverose by NMR* (month 7-17)	SPECTRAL
3.3 *Alternative test development* (month 7-17)	TELA, TTZ

Task 3.1 Purification of Aloverose

Separation of Aloe vera pure juice in different fractions and characterization:

TTZ conducted an Ethanol precipitation (50%) of Aloe vera juice provided by SANTAVERDE. After elimination of the Ethanol and centrifugation, a precipitate and a supernatant were obtained and freeze-dried. The product obtained was sent to SPECTRAL for NMR analyse (task 3.2) and SZU for in vitro tests (see WP4). The samples presented problems of solubility, which led to inconsistent results in the case of the in vitro tests performed by SZU (WP4) or imprecise results in the case of the analyse with NMR (WP3, task 3.2)

The results of the NMR analyses were ready for the meeting in month 6, where the partner extensively discussed how to solve it, specially the problem of solubility. It was supposed that the in vitro tests would also face problems with solubility. However, SZU tested them anyway and so have already a first approach of the results.

Sample	**Aloverose content (%)**	**Aloverose content (mg/L)**
Aloe juice	13,7	956,3
Aloe precipitate	1,6	113,7
Aloe supernatant	3,9	275,2

Table 4: Aloverose content in lyophilisate measured by NMR Spectroscopy/ HPLC analysis

Content in Aloverose in precipitate was lower than expected (57%), since it was not possible to fully dissolve the sample in D2O.

The document describing the process explained above, as well as the characterisation of the obtained fractions can be found in *Annex XIII.*

Optimised separation of Aloe vera fresh gel, Aloe vera pure juice (filtrated) and Aloe vera powder in different fractions and characterization:

SPECTRAL took over the separation process and carried out the same Ethanol precipitation (50%), this time from 3 starting materials: fresh Aloe vera inner gel, Aloe vera juice and Aloe vera powder 200:1 (leaves provided by ALFAVERDE and the rest provided by SANTAVERDE) in order to obtain two different fractions of the Aloe materials, providing that the fractionation was performed from a powder. Therefore, the fresh juice as well as the juice had to be first freeze-dried.

When utilizing the fresh gel from the leave without applying enzymes, the material is very difficult to treat because of the structure of the gel. Freeze-drying lead to a sticky material from which it is not possible to conduct the ethanol fractionation.

Thus, SPECTRAL further performed the fractionation from the other 2 starting materials (juice and powder), taking into account the possibilities of optimisation suggested during the 6 month progress meeting after presentation of the results of the juice fractionation performed by TTZ.

For the freeze drying of the Aloe vera juice (about 2 Liter), it was necessary to filtrate it firstly, since otherwise the juice could not be dissolved. The filtrated juice was freeze-dried and the powder obtained was fractionated with the water-ethanol extract.

The Aloe vera powder was directly fractionated.

20 g of the 2 powders (freeze dried filtrated juice and powder) were diluted in 80 ml of 50%Ethanol solution. The insoluble part (precipitate) was separated by centrifugation. The ethanol of the clear supernatant was separated by a rotavapor. The obtained fractions were then freeze-dried.

About 50mg of the testing material were dissolved in 1 ml D2O and provided with 5mg of an intern standard NSA. A ^{1}H-NMR Spectra was measured from the prepared samples. The used NMR parameters are indicated in the Spectra. (see *Annex XI*, Deliverable 3.1, protocol for NMR analysis)

Content of Aloverose, malic acid, lactic acid and aloin between others were determined.

Table 5: Composition of the different fractions analysed by NMR spectroscopy

	Aloverose		Glucose		Malic acid		Lactic acid		Citric acid	
Powder	Content %	Content mg/l	Content %	Content mg/l	Content %	Content mg/l	Content %	Content mg/l	Content %	Content mg/l
Powder total	11,2	780,8	12,6	882,0	24,3	1700,2	0,4	28,9	0,0	0,0
Powder precipitate	1,1	78,3	1,4	98,5	49,0	3431,8	-	-	0,0	0,0
Powder ethanol soluble	13,9	975,6	14,0	979,8	17,0	1189,9	0,5	32,7	0,0	0,0
Juice										
Juice total	19,8	1386,5	17,1	1196,0	30,9	2163,1	-	-	0,0	0,0
Juice precipitate	3,1	218,5	3,5	246,3	42,6	2984,5	-	-	0,0	0,0
Juice ethanol soluble	17,9	1251,8	17,3	1211,9	22,3	1558,3	-	-	0,1	5,5

Apart from the fractions obtained, pure Aloverose as well as total juice freeze-dried (filtrated) and 2 more samples corresponding to freeze-dried Aloe vera whole leaf and freeze dried inner leaf were analysed by NMR and tested in vitro by partner SZU.

In order to characterize accurately the different fractions, the content in proteins was determined by Kjendahl method by partner TTZ. The figure below represents the results of this determination expressed in g in 100 g of sample.

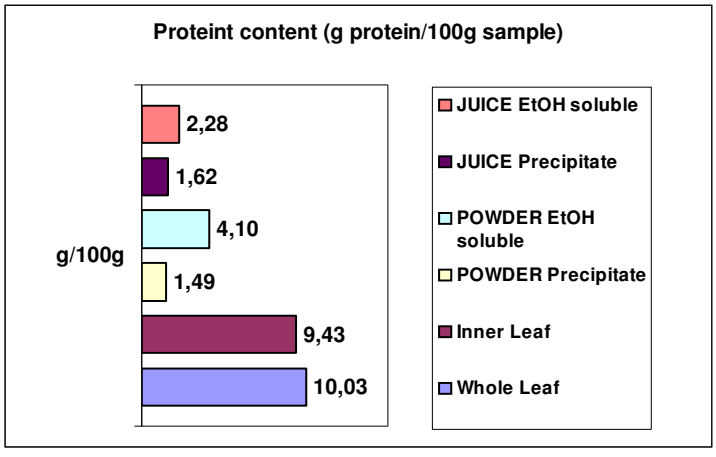


Figure 8: content in proteins measured in different fractions obtained from Aloe vera

Samples of the fractions were provided SZU conducted in vitro test of the fractions in order to assess their cytotoxicity and their protective effects against standard tenside and UV radiation (see WP4).

After the analyse of the obtained fractions regarding composition (through NMR), protein content, cytotoxicity and protective effect against tenside and UV radiation, no more sample was available. Thus, partner SPECTRAL performed the fractionation again, in order to obtain enough amount of sample for the development of the cosmetic product, being this sufficient to be able to perform the dermatological tests(WP4) with a sufficient number of tester for their statistical significance. This was discussed in the project mid-term meeting (see minutes *Annex XVIII*). It was agreed to incorporate the precipitate fraction, which should be mainly Aloverose, according to the available scientific literature.

Original powder	**Amount [%]***	**Amount [mg/l]***
Aloverose	**17,7**	**1240,0**
Glucose	**32,6**	**2284,9**
Malic acid	**9,9**	**695,2**
Lactic acid	**1,5**	**105,5**
Precipitate		
Aloverose	**7,2**	**503,7**
Glucose	**11,8**	**828,0**
Malic acid	**18,3**	**1281,9**
Lactic acid	**0,7**	**51,5**

Table 6: Content on main Aloe vera components in starting Aloe power and obtained precipitate powder

Partner TTZ performed a microbiological test of the obtained precipitate in order to assess the absence of any microbiological contamination in the sample. Prepared dilution series of the obtained precipitate diluted in water at 37°C did not show indeed any growth of mesophile aerobe when cultivated in standard-I-agar (15.0 peptone, 3.0 yeast extract, 6.0 sodium chloride, 1.0 D(+) glucose, 12.0 agar agar [g/L]). From each dilution duplicated were analysed.

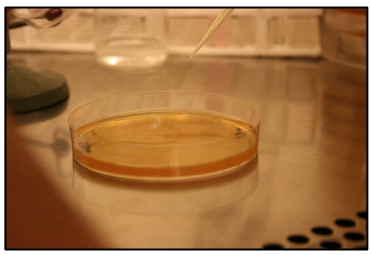


Figure 9: Microbiological test

General quality parameters

Some usual methods for food analysis were applied at freeze dried aloe gel powder as a

reference product in order to become acquainted with aloe and to characterise the aloe product. This was carried out by partner TELA

Among the analysed parameters, there were amino acids, fatty acids and various vitamins.

Table 7: pantothenic acid content in aloe vera products

vitamin	gel powder	Whole leaf powder	juice	gel powder rainbow	enzyme-solution
pantothenic acid	210µg/100g	494µg/100g	23,5µg/100g	469µg/100g	920µg/100ml

Aminoacids represent less than 0-1% in the analysed Aloe vera products. It is not therefore a significant compound of Aloe vera.

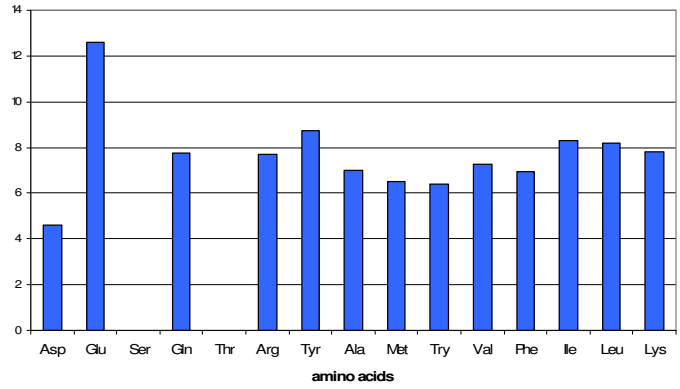


Figure 10: Amino acid pattern in Aloe vera

There is a very low fat content in Aloe vera. **Fatty acids** also represent less than 0, 1% in the products. C12:0 (lauric acid), [a little C16:0 (palmitic acid) and C18:0 (stearic acid)] were mainly found. No unsaturated fatty acids were found. The following table shows the fatty acids detected in the different products.

fatty acid	short	whole leaf powder in %	gel powder in %	juice in %
Dodecansäure	**C 12:0**	**85,48**	**94,35**	**94,95**
Tetradecansäure	C 14:0	0	0	0
Hexadecansäure	**C 16:0**	**8,22**	**0**	**0**
Octadecansäure	**C 18:0**	**6,3**	**5,65**	**5,05**
Eicosansäure	C 20:0	0	0	0
Hexadecensäure	C 16:1	0	0	0
Octadecensäure	C 18:1	0	0	0
Octadecadiensäure	C 18:2	0	0	0
Octadecatriensäure	C 18:3	0	0	0
Eicosensäure	C 20:1	0	0	0
Eicosadiensäure	C 20:2	0	0	0
Eicosatetraensäure	C 20:4	0	0	0
Eicosapentaensäure	C 20:5	0	0	0
Docosensäure	C 22:1	0	0	0
Docosadiensäure	C 22:2	0	0	0
Docosapentaensäure	C 22:5	0	0	0
Docosahexaensäure	C 22:6	0	0	0

Table 8: Content on fatty acids in Aloe vera materials

The content of **vitamins** was also determined. Only pantothenic acid could be detected in relatively high concentrations (for instance the content of panthotenic acid in calf lever or kidney is about 3-8 mg/100g, in fruits 0,1-0,3mg/100g.

vitamin	gel powder	whole leaf powder	juice
thiamine B1	detectable! < 0.1mg/100g	detectable! < 0.1mg/100g	detectable! < 0.1mg/100ml
riboflavin B2	n.d.	n.d.	n.d.
niacin B3	100µg/100g	200µg/100g	n.d.
pantothenic acid B5	210µg/100g	494µg/100g	23,5µg/100ml

Table 9: Content on different vitamins in different Aloe vera materials

The Luff-Schoorl method, based on iodine titration of excess copper, was applied for determination of reducing sugar. The results obtained clearly show that the saccharides are a very important component of Aloe vera.

sample	reducing saccharides
aloe vera gel powder	51%
aloe vera whole leaf powder	50%

Table 10: Percentage of reducing saccharides in Aloe vera determined by Luff-Schoorl

Since the determinations of amino acids, fatty acids and most vitamins gave no special results for aloe, it was determined, that aloe contains relatively high concentrations of pantothenic acid. It was tested whether these relatively high amounts could be a result of the enzyme solution used for the partial hydrolysis of polysaccharides in aloe products for the cosmetics industry. But since only relatively low concentrations of enzymes are generally used for the juices, we can conclude that panthotenic acid is a natural component of aloe vera products.

Task 3.2 Structural analysis of Aloverose by NMR

SPECTRAL performs the NMR analysis by means of an innovative, electronic process which is working via Gauss pulses. This additional measure is enabling the analysis to be carried out without drying and with the addition of 20% D2O only.

If the aloe vera gel analysed contains natural or natural impurities, this can be detected by means of the NMR method. Decomposition products give information about period of time elapsing between the leaves being harvested and processed and the age of the raw material.

The Aloe Vera leaf samples provided by the cultivators (ALFAVERDE, QUIVERA, FARO and POULIMENOS) to SPECTRAL were chemically analysed. The content on Aloverose as well as other compounds contained in the Aloe vera was determined by means of the NMR analysis. The results were provided to TTZ in order to estimate the influence of soil conditions; plant clone, cultivation methods and climate on the yield of Aloverose (see WP1).

From the leaf samples provided, the hole Leaf (WL) as well as the Inner Leaf or gel (IL) were analysed.

The whole leaf includes the outer skin of the leaves, from which a yellow, bitter fluid seeps out and dries out into an oleoresin. Major compounds of this resin are anthrol derivatives e.g. Aloin or Barloine, Isobarbaloin, Chrysophanic acid, etc. (see Fig. 10 beside).

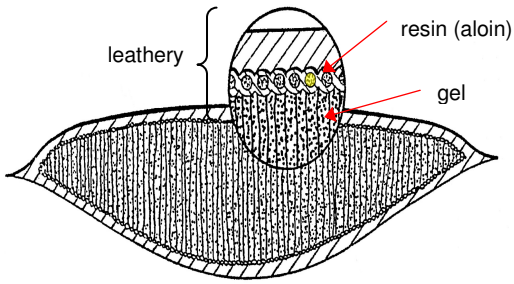


Fig 11: Internal structure of the Aloe Vera

The Aloe vera inner leaf is constituted by an inner transparent water-like gel, which is obtained by filleting the thick leaf. The gel is colourless, gelatine-like, with hair-like connective fibres and consists of >99% water. In the solid part of the gel, the three main compounds are glucose (a monomeric sugar constituent), malic acid (a preliminary stage for sugar in the plant's organism) and the polysaccharide "Acemannan", the core of Aloe Vera. The word Acemannan is a term generally used for a specific range of molecular weight polysaccharide, and actually is a term trademarked by Carrinton Laboratories in the U.S. The same compound can be found with different names such as Carrysin and **Aloverose**, both trademarks. Aloverose is a linear polysaccharide composed of (1, 4)-linked mannosyl residues, with acetyl groups and some chains of galactose. The structure of this polysaccharide has not been completely identified yet and only an empiric structure is available (see fig 9 on the next page).

The three main components of the fresh Aloe vera (Aloverose®, gl ucose and malic acid) are

all detectable by 1H-NMR spectroscopy. These compounds are markers for good material.

High amounts of lactic acid indicate a bacterial degradation (Lactobacillus). Succinic and fumaric acid are produced by Aloe Vera's own enzyme system. Acetic acid and formic acid are caused by hydrolysis of Aloverose® and thermal degradation of glucose during storage.

Aloverose is a polysaccharide, but not all polysaccharides in Aloe Vera are Aloverose. Aloverose is partly acetylated polymannose (chemical structure see Figure 9). NMR distinguishes between Aloverose and other polysaccharides selectively. It defines its total amount independent of the chain length and the molecular weight. Acemanan® defines a specific molecular weight fraction.

There are eight different configuration possibilities for the acetyl groups within a monomeric unit. The acetyl groups (Ac) are possibly a periodic sequence arranged like a DNA (three-dimensional) structure and can be interpreted as an Aloe Vera genetic fingerprint. NMR spectroscopy uses the acetyl proton signals as a fingerprint for identification and quantification (see Figure 10). The method is validated.

The amount of Aloverose changes because Aloe Vera is a natural material. From experiences SPECTRAL knows its amount in fresh Aloe Vera gel varies between 5% and 20% w/w of dry matter.

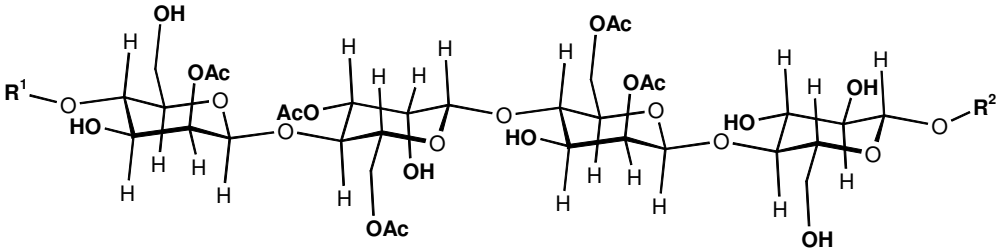


Figure 12: Theoretical chemical structure of the Aloverose

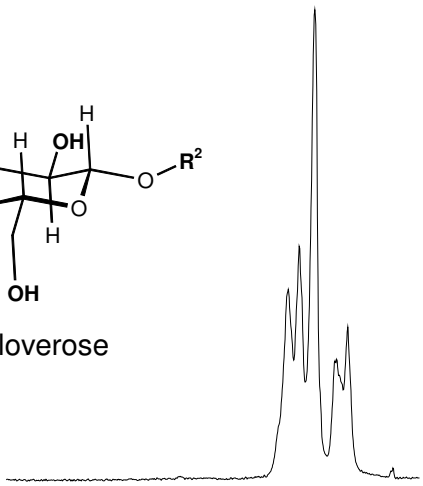


Figure 13: Acetyl proton signal

To distinguish between Aloe Vera Gel (GEL) and Whole Leaf (WL) three marker had been detected by 1H-NMR spectroscopy, named Whole Leaf Marker 1, 2 and 3 (WLM). The presence of these chemical substances in any Aloe Vera product indicates the use of whole leaf material. In future statistically evaluated data will enable not only to show the presence or absence but even the quantity of WL.

The WHL are synthesized in a biochemical reaction taking place in the rind as a part of the well known and well documented citric acid cycle. The chemical structure is elucidated. It is from the same family as other fruit acids in Aloe Vera, similar to malic acid. Therefore there is no possibility to remove the WLM from an Aloe Vera product.

A protocol for the Aloverose purification as well as for the NMR analyse (Deliverable 3.1) can be found in *Annex XI.*

Task 3.3 Alternative test development

TELA, with support of TTZ, developed and set-up an alternative and cheap analysis method to verify and quantify Aloverose in the extracts from Aloe Vera gel, as well as in the end-products produced in WP 4. Thereby HPLC technnique was selected, which has the potential of automatisation. Directs analysis of Aloverose as well as analysis of sugar spectrum after hidrolysis were tested and evaluated according feasibility.

Gained measurement results of the different extracts and end-products were correlated to NMR results of task 3.2 to investigate in the developed method is suitable as sensitive and reliable quick test for quantification and certification of Aloverose.

Partner TELA with the support of TTZ tried different approaches for the development and optimisation of the method. The complete report can be found in Annex XVI (Deliverable 3.4 Report on the Aloe certification quick-test). The quantification of mannose as product of polysaccharide hydrolysis can serve as a criteria for aloe quality. TELA carried out a complete hydrolysis of the polysaccharides in the samples to gain monomeric mannose. The glycosidic and the acetylic bond are destroyed. The monomer gained after the hydrolysis is the mannose which is measured as chlorinated anion and it is evaluated over the extracted ion traces. With every measurement an external calibrating straight line is determined. A defined content of mannose in the samples and the Aloverose standard is determined by means of this calibrating straight line.

The mannose content in the samples is evaluated by a HPLC-MS measurement before and after the hydrolysis. The difference announces the mannose part which arose from the hydrolysis of the Aloverose. Preparation of samples:

The samples were extracted from the cosmetic products developed under WP4. 3 samples were used to test this method: the 3 versions of the cream developed by partner GRADIENS incorporating different percentages of Aloe vera. Additionally, pure Aloe vera juice was also analysed.

- weight 1 g of the sample
- add 2ml chloroform, shake vigorously until the sample is completely dissolved
- add 10ml water, shake for 2 h
- centrifuge
- use the aqueous phase for the hydrolysis

For the hydrolysis of Aloverose following conditions were adopted

- add 4ml of concentrated HCl to 1ml of the aqueous phase
- 2,5h ultrasonic bath at 80°C

- dilution with HPLC-water
- measurement of mannose monomer before and after hydrolysis and comparison

Development of a HPLC-MS method:

- Testing different saccharide measurement methods (e.g. ion chromatography). Partner TTZ applied Ion Chromatography for the quantification of mannose in the same samples. The separation was performed in a CarboPac PA100 column with the mobile phase Latexpolymer Ethylvinylbenzol. However, the quantification of mannose molecules by this means conducted to inconsistent results.
- Measurement of mannose as chlorinated anion

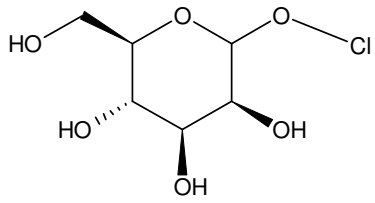


Figure 14: mannose as chlorinated anion

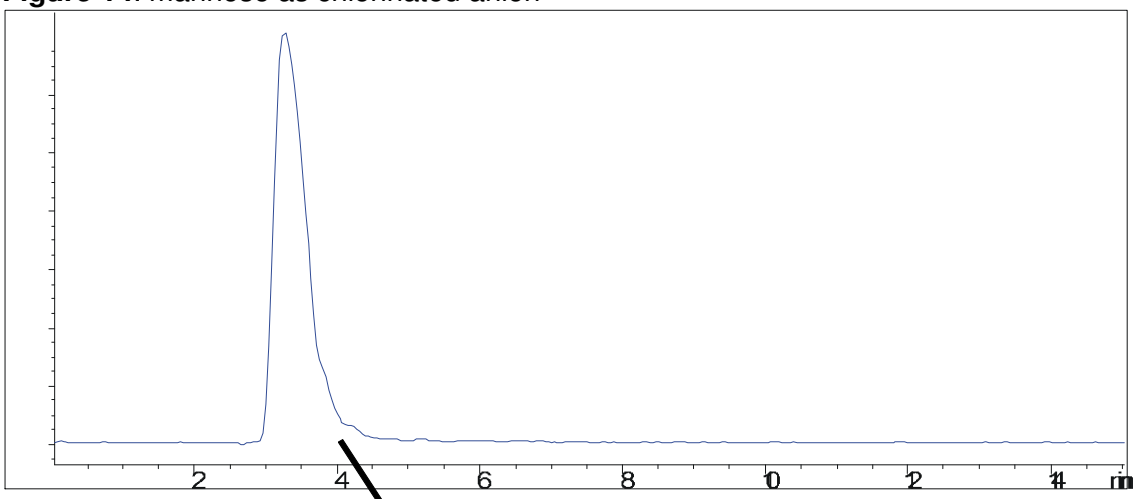


Figure.15: chromatogram of mannose

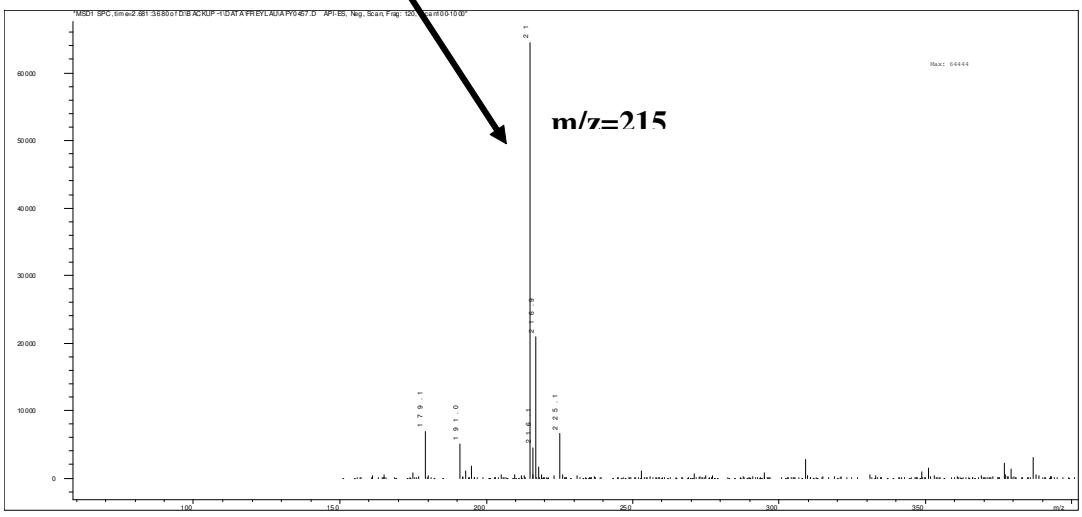


Figure 16: Mass spectrum of mannose, the main component found in hydrolysed aloe products; m/z:215 is an adduct of chlorine (mode: electrospray, negative ionisation)

This method was applied for the three creams containing different percentages of Aloe vera pure juice developed by partner GRADIENS in WP4. The complete hydrolysis led to the following results:

sample	mannose before hydrolysis	mannose after hydrolysis
juice	63	530400
creme 11%	193	4576
creme 23%	371	15600
creme 36%	502	22100

Table 17: results of complete hydrolysis, [µg/100g]

Subtracting the amount of mannose before the hydrolysis to the amount of mannose after hydrolysis we obtain the amount of mannose derived exclusively from the hydrolysis of Aloverose. This hydrolysis of the polysaccharides and subsequent determination of mannose content can be used for qualitative assurance of aloe vera products. However, further empirical studies on different aloe vera products are necessary.

The extraction of the Aloverose and the complete hydrolysis can carried out in a simple and not very time-consuming way. Accordingly, the samples can be prepared fast and analysed fast. The special analytic method at which the saccharide molecules are chlorinated is also simple and can be applied with adequate technique without problems. The evaluation with the module Extracted Ion provides the analyst the chlorinated saccharide molecules only and over the retention time. A further safety that it has to be the chlorinated monomer is given to the analyst.

The aim of this task was to develop a quick, easy and cheap HPLC-MS method to characterise aloe vera samples. We tried to find a typical compound of aloe vera, which can be used as quantifier and qualifier. Different analysis methods were applied to find such a compound. But it was not easy to find such a compound which is typical for aloe vera and which could be easy detected. At the end the monomeric saccharide mannose was used as typical substance to identify and quantify aloe vera cosmetic products.

In pararel to the application of this HPLC-MS method envisaged in the AMAY project, partner TTZ tried a different approach using Ion Chromatography for the quantification of mannose in the same samples. The separation was performed in a CarboPac PA100 column with the mobile phase Latexpolymer Ethylvinylbenzol. However, the quantification of mannose molecules by this means conducted to inconsistent results and therefore they are not included in this report. The partner in charge suspects it is due to the sample pre-treatment, which might not be appropriate for the ion chromatography.

The partners involved in the task (TELA, TTZ and SPECTRAL) discussed other possibilities for quantification of Aloverose and different ways of pre treatment. One possibility would be the

application of size exclusion chromatography. This approach can not be realised in the frame of the AMAY project and the partner involved in the project do not possess the necessary technical resources (Size exclusion chromatographer). However, the mentioned partners are planning research collaborations together with further partners working on such technique. It is still questionable if such technique represents an advantage compared to the existing NMR and if it is economically feasible.

Achievements

Tasks are accomplished. TELA developed the protocol for carrying out the HPLC-MS analysis (D 3.1), which can be found in Annex XI.

The quantification of mannose as product of polysaccharide hydrolysis can serve as a criteria for aloe quality. TELA with support of TTZ developed a HPLC-MS method to characterise aloe vera samples as alternative to the NMR. The complete report on the Aloe vera certification quick-test (D 3.4) can be found in Annex XVI.

The protocol for carrying out HPLC analysis for Aloverose (D3.2) was prepared by SPECTRAL, as well as the report of the NMR analysis which was submitted to the coordinator when the analysis was finalized.

List of deliverables:

	Due Date	Actual Submission date	Lead Contractor
D 3.1 Protocol for the Aloverose purification and NMR analysis	February 2006	November 2005	SPECTRAL
D 3.2 Protocol for carrying out HPLC analysis for Aloverose	September 2006	October 2006	TELA
D 3.3 Report on the NMR analysis of Aloverose	September 2006	October 2006	SPECTRAL
D3.4 Report on the Aloe vera certification quick-test	September 2006	February 2007	

List of Milestones:

	Due Date	Actual Submission date	Lead Contractor
M 3.1 Isolation of the Aloverose	September 2006	July 2006	SPECTRAL

Deviations/Remarks

It was agreed at the kick-off meeting to start earlier with task 3.1 of WP 3 (Purification of Aloverose), as well as the task 3.2 (Analysis by NMR) as soon as the samples of Aloe vera leaves had been delivered by the growers.

TELA also started in month 1 with the characterization of the Aloe material.

The three tasks lasted some moths more than scheduled due to technical reason which have to be solved in order to perform the work in an appropriate way.

2.4 Work Package 4: "Project Development and Evaluation"

WP Leader: GRADIENS

The aim of the WP4 is to develop cosmetic products containing standardised amount of the active compound and to test the product regarding safety and efficacy.

Task 4.1 Development of cosmetic products

Three different cosmetic products were developed within this task.

- The under WP3 obtained Aloe vera fraction was introduced in a basis cream formulation developed by Santaverde. TTZ took over the development of the cream. Due to technical difficulties, a second product was developed using a more stable formulation
- GRADIENS developed another basic cream formulation and developed 3 different versions of the cream including 3 different concentrations of Aloe vera pure juice.

This procedure was agreed by the project partners during the mid-term meeting (see mid-term meeting minutes, *Annex XVIII*). The aim of the production of the different products containing the isolated Aloe vera fraction one, and the pure juice the other, was to permit the comparison of the effects of the isolated fraction versus the pure juice with the results obtained after dermatological testing (task 4.2). Both can interact with different components from the skin and act as active molecules in the final cosmetic formulations.

Production of a cream (natural cosmetic) incorporating the under WP3 isolated Aloe vera fraction: partner TTZ

Partner TTZ carried out the production of the cream incorporating the Aloe vera fraction (powder) obtained in WP3. In order to be able to assess any effect on the human skin when testing the cream in human volunteers later on (WP4) derived of the Aloe vera fraction and not of other ingredients with possible effects on the skin, a simple formulation was chosen. Substances such as polysaccharides and any ingredient with hydrating or any other positive effects were avoided. The formulation of the cream incorporating the isolated Aloe vera fraction is shown below:

Phase A	**INCI name**	**%**
Axol C62	Glyceril Stearate Citrate	2,50
Tegin M	Glyceril Starate	3,50
Lanette O	Cetearyl Alcohol	3,50
Tegosoft CT	Caprylic/Capric Triglyceride	20,00
Dem. Wasser	Aqua	63,10
Dermofeel 700 B	Sodium phytate	0,70
Dermofeel PA-3	Glyceriyl Caprylate	0,10
Dermosoft GMCY	Parfum	1,00
Phase B		
Hops extract	humulus lupulus	0,10
Ethanol 96 % ig unvergällt	alcohol	5
Purasal S/HQ 60	Sodium lactate	5

Table 12: Composition of the cream developed by TTZ (natural cosmetic formula)

A placebo cream (not containing the Aloe vera fraction powder) was also produced. The production of the placebo cream was in views of the dermatological testing, since the effect of the Aloe vera fraction should be tested again placebo to be able to assess an effect of the ingredient on the human skin.

Phase A	**INCI name**	**%**
Axol C62	Glyceril Stearate Citrate	2,50
Tegin M	Glyceril Starate	3,50
Lanette O	Cetearyl Alcohol	3,50
Tegosoft CT	Caprylic/Cpric Triglyceride	20,00
Dem. Wasser	Aqua	58,10
Dermofeel 700 B	Sodium phytate	0,70
Dermofeel PA-3	Glyceriyl Caprylate	0,10
Dermosoft GMCY	Parfum	1,00
Aloe Fraction (Powder)		0,50
Phase B		
Hops extract	humulus lupulus	0,10
Ethanol 96 % ig unvergällt	alcohol	5
Purasal S/HQ 60	Sodium lactate	5

Table 13: Composition of the placebo cream developed by TTZ (natural cosmetic formula)

The aloe vera fraction (powder) was tested microbiologically according to a general procedure to determine mesophile aerobe (§35 LMBG). Standard-I-Agar (15.0 peptone, 3.0 yeast extract, 6.0 sodium chloride, 1.0 D(+) glucose, 12.0 agar agar [g/L]); autoclave: 15 min at 121°C was used as culture medium (dry medium). Two series of 7 dilutions (duplicate) were prepared and plating was performed under clean bench. The dilution series were prepared within 20 min after homogenisation and sterile test tubes were used for dilution. After sedimentation (not longer then 15 min) of the bigger particles, 1 mL will be taken out of the aqueous phase without previously stirring and added to 9 mL sterile NaCl (8.5 g NaCl/ L). → 1*102, and well stirred at Vortex. 1 mL of 1*102 dilution was taken for the next dilution step in 9 mL NaCl (1*103) and so on. (7 decimal dilutions were prepared in total). From each appropriate dilution step 1 mL was pipetted into a sterile petri dish with 15 to 20 mL of the sterile culture medium. The sample was distributed thoroughly on the medium. The petri dishes were incubated up side down 72 hours at 30°C.

The formula applied for analysis is shown below:

$$\overline{C} = \frac{\sum c}{n_1 \times 1 + n_2 \times 0{,}1}$$

C = Weighted arithmetic average of colonies

∑C = Lowest analysable plate count plus amount of colonies of next higher dilution

n1 = Amount of plates with lowest analysable dilution

n2 = Amount of plates with next higher dilution

However, in our analysis that followed incubation, no formula has to be applied since only one colony could be counted in one of the Petri dishes (dilution 1*105 but not in the duplicate).It was assumed that this isolated colony was not due to any contamination of the powder but came from the environment.

Even not having any probe of anaerobic microbiological contamination and in order to assure the complete absence of microorganisms, the powder diluted in the alcohol was filtrated before its incorporation in the cream formulation (filter unit of 0,2nm).

Figure 18: Filter

The production of this cream was carried out by TTZ. The device used for the mixture and homogenization of the ingredients was *Mixer Becolab-Mini RW 2,5* (load volume: 0,5 - 2 L)

Procedure:

Phase A was heated up to 85 ° C while mixing and homogenised at 3000 U/ Min during 10 minutes. Then, still mixing, it was cooled down to 30℃. Phase B was added to phase A when the temperature reached 30℃. The mixture was cooled down to 20℃ while mixing it and at las, it was homogenised at 2.000 U/Min for 2 minutes.

The cream was filled in 375 ml cream tins and sent to SZU for testing.

Figure 19: Becolab mixer

Figures 20 and 21: Cream incorporating the Aloe vera fraction and placebo

The cream was dermatologically tested against placebo by partner SZU. (WP4, see task 4.2 later on) , However, only two tests could be carried out before the cream lost stability and broke in two phases. Although partner SZU tried to recover the emulsion by homogenizing the cream again, it was not possible further testing the cream since it would not absorve properly in the human skin.

Thus, the partners agreed on producing a new cream incorporating the Aloe vera powder fraction. Since the problem of stability was likely due to a problem in the recipe developed, which incorporated only natural ingredients, and taking into account that the amount of power produced (WP3) was rather limited, the partners decided to use an stable basis cream (not natural cosmetic) where they could easily incorporate the powder fraction. This would permit assessing an effect of the Aloe fraction on the skin, minimising the risks of low stability of the cream emulsion.

Remark:

Natural cosmetic producers like the SMEs involved in the AMAY project (e.g. SANTAVERDE) use their raw material as basis and main ingredient of their products. For instance, in the case of Aloe vera in the development of natural cosmetics, pure Aloe vera juice is often used as the water phase where many other ingredients are included, and this phase represents a high percentage of the formulation, sometimes even up to 100%. Therefore the difficulty in developing a good and stable formulation for the AMAY product, where the main raw material (Aloe vera poder) represent a very low percentage of the total formulation and the rest are other natural ingredients at a higher percentage. As a result, the cream might present problems of stability, as occurred to the developed formulation.

The developmet of a better formulation of a natural cosmetic including the Aloe vera fraction might be possible counting with the neccesary time and resources. The product will requires a high degree of optimisation . The extraction of the Aloe vera powder fraction is a long-lasting process with a low yield. Due to the scarce amount of powder left after the first cream production and the short time left until the end of the project, it would have not been possible for the partners to achieve a new extraction, natural cosmetic formulation and trials, product development and testing. For the reasons explained, the partners agreed on incorporating the Aloe powder fraction in a normal (not natural cosmetic) and stable commercial basis cream, making sure this way that they could be able to test the effect of the Aloverose on the skin without other factors interfering.

Production of a skin milk (not natural cosmetic) incorporating the under WP3 isolated Aloe vera fraction: partner TTZ

A very simple formulation of O/W Skin milk (Oil/Water-Skin milk) was selected for the incorporation of the Aloe powder fraction (Aloverose). Like the former cream, the skin mild was produced in the *Mixer Becolab-Mini RW 2,5 (see Figure 13)*.

Phase	Ingredient	INCI name	%
A	Hostaphat® KL 340 D	Trilaureth-4 Phosphate	1
	White vaseline	Petrolatum	4,5
		Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben	1
	Nipaguard® PDU	Ammonium Acryloyldimethyltaurate/VP Copolymer	1,3
B	Aristoflex® AVC	Aqua	91,7
C	Water		0,5
	Aloe vera powder		

Table 14: Composition of the skin milk (non natural cosmetic formula)

Formula of the placebo

Phase	Ingredient	INCI name	%
A	Hostaphat® KL 340 D	Trilaureth-4 Phosphate	1
	White vaseline	Petrolatum	4,5
		Propylene Glycol (and) Diazolidinyl Urea (and) Methylparaben (and) Propylparaben	1
	Nipaguard® PDU	Ammonium Acryloyldimethyltaurate/VP Copolymer	1,3
B	Aristoflex® AVC	Aqua	92,2
C	Water		

Table 15: Composition of the placebo skin milk (non natural cosmetic formula)

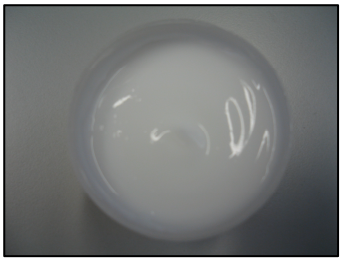
Procedure:

Vaseline was melted and all ingredients excepting the Aloe vera powder fraccion were mixed together in the Mixer at 0,8 m/s and warmed up to 80 ºC and homogenised at 24 m/s for 10 minutes. The high temperature was necessary to achieve a stable emulsion. The mixture was cooled down to 30ºC while pumping it with 8 m/s. Then, the aloe vera powder was added and the mixture was homogenised again at 24 m/s for 6 minutes. After this, it was cooled down to 20ºC while pumping at 8 m/S and filled into small beakers (75 ml). The result was a skin milk rather liquid than creamy but stable.

The Aloe vera powder fraction was not added at a high temperature since the heat could harm the structure of the polysaccharide Aloverose. In order to maintain its structure intact and test the effect of the molecule on the human skin later on, the mixture of ingredients was cooled down to 30ºC.

The placebo was produced in the same way but no Aloe powder was added.

Figures 22, 23 and 24: Mixer at different steps on the production: mixture, cooling down and homogenisation



Figures 25 and 26: O/W skin milk and placebo (marked with the red cross on the right side)

Production of a cream (not natural cosmetic) incorporating the under WP3 isolated Aloe vera fraction: partner GRADIENS

The cream produced by GRADIENS should contain Aloe vera pure juice (not fractionated Aloe vera, inner part of the leave, treated with enzymes to liquidize it and no further treatment)

Three versions of the cream containing different percentages of juice (11%, will be produced

Version 1. (11%)

Requested materials and amounts for producing 100 g Version 1. product			
Water gel phase			**73,13 g**
	material	*amount*	
	Carbomer	0,56	
	Aqua	56,3	
	Alcohol	16,08	
	Methyl Paraben	0,2	
Bulk solution with Aloe			**14,7 g**
	material	*amount*	
	Polysorbate 60	1,32	
	Aloe Vera	4,22	
	Glycerin	3,04	
	Propylene Glycol	2,71	
	Alcohol	3,21	
	Methyl Paraben	0,2	
Juice			**7,44 g**
	material	*amount*	
	Aloe Vera	7,44	
Base			**2,44 g**
	material	*amount*	
	Triethanolamine	0,54	
	Aqua	1,9	
Fragrance			**1,07 g**
	material	*amount*	
	Lavandula Angustifolia	1,07	
Additive			**1,22 g**
	material	*amount*	
	Cetyl Alcohol	1,22	

Table 16: Composition of the cream produced by GRADIENS containing 11% Aloe juice

Version 2. (23%)

Requested materials and amounts for producing 100 g Version 2. product			
Water gel phase			**54,54 g**
	material	*amount*	
	Carbomer	0,42	
	Aqua	41,98	
	Alcohol	11,99	
	Methyl Paraben	0,15	
Aloe gel phase			**15,68 g**
	material	*amount*	
	Carbomer	0,12	
	Aloe Vera	12,07	
	Alcohol	3,45	
	Methyl Paraben	0,04	
Bulk solution with Aloe			**14,12 g**
	material	*amount*	
	Polysorbate 60	1,26	
	Aloe Vera	4,05	
	Glycerin	2,92	
	Propylene Glycol	2,61	
	Alcohol	3,09	
	Methyl Paraben	0,19	
Juice			**7,14 g**
	material	*amount*	
	Aloe Vera	7,14	
Base			**2,81 g**
	material	*amount*	
	Triethanolamine	0,62	
	Aqua	2,19	
Fragrance			**1,03 g**
	material	*amount*	
	Lavandula Angustifolia	1,03	
Additive			**4,68 g**
	material	*amount*	
	Cetyl Alcohol	4,68	

Table 17: Composition of the cream produced by GRADIENS containing 23% Aloe juice

Version 3. (36%)

Requested materials and amounts for producing 100 g Version 3. product			
Water gel phase			**32,74 g**
	material	*amount*	
	Carbomer	0,25	
	Aqua	25,2	
	Alcohol	7,2	
	Methyl Paraben	0,09	
Aloe gel phase			**32,74 g**
	material	*amount*	
	Carbomer	0,25	
	Aloe Vera	25,2	
	Alcohol	7,2	
	Methyl Paraben	0,09	
Bulk solution with Aloe			**13,16 g**
	material	*amount*	
	Polysorbate 60	1,18	
	Aloe Vera	3,77	
	Glycerin	2,72	
	Propylene Glycol	2,43	
	Alcohol	2,88	
	Methyl Paraben	0,18	
Juice			**6,66 g**
	material	*amount*	
	Aloe Vera	6,66	
Base			**2,84 g**
	material	*amount*	
	Triethanolamine	0,63	
	Aqua	2,21	
Fragrance			**0,96 g**
	INCI név	*mennyiség*	
	Lavandula Angustifolia	0,96	
Additive			**10,90 g**
	material	*amount*	
	Cetyl Alcohol	8,72	
	Polysorbate 60	2,18	

Table 18: Composition of the cream produced by GRADIENS containing 66% Aloe juice

Version 4. (Placebo)

Requested materials and amounts for producing 100 g Version 4. product			
Water gel phase			**71,37 g**
	material	*amount*	
	Carbomer	0,55	
	Aqua	54,93	
	Alcohol	15,69	
	Methyl Paraben	0,2	
Bulk solution with water			**14,34 g**
	material	*amount*	
	Polysorbate 60	1,28	
	Aqua	4,12	
	Glycerin	2,96	
	Propylene Glycol	2,65	
	Alcohol	3,14	
	Methyl Paraben	0,19	
Juice			**7,26 g**
	material	*amount*	
	Aqua	7,26	
Base			**2,38 g**
	material	*amount*	
	Triethanolamine	0,53	
	Aqua	1,85	
Fragrance			**1,05 g**
	material	*amount*	
	Lavandula Angustifolia	1,05	
Additive			**3,6 g**
	material	*amount*	
	Cetyl Alcohol	1,2	
	Maltodextrine	2,4	

Table 19: Composition of the placebo cream produced by GRADIENS

Procedure:

Each phase was mixed separately, bulk solution (with or without Aloe) and the base. The different phases and solutions were mixed together in a requested ratio and finally the fragrance was added to the cream. The cream was incorporated in tubes (75 mg each tube) and sent to partner SZU for dermatological testing.

Figure 27 and 28: Final cosmetic product (3 versions and placebo) produced by GRADIENS

Scientific workshop on cosmetic product development

An internal workshop for the AMAY project partners was organized by GRADIENS together with TTZ in April 21st 2006 at Gradiens facilities in Budapest (Hungary) alongside to the mid-term meeting. After the presentation of cosmetic applications of the Aloe vera and its benefits, the partners discussed together different product formulations to work during the workshop, after which the attendants worked practically in the laboratory. This was followed by a discussion about product development and dermatological tests of the products within AMAY. Detailed information of the workshop can be found in the workshop minutes (see *Annex XIX*)

Figure 29 and 30: Dr. Keve from Gradiens shows the participants some of the experiments performed

Task 4.2 Dermatological testing of cosmetic products:

The start of WP4 is due in month 16. However, it has been brought forward in order to assess the safety and the protective effect of different Aloe vera fractions in vitro before incorporating the development of the cosmetic product containing the Aloe vera fraction and its subsequently tests in vivo.

Effects of Aloe vera and its fractions evaluated in skin cell systems in vitro (cytoprotective/photoprotective effects)

The different isolated Aloe vera fractions were tested in vitro in order to assess their ***cytotoxicity*** and their ***protective effects against standard tenside*** (Sodium Dodecyl Sulfate- SDS),

The aims of these tests were to detect the most skin-friendly fraction on the basis of highest non-cytotoxic concentration.

The cell line 3T3 NRU Balb/c mouse fibroblasts (free from mycoplasma) were used for tests. They were exposed during 24 hours to different concentrations of the fractions in culture medium (DMEM supplemented with antibiotics and 10% of inactivated calf serum)

The highest non-cytotoxic concentration was determined for each test material. The viability is expressed as % of control (viability>80% of control)

The results of both tests performed on the first batch of fractions were not consistent and statistically significant. The main reason was poor solubility in the culture medium and stability of the samples. The results are thus not shown in this document but they can be found in *Annex XIV*.

The second batch of samples, produced by an optimised method (and therefore with a better solubility in the culture medium), conducted to the following results:

The results of cytotoxicity tests in cell culture are summarized in Figure 31. The test material **Aloverose exhibited the lowest cytotoxicity** (no cytotoxic effects up to the concentration of 10 000 µg/ml). Although the other samples exhibited relatively higher cytotoxicity, the highest non-cytotoxic concentrations of 2500 and 5000 µg/ml represent extremely low cytotoxicity of the tested materials. The results suggest **very good compatibility of the tested samples with human skin or mucosa in vivo**.

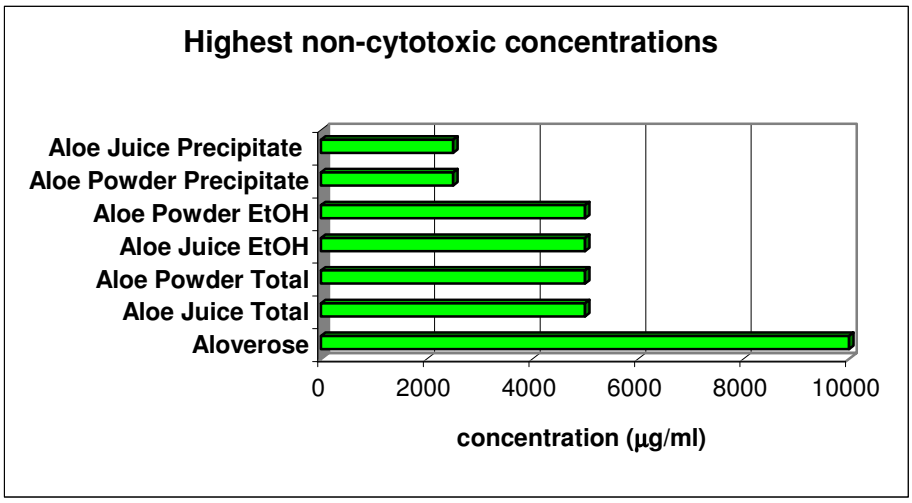


Figure 31: Highest non-cytotoxic concentrations

In a subsequent set of experiments, the protective effect of the tested samples against SDS, that is regularly present in formulations of cleaning products and represents risk of skin irritation, was assessed. The evaluation of protective effects of Aloe vera fractions against SDS cytotoxicity revealed that Aloverose, Aloe Juice Total, Aloe Powder Total and Aloe Powder EtOH Soluble exhibit protective effects and increase the viability of the cell culture at least in one distinct concentration. The most effective concentrations are summarized in Figure 13.

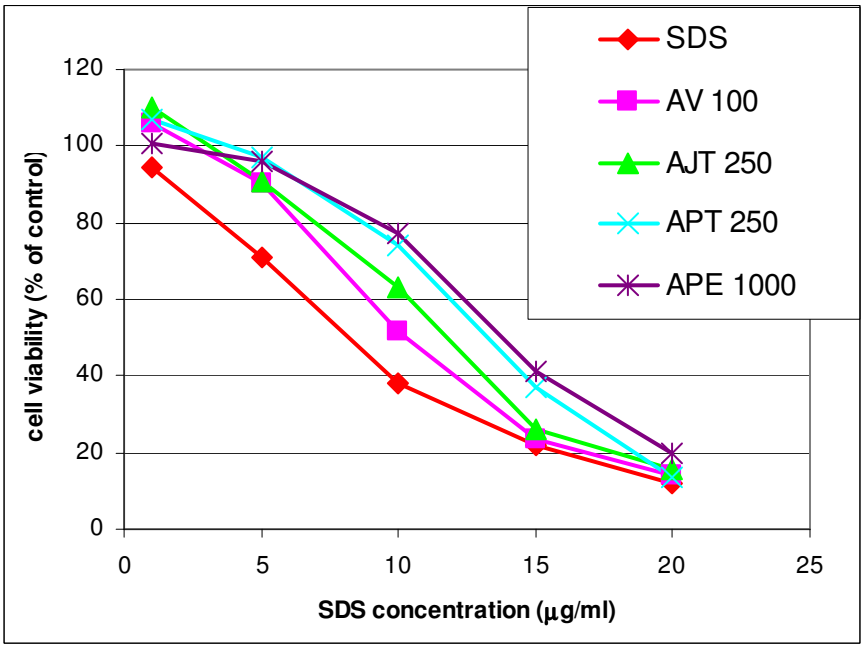


Figure 32: Protective effects of Aloe Vera fractions against cytotoxicity of SDS

The in vitro test reports constitute the *Annex XIV* of this report

Cytotoxicity and protective effects of malic acid, glucose, Aloverose and Aloe juice

As different effects are linked to different compounds from Aloe Vera, there are a lot of questions left open today concerning the real effects of Aloe Vera on human health/skin[2]. Aloe Vera has been used therapeutically certainly since Greeks and Egyptians times and perhaps long before. Nonetheless, there is some confusion concerning the effectiveness of the single plant compounds. While some reports state that the most healing benefit comes from the compound *Aloverose (*the irregularly acetylated polymer of the sugar mannose from the inner gel[3]), most of the Aloe companies claim that the polysaccharide activity is synergistically enhanced due to the presence of large number of minor compounds of the gel. However, there are many examples in the literature of polysaccharides exhibiting pharmacological and physiological activities without synergistic help from other compounds. For example, the anti-tumour activity of polysaccharides has been widely reported[4].

Aloe juice contains 99% water and 1% dry matter. Dry matter may consist of 10-30% malic acid (MA), 10-30% glucose and 5-25% Aloverose (AV)[5].

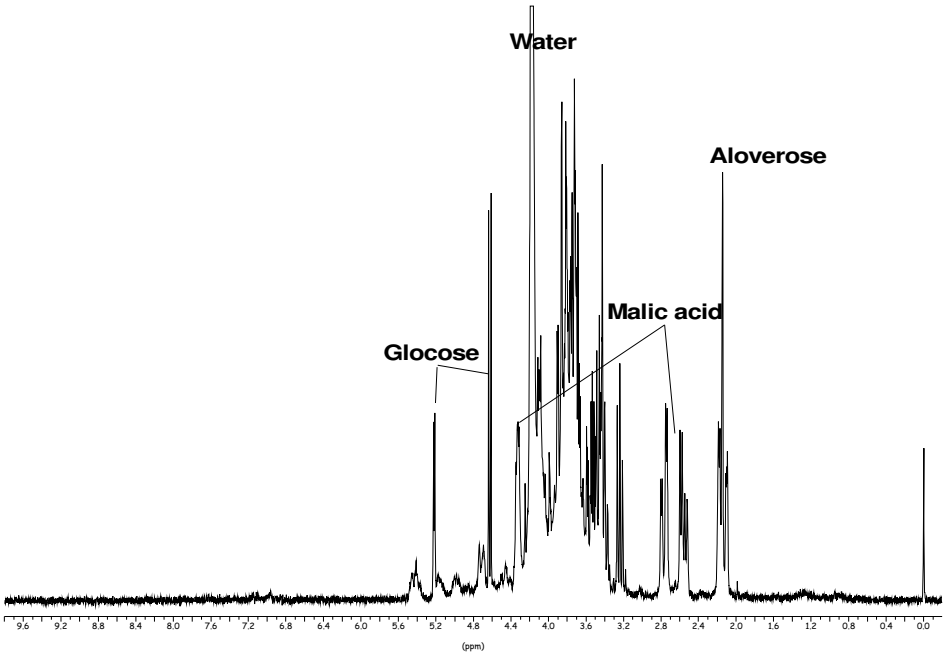


Figure 33:.Aloe vera chromatogram by NMR analysis

[2] Reynolds, T.; Dweck, A.C. (1999): *Aloe Vera leaf gel: a review update*. Journal of Ethnopharmacology, vol. 68, pp. 3-37

[3] R.L. Whistler, Purdue University

[4] Kimoto and Watanabe, 1987; Waldron and Selvendran, 1992

[5] Diehl, B.; Teichmüller, E.E.; (1997) *Aloe vera, qualitäts-und Identitätsprufung.* SÖFW-Journal, 15(1997), 1015-1018

Partner SPECTRAL provided partner SZU with alfa-D-glucose, 99% pure Aloverose and D1-malic acid in order to assess in vitro the effect of the 3 compounds separately and also in different combinations. In vitrotoxicity, as well as protective effect against standard tenside (Sodium Dodecyl Sulfate SDS) and toxic effects of UVA radiation. The complete test reports on the effect of the 3 substances is entirely attached to this report as Annex XX. The results of the experiments are summarized in the following pages.

1. Assessment of the protective effects of Aloverose against toxic doses of UVA irradiation

The possible protective effects of Aloverose, when applied to the cell culture in the preincubation period, during irradiation and in the postincubation period, were assessed in a set of experiments. Data of all experiments document a promoting effect on cell growth of skin fibroblasts. In general, higher concentrations of Aloverose (1000, 2500 µg/ml) have exhibited better protective effects comparing to the lower dose (100 µg/ml). Application of Aloverose during the preincubation period did not significantly improve the cell viability even if high concentrations were used. Significant protective effect of Aloverose was detected if Aloverose was added during irradiation and/or in the postincubation phase. Moreover, the protective effect was relevant to Aloverose concentrations employed. During the postincubation period, Aloverose increases viability only in combination with Aloverose applied during irradiation.The best protective effect of Aloverose was revealed when Aloverose was present in the cell culture, both during the irradiation and the postincubation period

2. Assessment of the in vitro cytotoxicity of the test materials and their protective effects against standard tenside (Sodium Dodecyl Sulfate SDS) and toxic effects of UVA radiation.

The possible protective effects of glucose and malic acid against the commonly used tenside SDS were assessed. Glucose and malic acid do not significantly increase the viability of cells exposed to SDS. Due to extremely low pH (pH 4 in the concentration 2500 mg/ml, pH 5 at 1000 mg/ml) malic acid can be applied to the cell culture only in lower concentrations.

The protective effect of malic acid against cytotoxic doses of UVA radiation was evaluated. MA, when applied during irradiation, slightly increases viability of the cells, during postincubation period the most effective concentration seems to be MA 10 µg/ml. The concentration MA 25 µg/ml is too high, after irradiation the viability of cells incubated with MA 25 µg/ml is decreasing.

3. Assessment of protective effects of Aloe Juice against standard tenside (Sodium Dodecyl Sulfate SDS) and toxic effects of UVA radiation

The protective effect of the new Aloe Juice sample against SDS, a tenside that is regularly present in formulations of cleaning products and represents risk of skin irritation, was assessed. The experiments revealed that Aloe Juice, particularly in higher concentrations, exhibits protective effects and increases significantly the viability of cells exposed to SDS.

The possible protective effect of Aloe Juice against slightly cytotoxic dose of UVA radiation was assessed. Unfortunately, no effect of the the two tested concentrations (100 and 250 µg/ml) was observed, when applied during irradiation or in the postincubation period.

4. Assessment of cytotoxicity and protective effects of different ratios of the three main Aloe Vera components (Malic Acid/Glucose/Aloverose) against standard tenside (Sodium Dodecyl Sulfate SDS).

The cytotoxicity of the Simulated Aloe Juice samples is clearly related to the malic acid and glucose content.

The content of 30% of malic acid seems to be too high, the pH value of the samples after dilution simulating the aloe juice is extremely low (pH = 4 at 100% dilution, pH = 5 at 50% dilution). The optimum content of malic acid was found to be 10% as the samples No. 1, 4 and 7, containing 10% of malic acid, exhibit the lowest cytotoxicity.

The highest cell viability was found for samples with the highest content (30%) of glucose regrdless of the content of Aloverose.
The optimal combination with lowest cytotoxicity was identified:
30% glucose – 10% malic acid – 5-25% Aloverose.

All the test samples, when used in non-cytotoxic dilutions, exhibited protective effects against SDS at least in two distinct concentrations of SDS. Most of them increase the cell viability even without the presence of SDS. The most effective combination of malic acid, glucose and Aloverose against the cytotoxicity of the model irritative tenside was demonstrated for samples No. 4 and 7. These samples simulating aloe juice contain the lowest concentration of malic acid (10%), the highest content of glucose (30%) and the higher concentrations of Aloverose (15% - 25%).

The protective effects of these samples against UVA radiation could not be tested, as Aloverose was available for testing in a very limited amount.

Biological effects of Aloe vera incorporated in cosmetic products assessed by dermatological studies in human volunteers (clinical / instrumental studies)

Partner SZU implemented the following studies:

Clinical studies:

- skin compatibility (repeated application closed patch test)
- hypoalergenicity (repeated insult patch test)
- compatibility with sensitive skin (repeated application open test)
- anti-inflammatory effect (inhibition of solar erythema development)

Instrumental studies:

- skin hydration (corneometry)
- skin surface sebum (sebumetry)
- skin surface pH (skin pH measurement)
- skin elasticity (cutometry)
- anti-wrinkle effect (digital image analysis)

Tested cosmetic products:

O/W Skin-milk developed by TTZ

- AMAY Cream (aloe vera precipitate powder 0,5%)
- AMAY Placebo

Cream developed by GRADIENS

- Aloe Vita Cream version 1 (11% aloe juice)
- Aloe Vita Cream version 2 (23% aloe juice)
- Aloe Vita Cream version 3 (36% aloe juice)
- Aloe Vita Cream version 4 (placebo)

Skin compatibility

Repeated application closed patch test

- closed patch test application
- repeated application for 5 days
- assessment of skin reaction (erythema, dryness, oedema)

Number of volunteers: 30

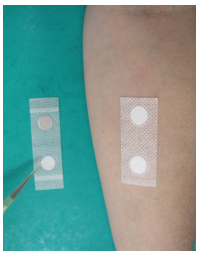


Figure 34: closed path test

Conclusion:

excellent skin compatibility

no adverse skin reactions / irritation were recorded

Hypoallergenic claim

- induction phase: repeated closed patch test application for 3 weeks
  latent phase - 2 weeks without application
- challenge phase: one application for 24 hours
- assessment of reactions (erythema, dryness, oedema)

number of volunteers:

40 for AMAY Cream and 40 for AMAY Placebo

60 for each Aloe Vita creams 1-4

Conclusion

hypoallergenic claim confirmed

no adverse skin reactions, no itching / burning recorded

Compatibility with sensitive skin

Repeated application open test

- application on the volar forearm
- repeated application 4 weeks (twice a day)
- assessment of reactions (erythema, dryness, oedema)

number of volunteers with sensitive skin:

40 for AMAY Cream and 40 for AMAY Placebo

60 for each Aloe Vita creams 1-4

Conclusion

„*intended for sensitive skin*" claim confirmed

no adverse skin reactions, no itching/burning recorded

Anti-inflammatory effects

- induction of reversible skin inflammation due to UV irradiation (solar simulator)
- application of the tested samples immediately after irradiation and subsequently twice a day (5 days)
- recording of erythema reactions
  grading 0, 1, 2, 3, 4;
  from no to severe erythema
- assessment of solar erythema inhibition

Number of volunteers: 20

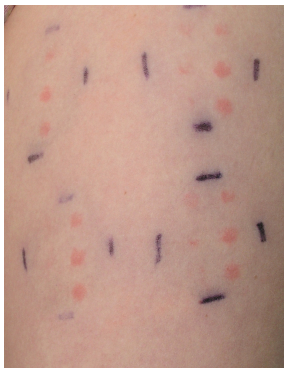
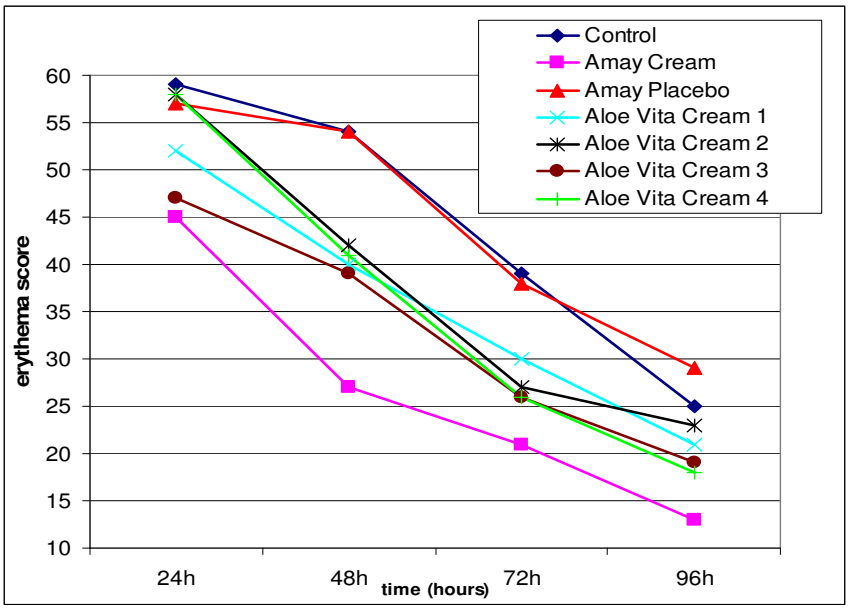


Figure 35: erythema reaction on skin

Figure 36: Results of anti-inflammatory effect test



Conclusion

anti-inflammatory effect proved all aloe vera creams, AMAY Cream best

Skin hydration

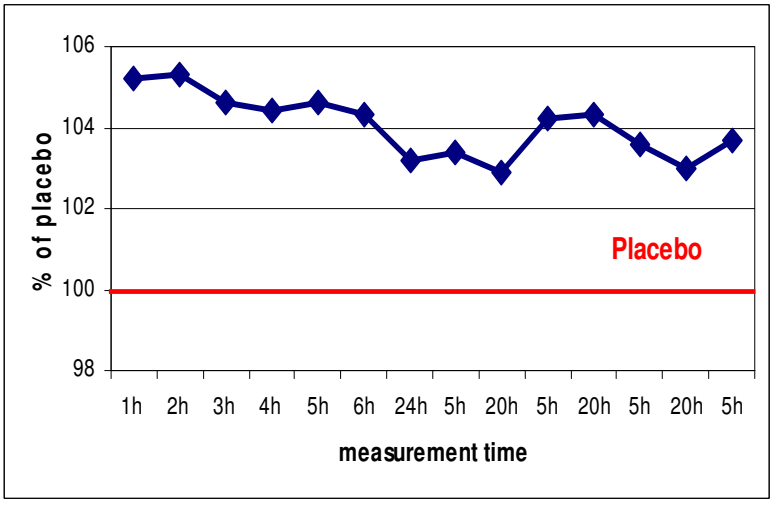
- repeated application on the volar forearm twice a day (5 days)
- measurement twice a day by means of Corneometer CM 820 (Courage-Khazaka)
- placebo = control

Figure 37: Corneometer

Number of volunteers: 30

AMAY cream

Figure 38: Results of skin hydration test with the skin milk developed by TTZ

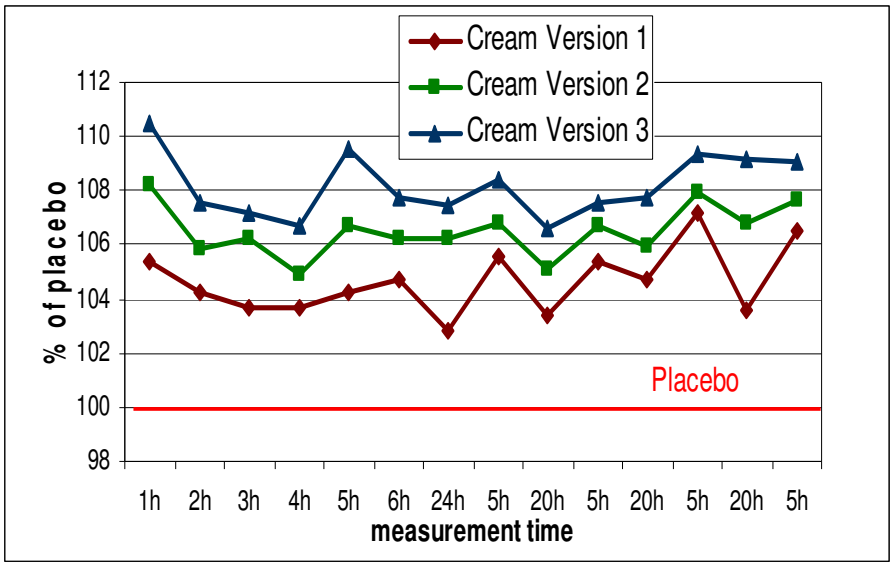


Conclusion:

Significant hydrating effect attributed to aloe vera content

ALOEVITA cream

Figure 39: Results of skin hydration test with the creams developed by GRADIENS



Conclusions:

Significant hydrating effect dependent on aloe vera juice content

Skin sebumetry

- single application on the volar forearm
- measurement before application and 1 to 6 h after application by means of Sebumeter SM 810 (Courage-Khazaka)
- control = untreated skin

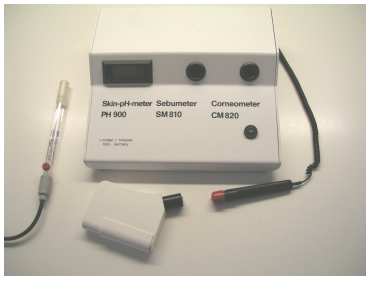
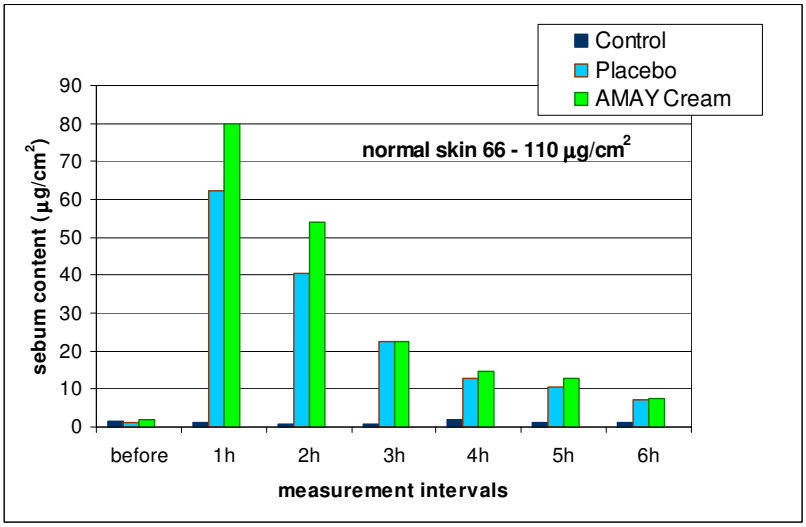


Figure 40: Sebumeter

Number of volunteers: 30

AMAY cream

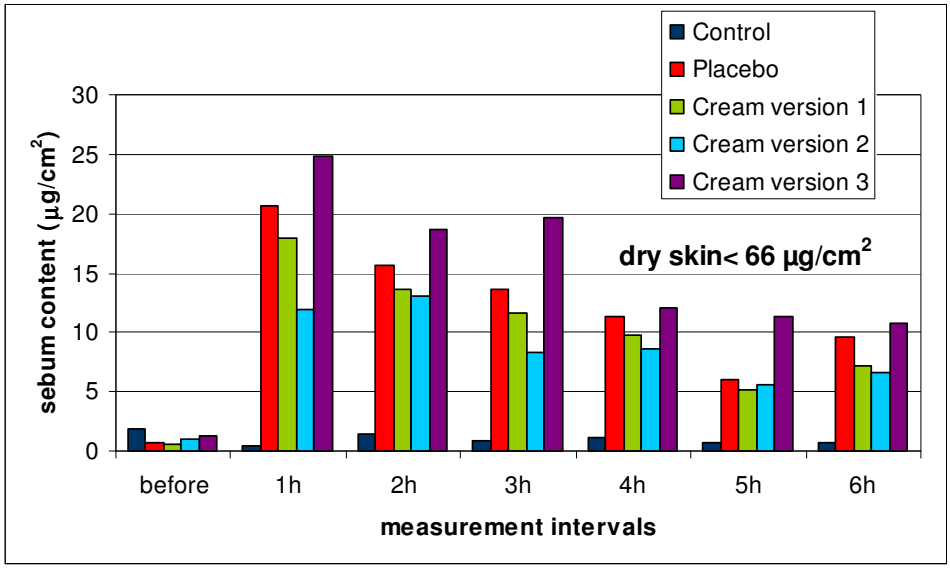
Figure 41: Results (sebum content mg/cm2) of skin sebumetry test for the skin milk developed by TTZ



Conclusion:
no significant increase of sebum content

ALOEVITA creams

Figure 42: Results (sebum content mg/cm2) of skin sebumetry test for the creams developed by GRADIENS



Conclusion:
no significant increase of sebum content

Skin surface pH

- single application on the volar forearm
- measurement before application and 1 to 6 h after application by means of Skin-pH-Meter PH 900 (Courage-Khazaka)
- control = untreated skin

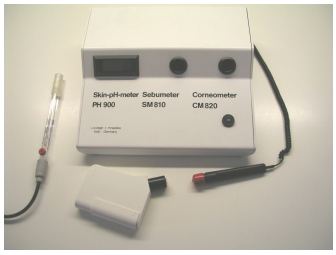
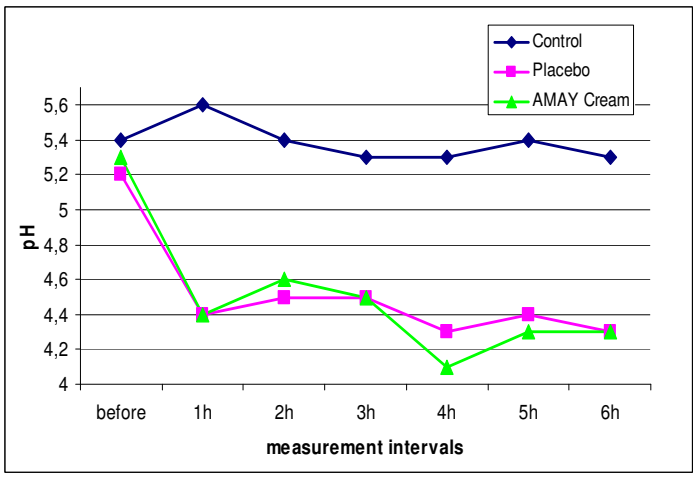


Figure 43: Skin pH-meter

Number of volunteers: 30

AMAY cream

Figure 44: Results pH values od the skin milk developed by TTZ

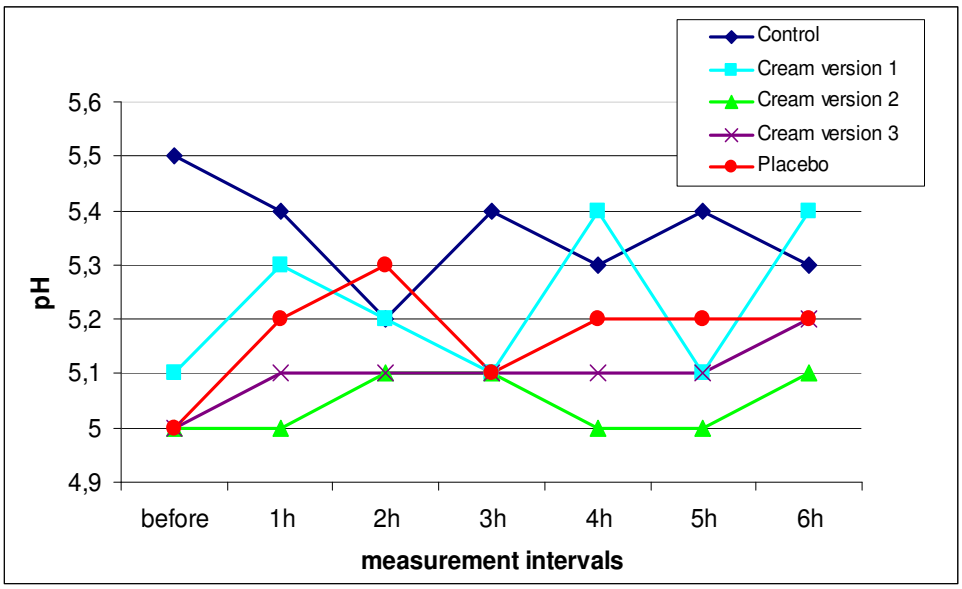


Conclusions:

both products induce lower skin surface pH supporting skin barrier functions

ALOEVITA creams

Figure 45: Results pH values of the creams developed by GRADIENS



Conclusions:

pH values not influenced significantly

Skin elasticity

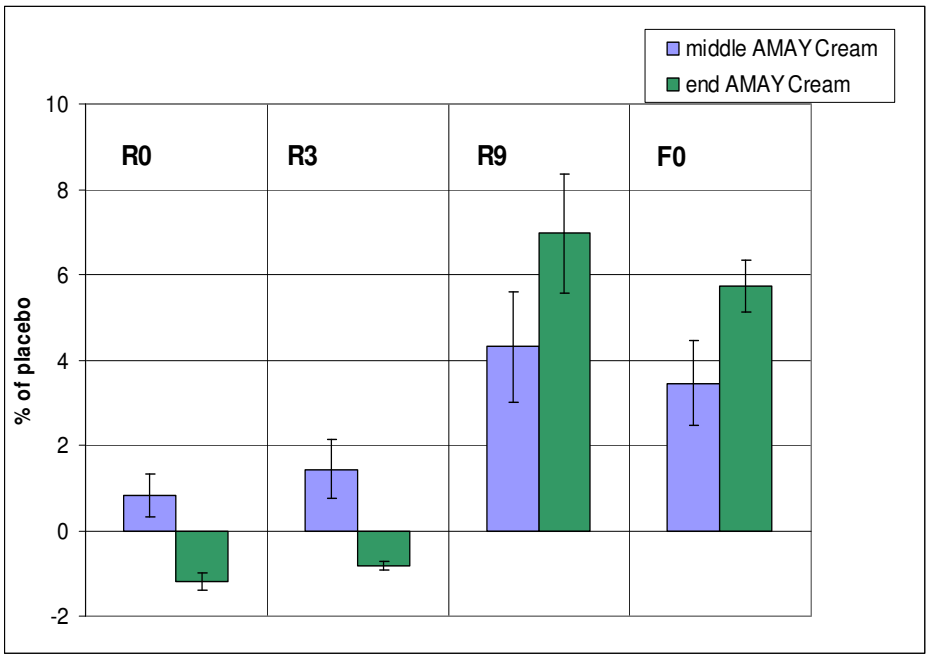
- repeated application on the volar forearm twice a day (5 days)
- instrumental measurement in the middle and at the end of the study by means of Cutometer SEM 575
  (Courage-Khazaka)
- measured parameters R0, R3, R9, F0
  characterize overall elasticity,
  fatigue and viscous coefficient of the skin
- placebo = control

number of volunteers: 30

Figure 46: Cutometer

AMAY cream

Figure 47: Results of the skin elasticity test for the skin milk produced by TTZ

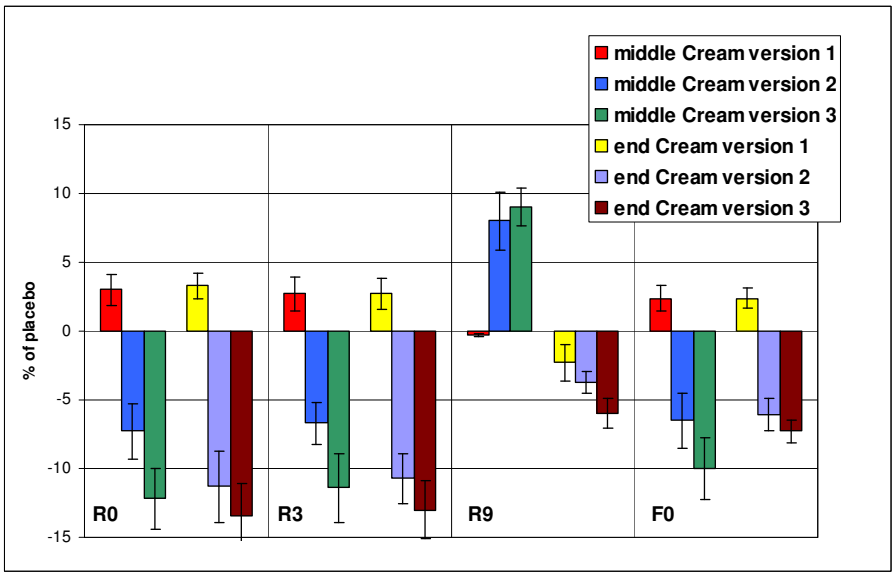


Conclusion:

slight improvement of overall elasticity (parameters R0, R3)

ALOEVITA creams

Figure 48: Results of the skin elasticity test for the creams produced by GRADIENS



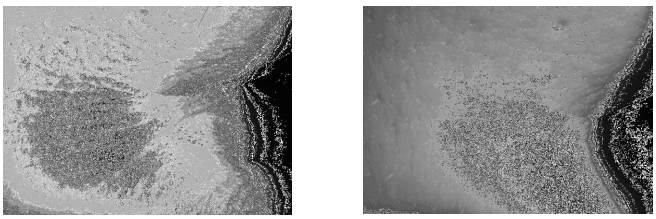
Conclusions:

Creams version 2, 3 significantly improve all elasticity parameters

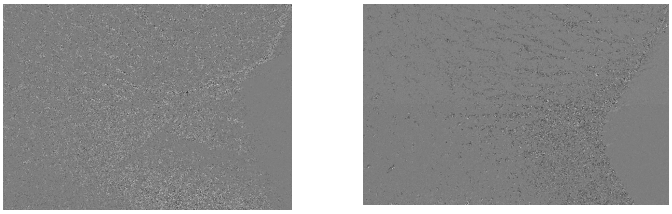
Anti-wrinkle effects

- repeated application on the face around the eyes twice a day, 4 weeks
- digital photographs of skin relief before the start and at the end of the study
- conversion of photographs into digital skin relief images
- digital image analysis by LUCIA Measurement SW

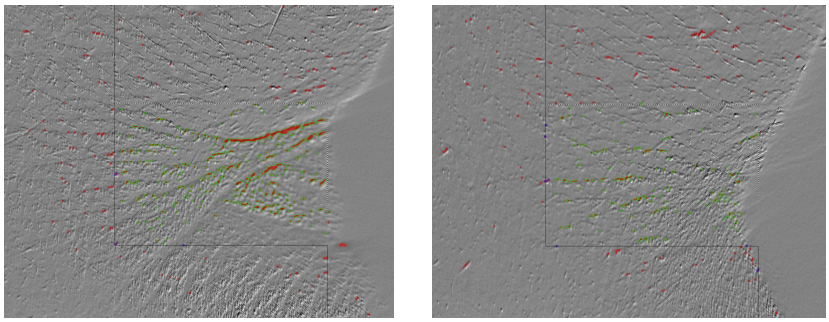
tested products: AMAY Cream and Placebo, Aloe Vita Cream version 3 and 4 (placebo)
number of volunteers: 20 for each sample



Figures 49 and 50: Digital images before and after application.



Figures 51 and 52: Skin relief images before and after application



Figures 53 and 54: LUCIA Measurement image before and after application

Table 20: Results changes in wrinkle parameters (%)

	ALOEVITA cream V3	ALOEVITA cream V4	AMAY cream	AMAY placebo
Reduction of wrinkle area	57%	42%	56%	48%

Conclusion
the proved anti-wrinkle effect is related to aloe vera content in Aloe Vita Cream version 3 and AMAY Cream

Results of dermatological tests:

As a conclusion after the dermatological tests performed, we can afirm that cosmetic creams with Aloe vera

- are **hypoallergenic**
- are **suitable for sensitive skin**
- exhibit **anti-inflammatory effect**
- **improve physiological skin parameters** (hydration, elasticity, skin pH)
- exhibit **anti-wrinkle effect**

The reports corresponding to all tests performed by partner SZU with human volunteers can be found in Annex XXV (D4.3 Dermatological testing results of final products)

Test of anti-cellulite effect of an Aloe vera gel:

Additionally, a cosmetic product containing 75-100% of Aloe vera gel was tested by SZU. The product is an anti-cellulite gel from SANTAVERDE, in which we wanted to assess the anti-cellulite effect complementary to the other effects to assess in the developed products.

The anti-cellulite effect was assessed by:

- measurement of thigh circumference (in cm)
- skin hydration (Corneometer)
- skin elasticity (Cutometer)
- visual evaluation of cellulite severity images (digital photography)
- questionnaire (self-assessment)

The tests were performed during 4 weeks in 30 healthy volunteers, females, age 26-59. The product was applied on one thigh twice a day after 5 min massage with a brush. As control, the second thigh was only massaged without product application.

An increase of the degree of skin hydratation was demonstrated. A statistically significant improvement of all viscoelasticity parameters was demonstrated comparing to the control sites massaged with the brush only. A visual clinical assessment of the photo documentation revealed a significant improvement of the cellulite appearance during the 4 week study. The product application resulted in a significant increase of thigh circumference at the site of application of the product pre-treated with a brush massage compared with the control sites.

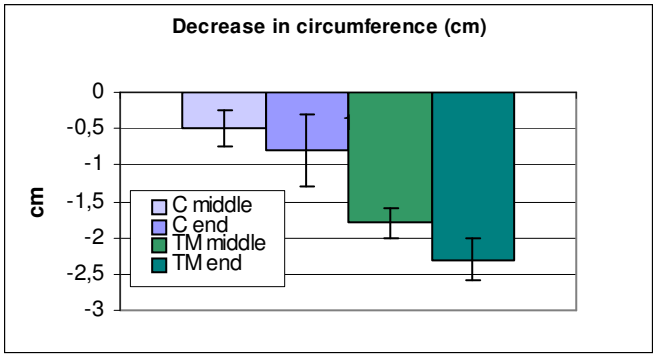


Figure 55: Measurement of thigh circumference

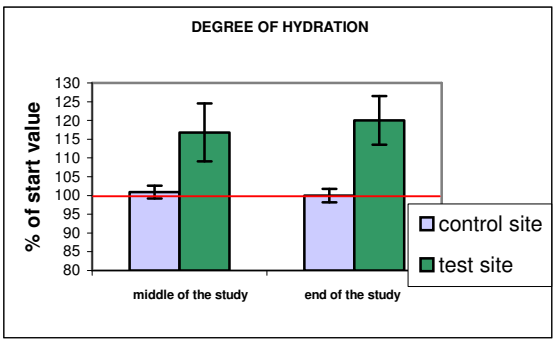


Figure 56: Skin hydration

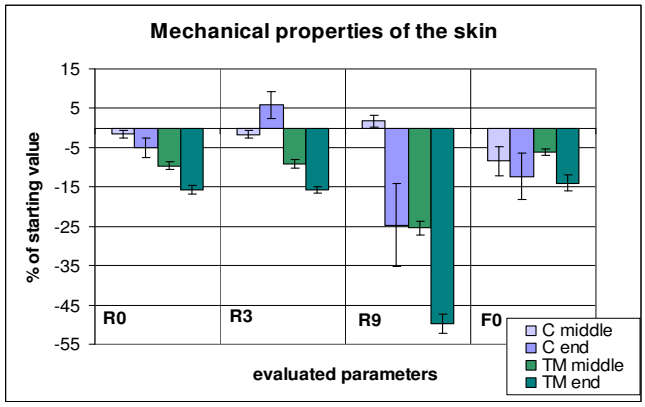


Figure 57: Skin elasticity

The visual evaluation of the cellulite severity[6] was a blind-study – an independent dermatologist evaluated digital images taken before the start and at the end of the study. This showed the following results: 66.7% improved 1 grade, 20% improved 2 grades. In 13,3% of the cases, no changes were found.

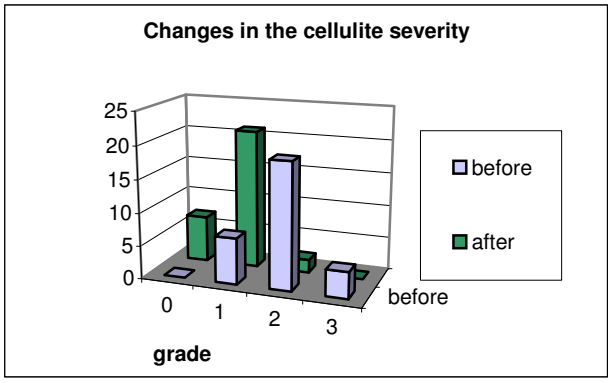


Figure 58: visual evaluation of cellulite severity images

[6] Grading of cellulite severity:

0 no changes, smooth surface

1 dimples visible only when pressed

2 orange peel skin appearance, visible minor dimpling when standing

3 moderate to severe dimpling on the whole test area

The results of the subject self-assessment questionnaire confirmed the data obtained by instrumental methods. 50% of the volunteers reported an improvement in cellulite appearance. 66.7% reported less rough, more silky skin, while 33,3% found a better contour of thigh. None of the volunteers reported any adverse effects (itching, burning).

The complete tests reports for the anti-cellulite gel can be found in *Annex XVII*

Volunteers' self-assessment of anti-wrinkle and anti-cellulite effect of cosmetic products

Additionally, partner TOPORIINA carried out a volunteers' self-assessment of anti-cellulite effect and anti-wrinkle effect of Aloe Vera in their beauty salons (see *Annex XXVI*).

The anti-cellulite study involved 50 volunteers. To make results more comparable with the study made by partner SZU (Test Report dated 6.1.2006) TOPORIINA used the same questions in the questionnaire. The results of this self-assessment questionnaire confirmed the results with SZU's study: after 4 weeks of treatment with the test gel the product was reported to show improvement in cellulite appearance by 43,5 % of the volunteers.

Additionally, TOPORIINA performed another test of one the product developed by GRADIENS (cream incorporating 23% the juice of the Aloe) to assess an anti-wrinkle effect against placebo. The study involved 50 volunteers and had duration of 4 weeks. 25 volunteers used the cream while the other 25 were provided with the placebo cream. The cream was applied twice a day (i.e. in the morning and in the evening) on the 'wrinkle area' of the clean face and décolleté. The results of this self-assessment questionnaire do not show much positive anti-wrinkle effect, especially when you take into consideration the results with placebo creams. A decrease of wrinkles was reported to be better with placebo cream: 66,6 % of the volunteers that had been using the placebo cream said that the product had some or a lot of anti-wrinkle effect, whereas 41,7% of the volunteers that had been using the Aloe cream affirmed the product had some or a lot of effect.

When looking at these results it has to be taken into account that it is a subjective evaluation and that the consumer expectations play a very important role in the individual's judgement towards the product.

Achievements

Different Aloe vera fractions obtained in WP3 were tested in-vitro in skin cell systems with regard to their cytotoxicity and their protection effect against a standard irritating agent (SDS). The tested materials represent extremely low cytotoxicity of the tested materials. The results suggest very good compatibility of the tested samples with human skin or mucosa in vivo.

In-vitro tests of different ratios of Aloverose, malic acid and glucose regarding their cytotoxicity, as well as their protective effect against tenside and UV threw some interesting results of the synergy of the 3 main components of the Aloe vera gel. Further research on the effect of these components in vivo might be useful to completely understand their synergy.

Three different cosmetic products were developed. One contained the isolated Aloe vera fraction (Aloverose) and was a natural cosmetic formulation. Some tests were performed until the cream became unstable. A second cosmetic product was developed that also incorporated the powder fraction. A non natural cosmetic formula was used in order to gain in stability and be able to perform all envisaged dermatological tests on human skin. The third product was produced with the pure gel (juice) in 3 different versions with 3 different percentages of juice. For all 3 products a duplicate not containing Aloe vera was produced, to be used a placebo for the dermatologic tests.

The in-vivo tests of the biological effects of the creams developed show that they are hypoallergenic, suitable for sensitive skin, exhibit anti-inflammatory effect, improve physiological skin parameters (hydration, elasticity, skin pH) and exhibit anti-wrinkle effect.

The dermatological tests of the Aloe vera anti-cellulite product show a significant improvement of this skin disorder when the product is applied in a thigh after massage with a brush comparing to the control (massaged only, without product application).

List of deliverables:

All deliverables from WP4 have been achieved at due time or slightly sooner or later.

	Due Date	Actual Submission date	Lead Contractor
D 4.1 Recipes of novel cosmetic products containing Aloverose	December 2006	January 2007	GRADIENS, TTZ
D 4.2 Samples of novel cosmetic products containing Aloverose	December 2006	January 2007	GRADIENS, TTZ
D 4.3 Dermatological testing results of final products	April 2007	March 2007	SZU

List of Milestones:

	Due Date	Actual Submission date	Lead Contractor
M 4.1 Delivery of cosmetic products fulfilling the dermatological testing requirements	April 2007	March 2007	GRADIENS

Deviations/Remarks

The tested anti-cellulite gel was not a product developed under the frame of the AMAY project, but formerly developed by the SME partner SANTAVERDE. Taking into account the high content of pure aloe vera juice contained in it, the partners considered its testing and the subsequently results are of the highest interest for the AMAY partners, being able to assess an additional effect of the Aloe vera (anti-cellulite) derived from its high content in Aloverose. RTD partner SZU agreed on carrying out the tests. The performance of this activity in no case means the negligence of any important activity of the AMAY project (deliverables, milestones, work-packages).

Partner SZU also tested alfa-D-glucose, 99% pure Aloverose and D1-malic acid in order to assess in vitro the effect of the 3 compounds separately and also in different combinations. In vitrotoxicity, as well as protective effect against standard tenside (Sodium Dodecyl Sulfate SDS) and toxic effects of UVA radiation. The project partners consider these tests important to understand the synergist effect of the main components contained in the Aloe vera gel (Aloverose, glucose and malic acid) in comparison to the effect of Aloverose isolated.

The development of cosmetic products by partner TTZ was not contemplated in the DoW but allocated to GRADIENS. GRADIENS carried out the development of a cosmetic product as defined in the contract. The development of a second product was taken over by TTZ due to technical issues that could not be solve by partner GRADIENS. All partners agreed on this at the project mid-term meeting.

2.5 Work Package 5: "Project management, dissemination and pre-exploitation measures"

WP leader**: SANTAVERDE**

The objective of this WP is to ensure effective Project Management and co-ordination, to ensure that the partners work as a cohesive team in the tasks assigned to them in WPs 1-4, as well as to communicate to the public and companies outside the consortium

Task 5.1 General project management

A specific and detailed description about actions concerning management can be found later on in this report (Section 3 - Consortium management)

Task 5.2 Dissemination strategy and knowledge protection

Dissemination activities developed during the first year include the design and update of the AMAY website (http://www.insidealoe.de), which contains extensive public information about the project. SANTAVERDE was in charge of the webpage construction The AMAY website is linked to all partners' websites. Some of the partners´ website contains additionally some information about the AMAY project in the respective languages.

Figure 59: AMAY website

In addition, further dissemination tools have been developed by TTZ, always after unanimous revision and subsequent approval by all partners:

AMAY flyers have been designed for being used as general dissemination tool and in fairs, seminars, conferences and other dissemination activities. The flyer constitue a project deliverable (D5.3) and can be found in *Annex XXVIII*

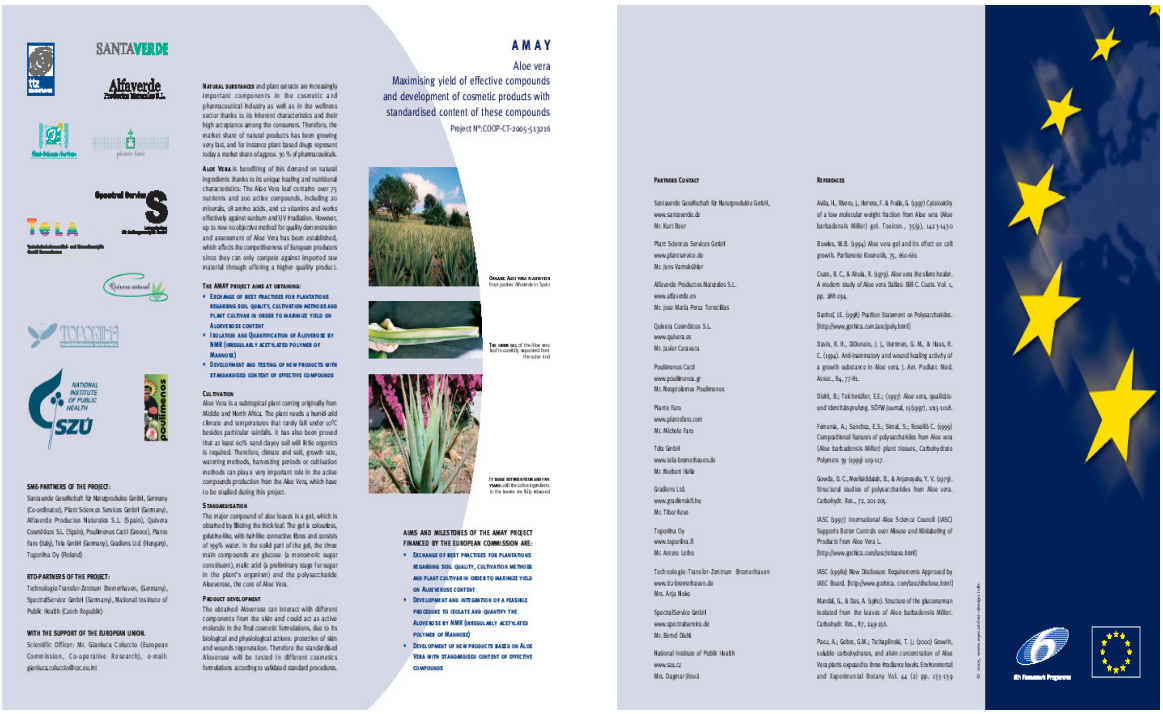


Figure 60 AMAY Flyer

Events attended by AMAY partners

Partner ttz attended the ISA (International Sea buckthorn Association) Conference in Beijing 2005, gathered together many natural cosmetic companies, which not only produced Sea Buckthorn based cosmetics and food products, but in many cases also based on Aloe Vera. Therefore, dissemination of the AMAY project was possible at this conference.

ISA 2005 International Conference on Sea Buckthorn

Beijing (China) - 26-29th September 2005

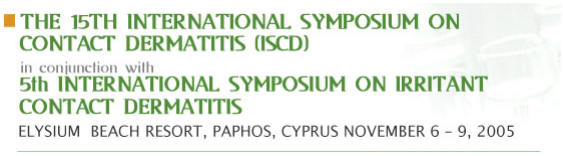
Partner SPECTRAL presented and hosted the 4th Aloe Vera seminar in September 2005 in Cologne. The seminar was exclusively given for Spectral Service customers, presenting the latest progress in analytical procedures, definition and fixing of quality specifications of Aloe Vera products and providing a communication platform between purchasers and producers.

Aloe Vera Seminar

Cologne (Germany) –12th September, 2005

www.spectralservice.de

In November 2005, partner SZU attended the International Symposium on Contact Dermatitis, an international forum of specialists in the field of clinical experimentation. This meeting offered the opportunity to discuss human skin compatibility and efficacy studies design, technique and interpretation of results in regard to the tested Aloe Vera components in the AMAY project.



In February 2006 partners ttz and Santaverde attended the BioFach fair, the world largest and most important fair for the organic sector (food and cosmetic products), where the AMAY project objectives were disseminated among interesting attendances. It was a chance to get in direct contact to the global trade and becoming aware of the needs of tomorrow's customers with innovative ideas, creations and concepts, as AMAY is.

BioFach (International fair of organic products)

Nürnberg (Germany) –26-29th February 2006

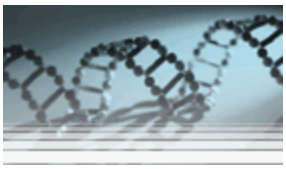
http://www.biofach.de

In March 2006, SZU partner took part in the European Tissue culture Society Meeting, held in Verona, in order to discuss and gain information on the mechanisms of cell cytotoxicity, mainly the beneficial effects attributed to Aloe Vera, the effect of plant extracts against cell membrane damage or the relevance of protective effects assessed in vitro and proved in vivo.

Additionally, partner ttz visited the Anuga Foodtec and got in contact with international representatives of the food and drink technology. The Anuga Foodtec is the leading information and acquisition platform of the food industry, and offers experts and professionals coming from the commercial management and the engineering and construction field the opportunity to exchange knowledge about food innovation trends.

Anuga FoodTech
Cologne (Germany) - April 4-7th 2006
http://www.anugafoodtec.com

Partner TELA attended the Analytica 2006, which every two years offers professionals in the fields of environmental, food and industrial analytics, biochemistry, biotechnology, molecular biology, medicinal diagnostic and pharmacology an international concourse to exchange know-how on new products and technologies.



Analytica (20th International Trade Fair and Analytica Conference)
Munich, Germany –April 25-28th , 2006
http://www.analytica-world.com

Partner TTZ also visited the **Vitafoods International 2006** in Geneve, which is one of the leading European exhibition for the **nutraceutical industry**. Over 300 of the leading suppliers from 39 countries converged at this event, which is a truly International event. Vitafoods International 2006 Exhibition combined the areas of nutraceuticals, cosmeceuticals, functional foods and drinks presented by manufacturers, distributors, marketing professionals, product developers and scientists. The Finished Products Expo run alongside Vitafoods International and welcomed over 60 leading suppliers of dietary supplements and functional foods & drinks.

Vitafoods International 2006
Geneve, Switzerland- May 9-11th, 2006
http://www.vitafoods.eu.com/

In May 2006 the 3rd **Central European Congress on Food** (**CEFood 2006**) took place in Sofia, Bulgaria, providing an open international forum where academics and stakeholders from across Europe and around the world can propose knowledge-based solutions of the current problems by presenting and discussing recent research findings, developments and trends in the sustainable production, preservation and supply of healthy and high-quality

foods and beverages. The congress gave scientists and practitioners an opportunity to meet colleagues and share ideas that may shape food research and engineering for years to come. TTZ was present at this Congress and carried out some dissemination of the AMAY project.

CEFood 2006

Sofia, Bulgaria- 22-24 May 2006

http://www.cefood.org/

In September 2006, partner TTZ visited the Intercool fair. **Intercool** one of three agreed food fairs Exhibitors (InterMopro, InterCool and InterMeat), **International Trade Fairs for Dairy Products, Frozen Food and Ice Cream as well as Meat and Processed Meat**. More than 965 firms presented a wide range of products and offered experts from the retail trade new impulses for designing their ranges.

InterCool 2006

Düsseldorf, Germany – 24-27 May 2006

http://www.intercool.de

In June 2006, partner SZU attended the 13th Congress on Alternatives to Animal Testing and 10th Annual Meeting of MEGAT - Middle European Society for Alternative Methods to Animal Testing, where new and progressive alternative methods are presented by leading European scientists. The possibility of testing beneficial effects of ingredients, particularly Aloe Vera, and the objectives of AMAY project were discussed with experts in the field of in vitro methods.

Partner SPECTRAL organized the 1st Aloe vera Symposium July 2006 in Cologne. The symposium gathered many experts who gave presentations on different topics related to Aloe vera (analytic, pharmacologic research, botany, etc). Partner TTZ presented the AMAY project to the audience.

1st Aloe Vera Symposium

Cologne (Germany) –21st July, 2006

www.spectralservice.de

The 14th International Workshop on In Vitro Toxicology, held in October 2006, is a world conference held every two years, which offers researchers from academia and industry a forum to improve knowledge and discuss new concepts and opportunities in the in vitro technology. The scientific programme included state-of-the-art lectures, workshops, original communications and poster sessions, where partner SZU had an opportunity to gain new information and discuss some issues of the AMAY project..

IBA is the **International Trade Fair World Market for Baking**. IBA plays for the entire baking sector an important role and has leading significance as an exhibition event for bakers and confectioners. In the frame of this exhibition, partner TTZ performed some dissemination for the AMAY project. Between others, there is a potential for the application of Aloe vera for baking products.

IBA 2006
3.10. - 9.10.2006
http://www.iba.de

Partner TTZ attended the Life Sciences Forum 2007 in Munich. This represented an opportunity to do a wide dissemination of the AMAY project and to gain interesting contacts among the industry as potential end-users. Additionally, TTZ established some contacts with academic institutions that would be willing to collaborate in the project by providing the last state of research on the field. The Congress and accompanying exhibition formed a highly attractive meeting point for information from science and industry on latest results and emerging trends. It also served as an interactive platform for establishing new contacts to national and international partners and customers for future innovations. The event counted with the attendance of 1000 participants from 20 countries and more than 65 speakers from 11 countries. The accompanying exhibition counted 110 exhibitors

Forum Life Science 2007
Technical University of Munich, Garching
14. /15. February 2007

Partners SANTAVERDE and TTZ visited the BioFach Fair again in February 2007. With more than 45.520 visitors, 21% more than in 2006, BioFach represents the most important event in Europe for organic products and natural cosmetics. The two partners again had the chance to perform a wide dissemination of the AMAY project among the exhibitors and visitors

BioFach (International fair of organic products)
Nürnberg (Germany) –26-29th February 2006
http://www.biofach.de

A significant number of cosmetic companies Europe-wide could be potential users of the technology developed within the AMAY project. Since the beginning of the project, the project partners have carried out extensive dissemination among stakeholders. The project dissemination carried out in fairs and exhibitions has been particularly a very suitable scenario for the establishment of relevant contacts among the industry.

Publication of the scientific results is planned, provided that all SME partners fully agree on it.

Other dissemination measures are found in the deliverable "D5.5 Provisional Plan for using and disseminating the knowledge" attached to this report (*Annex XV*)

Achievements

	Due Date	Actual Submission date	Lead Contractor
D 5.1 Website	September 2005	August 2005	SANTAVERDE
D 5.2 First progress report (6 months)	October 2005	October2005	SANTAVERDE
D 5.3 Project Flyer	November 2005	August 2005	SANTAVERDE
D 5.4 Mid term review report	May 2006	May 2006	SANTAVERDE
D 5.5 Provisional Plan for using and disseminating the knowledge	May 2006	May 2006	SANTAVERDE
D 5.6 Second progress report (month 19)	November 2006	November 2006	SANTAVERDE
D 5.7 Final plan for using and disseminating the knowledge	April 2007	April 2007	SANTAVERDE
D 5.8 Final reports	April 2007	April 2007	SANTAVERDE

List of Milestones:

	Due Date	Actual Submission date	Lead Contractor
M 5.1 Mid-term assessment report delivered	May 2006	May 2006	SANTAVERDE
M 5.2 Final review delivered	April 2007	April 2007	SANTAVERDE

Deviations/Remarks

No deviations occurred

Deviations from the Work Program

No significant deviations from the original Gantt Chart planned have occurred. Only the time schedule has been slightly modified concerning the start of WP 2 (Comparison of the Aloe vera cultivation) and 3 (Purification and standardisation of active compounds). Both WP have been brought forward in order to start simultaneously with WP1 (Definition of requirements) and optimise the development of the foreseen tasks within the WPs regarding sampling time, sample delivery and collection of results. This was agreed by all involved partners at the kick-off meeting and permitted a large discussion at the 6 months technical meeting with the available results as well as at the mid-term meeting.

Additionally, WP4, scheduled from month 17, started in month 6. The partners involved in the development and the testing of the cosmetic products foreseen in AMAY agreed on starting the task as soon as the material produced in WP3, ingredient for the cosmetic product, would be available (see mid-term meeting minutes). The dermatological test of the products started subsequently right after the product production, also a bit sooner than foreseen in the workplan.

Although their involvement was not foreseen in the DoW, partner TTZ assisted the partners responsible of the tasks 4.1 and 4.2 (development of cosmetic products). TTZ possesses great experience in the field of cosmetic development and their assistance made possible to tackle the technical problems that had arise. This in no means implied the non compliance of any task, deliverable or milestone and did not displace other partner’s work.

Section 3 – Consortium Management

Many actions related to the management activities during the whole project duration have been undertaken. A very important action was the launching the AMAY project in order to introduce the partners to each other, establish links between work packages members and organise the upcoming tasks for the first months. For this purpose, a Kick-off meeting was organised by partners SANTAVERDE (and subcontractor BIOZOON) and TTZ, as described in the later in this section.

Other related actions derived from the management activities within the project have been the development of the 6 month interim activity report, corresponding to D 5.2 First progress report; the periodic report (mid-term report), corresponding to the Deliverable 5.4; the Second progress report (D 5.6) and at last the 2nd periodic report (Final report), which constitute the D 5.8 . After 6 project months a progress meeting among the partners involved into the running tasks was organised in order to discuss the situation and results so far and the required actions in the future. The project mid-term meeting was organized in month 12 (April 2006) and the final meeting at month 24 (end of March 2007).

During the whole duration of the project a continuous communication flow was maintained among the members of the Consortium. Fluent information share has been provided by meetings, telephone, fax, e-mailing and post. Furthermore, active discussions took place during partner meetings. SANTAVERDE, as general co-ordinator responsible of the overall management of the project, assisted by Biozoon and by TTZ, has been responsible of collecting all relevant results derived from the tasks leaders, meeting preparations, partner communication, and other administrative and financial issues as well as the scientific/ technical and IPR management.

A provisional plan for using and disseminating the knowledge (D5.5) was available after 12 months. A final version can be found attached to this report (D5.7).

In addition, support of all partners has been received for the elaboration of this activity report and all related documents to be presented to the European Commission. The project mid-term meeting was organized in Budapest (Hungary) at GRADIENS´ facilities in April 2006. The partners took the chance to organise a scientific cosmetic workshop alongside the meeting (see minutes in *Annex XIX*). The final meeting was hosted by partner SZU in Prague on 30th March 2007. The minutes of all official and technical meetings were compiled by the partner in charge and distributed to all partners for their information. The minutes of the kick-off, mid-term and final meeting can be found as annexes in this report (*Annexes I, XVIII, XXXII)*

Contractors/ Communication

Intensive communication activities took place between the consortium members during the project. Contributions from partners to the overall consecution of the project, active discussions and fluent information share has been provided within technical meetings, telephone conversations, fax, e-mailing and current post, specially between the workpackage leaders and the RTDs and the partners responsible of the technical and scientific work in the AMAY project.

In order to ensure a smooth running of the project and address any technical difficulties encountered along with the execution of the work, several meetings were organised when necessary, where problems were intensively discussed to find possible solutions:

During the progress of the project three general official meetings were held:

- **Kick-off meeting** *(15th April 2005)*

This meeting marked the beginning of the project and took place in Hamburg, Germany, at the coordinator's facilities (SANTAVERDE), on the 29th April 2005. It was attended by all partners of the AMAY consortium excepting ALFAVERDE and PIANTEFARO, who could not attend. The minutes of the meeting are attached to this report (*Annex I)*. During the event, the AMAY partners made clear the objectives of the work to be performed, discussed and accurately planned the tasks for the first 6 months.

- **6 month progress meeting**: *(22nd August 2005)*

TTZ hosted a meeting at their facilities in Bremerhaven (Germany) on the 22nd August 2005, 6 months after the beginning of the AMAY project. All partners involved in the tasks performed within the first 6 months attended this meeting (SANTAVERDE, TTZ, BIOZOON, PLANTSCIENCE, SPECTRAL and TELA). During this meeting the tasks performed by each partner during the first 6 months project life were presented by the responsible partner for discussion, technical problems were also discussed and further steps for the next 6 months were defined.

The minutes of the 6 month meeting can be found attached to this report as A*nnex VII.*

- **Mid-term meeting** *(20th April 2006)*

This meeting was held in Budapest, Hungary, at GRADIENS´ facilities, on the 20th April 2006. It was attended by most partners of the AMAY Consortium. The minutes of the meeting are attached to this report (*Annex XVIII)*. The results of the first project year work were presented and the work plan for the next year was extensively discussed.

A scientific cosmetic workshop for the project partners was held alongside the mid-term meeting on 21 April 2006. The minutes of the workshop can be found in *Annex XIX.*

- **Final meeting** *(30th March 2007)*

The project final meeting took place in Prague and was hosted by the RTD partner SZU on the 30th March 2007. The minutes can be found in *Annex XXXII.*

Additionally, several **technical meetings** took place between partners involved in specific tasks to exchange ideas, define procedures, solve problems and assure a cooperative supportive work between partners at any time.

- **Technical meeting 13th June 2005,**

This meeting took place in Hamburg (Germany) between partners SANTAVERDE, PLANTSCIENCE and TTZ. The aim of this meeting was to discuss the first available laboratory results from the soil and the leaves analyses.

- **Technical meeting 1st September 2005,**

Partner TTZ visited PLANTSCIENCE in Westerkappeln (Germany) with the aim of discuss the results of the soil analyses in combination with the leaves analyses to assess the results with the last data given by the growers about their plantations.

- **Technical meeting 13th September 2005.**

It took place in Prag (Czech Republic) at SZU´s facilities SANTAVERDE and TTZ visited SZU in order to discuss the performance of in vitro as well as in vivo test of the Aloe vera fractions which were being produced (WP3 and WP4), the quantitative requirements for samples an possible outcomes.

- **Technical meeting 28th November 2005**

SANTAVERDE, TTZ, SPECTRAL and Biozoon met in Hamburg (Germany). The aim of this meeting was to discuss the technical difficulties found by SPECTRAL and TTZ to work with some of the starting materials for the isolation of the active compounds as well as the further difficulties found during the in vitro tests by SZU, mainly caused by a problem of solubility. The involved partners developed a strategy to overcome these problems.

- **Technical meeting 15th May 2006**

Partners SANTAVERDE, TTZ and SPECTRAL met in Hamburg (Germany) in May 2006. The aim of this meeting was to discuss the running of WP4 dealing with the development of cosmetic products and their dermatological tests.

- **Technical meeting 10th April 2007**

Partners TTZ and SANTAVERDE met in Hamburg (Germany) in order to make clear some IPR issues and the publication of project results.

The minutes of some of the project technical meetings are included in *Annex XXII*

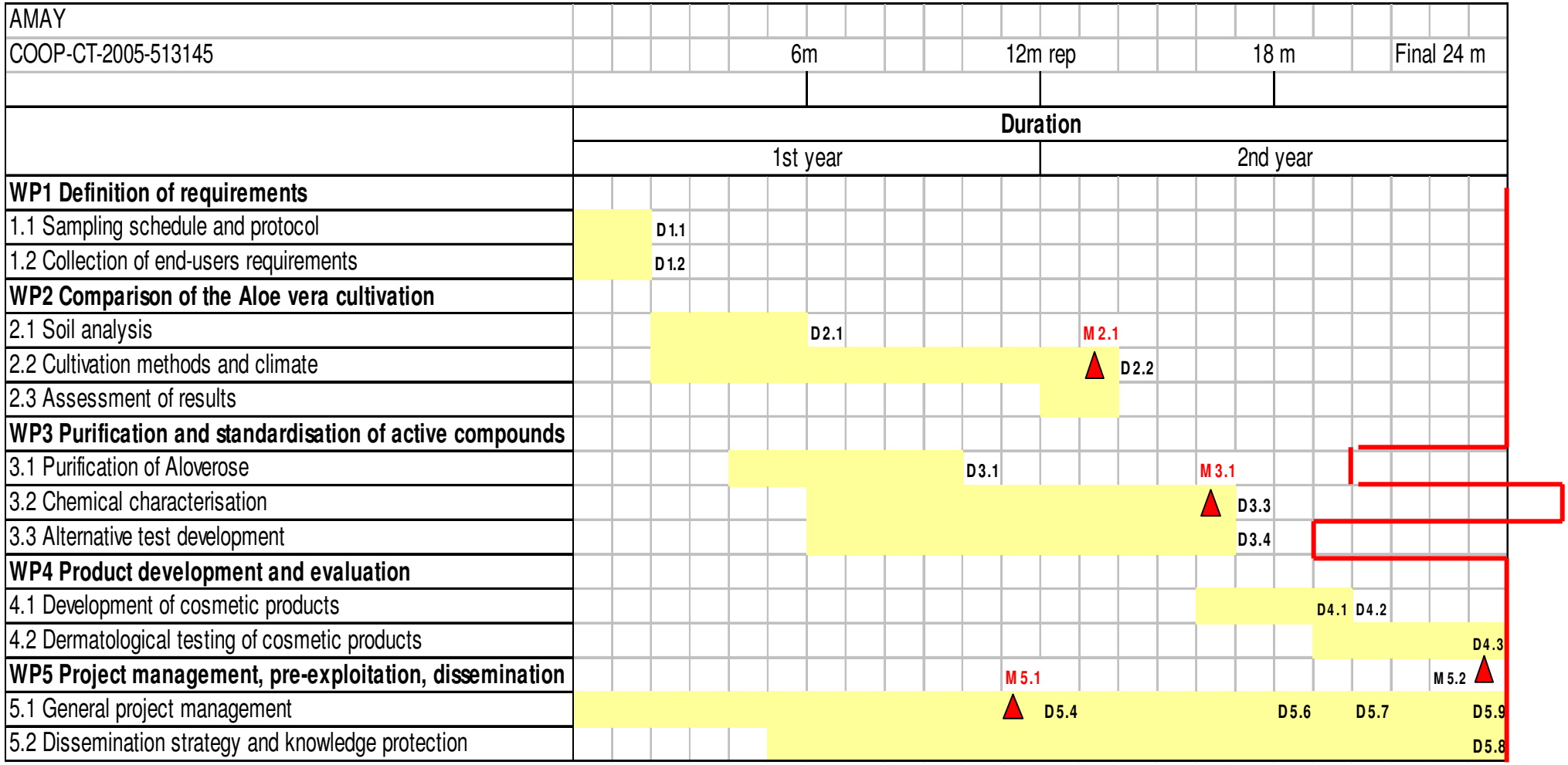
Project timetable and status

During the project, the consortium performed some rearrangements to the initial Work Plan in order to optimise the effectiveness of the work. The main modification, which is mainly related to the WPs timetable, was performed during the first 6 month of the project and already contemplated in previous reports. Such modification supposed the change of the scheduled timetable for WP2 and WP3. The partners agreed during the kick-off meeting that the related tasks could be brought forward since the test materials (Aloe vera leaves) could be available for testing soon and having the result of their analyse in an early stage would facilitate the performance of other tasks in both WP. Additionally, this permitted more results to present and therefore a richer discussion at the progress meeting after 6 months.

Additionally, WP4, scheduled from month 17, started in month 6. The partners involved in the development and the testing of the cosmetic products foreseen in AMAY agreed on starting the task as soon as the material produced in WP3, ingredient for the cosmetic product, would be available (see mid-term meeting minutes, *Annex XVIII*). The dermatological test of the products started subsequently right after the product production, also a bit sooner than foreseen in the workplan.

These rearrangements on the time schedule in no case meant the non performance of any activity, objectives, deliverables, milestones or budget envisaged in the AMAY project according to the DoW, they facilitated the work and permitted a better performance by all partners.

Graph 1 – Project Barchart and status.



The following tables list the deliverables and milestones to be developed during the project, which have been achieved.

DELIVERABLES:

Table 21: List of Deliverables

WP No	Del No	Deliverable name	Date due	Actual/Forecast delivery date	Lead contractor	Nature	Status
1	1.1	Sampling protocol	2	2	**SANTAVERDE**	R	✓
	1.2	End-users requirements	2	2	**SANTAVERDE**	R	✓
2	2.1	Soils analysis results	6	5	**TTZ**	R	✓
	2.2	List of different cultivation methods	14	12	**TTZ**	R	✓
3	3.1	Protocol for the Aloverose purification and NMR analysis	10	10	**SPECTRAL**	R	✓
	3.2	Protocol for carrying out HPLC analysis for Aloverose	14	17	**TELA**	R	✓
	3.3	Report on the NMR analysis for Aloverose	17	22	**SPECTRAL**	R	✓
	3.4	Report on the Aloverose certification quick-test	17	17	**TELA**	R	✓
4	4.1	Recipes of novel cosmetics products containing Aloverose	20	20	**GRADIENTS**	R	✓
	4.2	Samples of novel cosmetics products containing Aloverose	20	20	**GRADIENS**	O	✓
	4.3	Dermatological testing results of final products	24	24	**SZU**	R	✓
5	5.1	Website	6	4	**SANTAVERDE**	PU	✓
	5.2	First progress reports (after month 6)	7	7	**SANTAVERDE**	R	✓
	5.3	Project flyer	8	4	**SANTAVERDE**	PU	✓

	5.4	Mid term review report (after month 12)	13	13	**SANTAVERDE**	R	✓
	5.5	Provisional Plan for using and disseminating the knowledge	13	13	**SANTAVERDE**	R	✓
	5.6	Second progress reports (after month 19)	19	19	**SANTAVERDE**	R	✓
	5.7	Final Plan for using and disseminating the knowledge	24	24	**SANTAVERDE**	R	✓
	5.8	Final reports (after month 24)	24	24	**SANTAVERDE**	R	✓

Keys: R = Report; **O** = Other; **PU** = Public

MILESTONES:

Table 22: List of milestones

WP No	Mil No	Milestone name	Date due	Actual/Forecast delivery date	Lead contractor	Status
2	2.1	Assessment report	14	13	**TTZ**	✓
2	2.2	Samples of plants from the different Aloe vera cultivators to be analysed	14	14	**-**	✓
3	3.1	Isolation of the Aloverose	17	17	**SPECTRAL**	✓
4	4.1	Cosmetic product developed and tested	24	24	**GRADIENS**	✓
5	5.1	Mid term assessment report	12	12	**SANTAVERDE**	✓
5	5.1	Final report	24	24	**SANTAVERDE**	✓

Section 4 – Other Issues

4.1 CONTRIBUTION OF THE PARTNERS TO EVERY WORK PACKAGE

All partners, SMEs as well as RTDs worked together in close cooperation. Problems were discussed during the meetings and by telephone and e-mail.

The amount of work and resources of the single partners in the first period of the project was balanced. The work performed by the partners was in accordance to their human and technical resources.

The SMEs GRADIENS and TOOPORINA together with SANTAVERDE have contributed mainly with the definitions of the requirements (WP1). They set their expectations regarding the cosmetics to be developed in the AMAY project: product requirements, desired biological activity of the Aloe ingredient, its price and its application in cosmetic products. SANTAVERDE as coordinator is contact person for any queries and carries out the project management activities assisted by BIOZOON (WP5). They established the project website (WP5).

The SMEs ALFAVERDE, PLANTSCIENCE, PIANTEFARO, POULIMENOS and QUIVERA, all of them Aloe vera growers, are mainly involved in providing the samples for the analyses, planting the Aloe vera clones at different conditions and the discussion about the cultivation methods derived from the analyses results, for which they brought their broad experience in the cultivation of Aloe vera. All of them played a very important role in the project by providing material and information on their plantations and climatic conditions and they were strongly interested in the outcome of the project regarding cultivation recommendations to maximize the yield in bioactive compounds in Aloe vera. PLANTSCIENCE contributed with their extensive expertise in the field.

The three RTDs involved in AMAY (CZU, SPECTRAL and TTZ) contribute to the project with their knowledge and expertise as well as technical equipment. They all worked in close cooperation between them and with the SMEs. The three RTDs were mainly involved in the analyse of soil and Aloe vera leaves (WP1 and WP3), sample preparation purification of active compounds and their analyse (WP3) and dermatological testing (WP4). The three partners are also, like the rest of the partners, involved in WP1 and WP5. TTZ prepared the project flyers.

SANTAVERDE

WP1. "Definition of requirements":

SANTAVERDE developed in collaboration with TTZ the questionnaires addressed to cosmetic companies and growers in order to compile their respective requirements and prepared the "End user requirements" report (D1.1).

SANTAVERDE also played an important role in the extensive literature search that was

carried out by several partners in order to specifically define the requirements of the technical work to be developed.

WP3. "Purification and standardization of active compound"

SANTAVERDE provided the RTD partners TTZ and SPECTRAL and the SME TELA with different materials to perform the required experiments and analysis (e.g. aloe vera powder, aloe vera pure juice, etc).

WP4. "Product development and evaluation":

SANTAVERDE provided CZU with an anti-cellulite Aloe vera product to be tested regarding its hydrating and slimming effect.

They also developed the formulation for the cosmetic product incorporating the isolated Aloe vera fraction.

WP5. "Project management, dissemination and pre-exploitation measures"

SANTAVERDE contributed with their input to the "Plan for using and disseminating knowledge". SANTAVERDE carried out a wide range of dissemination actions for the AMAY project. SANTAVERDE was in charge of the project website which is online from month 3.

SANTAVERDE is the responsible for the overall management of the project assisted by BIOZOON. They have initiate intensive partner-to-partner communication and data transfer and monitored the activities in order to support smooth running of the project.. Personal contact was established by means of regular meetings. Three official meetings were organised: the kick-off meeting in Hamburg (Germany), the mid-term meeting in Budapest (Hungary) and the final meeting in Prag (Czech Republic) th. Additionally, several technical meetings were organized, for instance a 6-month technical meeting at TTZ facilities in Bremerhaven (Germany).

PLANTSCIENCE

WP1. "Definition of requirements":

PLANTSCIENCE actively contributed to the development of the sampling schedule and protocol together with TTZ, as well as to the development of the questionnaires for the collection of end-user requirements. They carried out an extensive literature search for the agronomic part of the project.

WP2. "Comparison of the Aloe vera cultivation"

They also supplied the growers ALFAVERDE, QUIVERA, POULIMENOS and FARO with a plant clone to be cultivated under different conditions.

PLANTSCIENCE actively collaborated with TTZ with their expertise in the assessment of the

laboratory results derived of the soil analyses and leaves analyses and their comparison with the different cultivation methods for the assessment of the cultivation characteristics in the Aloe vera active compounds

WP5. "Dissemination and Pre-exploitation measures"

PLANTSCIENCE contributed to the preparation of the draft of an exploitation plan and carried out dissemination of the AMAY project among their contacts

ALFAVERDE

WP1. "Definition of requirements":

ALFAVERDE contributed to the development of the testing schedule

WP2. "Comparison of the Aloe vera cultivation"

ALFAVERDE as Aloe vera grower contributed fulfilling the questionnaire addresses to the growers prepared by TTZ and SANTAVERDE with collaboration of PLANTSCIENCE where their cultivation methods and climate data where inquired.

They planted the plant clone provided by PLANTSCIENCE and they delivered the required soil and leaves samples for laboratory analysis to TTZ and SPECTRAL. Having being growing Aloe vera for many years now, ALFAVERDE took actively part in the discussion that followed the results of the soil and leave analysis in WP2.

Additionally ALFAVERDE provided RTD partner SPECTRAL with samples of sweet Aloe leaves for their analysis.

WP5. "Dissemination and Pre-exploitation measures"

ALFAVERDE contributed with their input to the "Plan for using and disseminating knowledge" and promoted the AMAY project among other farmers in the south on Spain.

QUIVERA:

WP1. "Definition of requirements":

QUIVERA contributed to the development of the testing schedule.

WP2. "Comparison of the Aloe vera cultivation":

QUIVERA as Aloe vera grower contributed fulfilling the questionnaire addresses to the growers prepared by TTZ and SANTAVERDE with collaboration of PLANTSCIENCE where their cultivation methods and clima date where inquired.

They planted the plant clone provided by PLANTSCIENCE and they delivered the required soil and leaves samples for laboratory analysis to TTZ and SPECTRAL

WP5. "Dissemination and Pre-exploitation measures"

QUIVERA contributed with their input to the "Plan for using and disseminating knowledge". QUIVERA received like all partners the project flyers and distributed them among their contacts in Spain. QUIVERA sells some of their products in small markets going around a wide territory, this gave them the change to do a good promotion of the project.

POULIMENOS:

WP1. "Definition of requirements":

POULIMENOS contributed to the development of the testing schedule

WP2. "Comparison of the Aloe vera cultivation":

POULIMENOS as Aloe vera grower contributed fulfilling the questionnaire addresses to the growers prepared by TTZ and SANTAVERDE with collaboration of PLANTSCIENCE where their cultivation methods and clima date where inquired.

They planted the plant clone provided by PLANTSCIENCE and they delivered the required soil and leaves samples for laboratory analysis to TTZ and SPECTRAL

WP5. "Dissemination and Pre-exploitation measures"

POULIMENOS received like all partners the project flyers and distributed many flyers among their contacts in Greece and abroad.

FARO:

WP1. "Definition of requirements":

FARO contributed to the development of the testing schedule.

WP2. "Comparison of the Aloe vera cultivation":

FARO as Aloe vera grower contributed fulfilling the questionnaire addresses to the growers prepared by TTZ and SANTAVERDE with collaboration of PLANTSCIENCE where their cultivation methods and climate date where inquired.

They planted the plant clone provided by PLANTSCIENCE and they delivered the required soil and leaves samples for laboratory analysis to TTZ and SPECTRAL

WP5. "Dissemination and Pre-exploitation measures"

FARO received like all partners the project flyers and continuously disseminated them among their contacts in Italy and abroad.

TELA:

WP1. "Definition of requirements":

TELA contributed to the requirements for the test to be development and setting up of an alternative and cheap analysis method to verify and quantify Aloverose in the extracts from Aloe vera gel (WP3)

WP3. "Purification and standardization of active compounds"

TELA started at month 7 with the development of the analysis method based on HPLC. Additionally, TELA applied some common food analysis methods to different Aloe vera materials in order to characterise them and developed a quality method to detect and determine the content of Aloverose in cosmetic products.

WP5. "Dissemination and pre-exploitation measures"

TELA contributed with their input to the "Plan for using and disseminating knowledge" and to the dissemination of the AMAY project at fairs and among their contacts.

GRADIENS:

WP1. "Definition of requirements":

GRADIENS contributed to the collection or requirements concerning the cosmetic requirements and desired effect of the final product by fulfilling the questionnaire provided at the beginning of the project and by active discussion at the kick-off meeting.

WP4. "Product development and evaluation":

GRADIENS developed the formulation and the cream incorporating pure Aloe vera juice, which was provided to SZU and TOPORIINA for test.

WP5. "Dissemination and Pre-exploitation measures"

GRADIENS contributed with their input to the "Plan for using and disseminating knowledge" and the dissemination of the project.

TOPORIIINA:

WP1. "Definition of requirements":

TOPORIINA contributed to the collection or requirements concerning the cosmetic

requirements and desired effect of the final product by fulfilling the questionnaire provided at the beginning of the project and by active discussion at the kick-off meeting.

WP4. "Product development and evaluation":

TOPORIINA carried out consumer tests of two cosmetic products in their beauty saloons to assess anti-wrinkle and anti-cellulite effect. TOPORIINA complemented the consortium with their marketing experience.

WP5. "Dissemination and Pre-exploitation measures"

TOPORIINA contributed with their input to the "Plan for using and disseminating knowledge". TOPORIINA received like all partners the project flyers and is continuously disseminating them.

TTZ:

WP1. "Definition of requirements":

TTZ developed together with SANTAVERDE the questionnaires for setting the requirements of the SMEs, as well as the sampling protocol in collaboration with PLANTSCIENCE.

WP2. "Comparison of the Aloe vera cultivation":

TTZ summarized the laboratory results carried out in task 2.1 (Deliverable 2.1, *Annex VI*) and compared them with the cultivation methods in the different plantations (task 2.2, Deliverable 2.2, *Annex VII*) and with the Aloverose content in the leaves analysed by SPECTRAL (WP3). The report resulting from this assessment constitute a milestone of the AMAY project (Milestone 2.1, *Annex VIII*)

WP3: "Purification and standardization of active compound"

TTZ performed the first fractionation from Aloe vera pure juice, treated some samples which presented problems of solubility and conducted the determination of protein content of the different Aloe vera fractions obtained.

TTZ closely worked with the other two RTDs in the project (partner SPECTRAL and SZU) for achieving a proper execution of the tasks.

WP4. "Product development and evaluation":

TTZ developed the creams incorporating the isolated Aloe vera fraction and provided them to SZU for subsequently testing.

WP5: "Dissemination and pre-exploitation measures"

TTZ contributed with their input to the "Plan for using and disseminating knowledge", especially with their wide dissemination activity carried out at fairs, congresses, conferences, etc.

SPECTRAL:

WP1. "Definition of requirements":

SPECTRAL have contributed to the WP1 by means of evaluating the requirements concerning the chemical analyses to be performed to define and discussing it with the rest of the partners involved.

WP2. "Comparison of the Aloe vera cultivation":

The Aloe vera leaves provided by the growers FARO, ALFAVERDE, QUIVERA and

POULIMENOS were analysed by SPECTRAL. They also contributed to this WP with an extensive discussion during meetings. SPECTRAL also tested the leaves from the plant clone provided to the growers at the beginning of the project.

WP3: "Purification and standardization of active compound"

SPECTRAL performed the fractionation of different Aloe vera materials for the isolation of active compounds and further optimised the process. They conducted the NMR analysis of the fractions as well.

WP5: "Dissemination and pre-exploitation measures"

SPECTRAL contributed with their input to the "Plan for using and disseminating knowledge" and perform dissemination of the project at fairs, conferences, etc and among their clients.

SZU:

WP1: "Definition of requirements":

SZU contributed to the collection or requirements concerning the cosmetic requirements as well as the dermatological tests to be performed and the desired effect of the final product.

WP4: "Product development and evaluation"

SZU tested in-vitro on skin cells the cytotoxic effect of different Aloe vera fractions as well as their protective effect against the effect of tensides. They also assessed in-vitro the effect of main components of Aloe vera alone and in combination at different rations. They performed the dermatological test with human volunteers to assess the biological effects of the isolated Aloe vera fraction and the pure juice incorporated to cosmetic products.
SZU provided their extensive expertise on in vitro and in vivo test of Aloe vera products

WP5: "Dissemination and pre-exploitation measures"

SZU contributed with their input to the "Plan for using and disseminating knowledge" and actively disseminated information about the AMAY project, mainly through conferences and congresses they attended.

Annexes

Annex I: Kick-off meeting minutes

Annex II: Questionnaire for the collection of the cosmetic end-users requirements companies

Annex III: Questionnaire for the investigation of cultivation methods

Annex IV: Sampling protocol (D1.1)

Annex V: Collection of end-users requirements (D1.2)

Annex VI: Literature review

Annex VII: Minutes 6 month progress meeting

Annex VIII: Soil analysis results (D 2.1)

Annex IX: List of cultivation methods (D 2.2)

Annex X: Assessment report on the influence of cultivation methods and climate in the Aloe vera active compounds (M2.1)

Annex XI: Protocol for the Aloverose purification and NMR analysis (D 3.1)

Annex XII: Protocol for carrying out HPLC analysis of Aloverose (D 3.2)

Annex XIII: Fractionation of Aloe vera juice and characterization of the fractions obtained

Annex XIV: Cytotoxicity and protective effects of Aloe vera fractions

Annex XV: Report on the NMR analysis of Aloverose (D 3.3)

Annex XVI: Report on the Aloeverose certification quick-test (D3.4)

Annex XVII: Assessment anti-cellulite effect of an Aloe vera gel

Annex XVIII: Minutes mid-term meeting

Annex XIX: Minutes cosmetic workshop

Annex XX: In-vitro tests Aloverose-glucose-malic acid

Annex XXI: Samples of plants from the different Aloe vera cultivators to be analysed (M2.2)

Annex XXII: Minutes technical meetings

Annex XXIII: Recipes of novel cosmetic products containing Aloverose (D4.1)

Annex XXIV: Samples of cosmetic products containing Aloverose (D4.2)

Annex XXV: Dermatological testing results of final products (D4.3)

Annex XXVI: Consumer tests anti-cellulite and anti-wrinkle self-assessment

Annex XXVII: First progress report (D5.2)

Annex XXVIII: Project flyer (D 5.3)

Annex XXIX: Mid-term review report (D5.4)

Annex XXX: Provisional plan for using and disseminating the knowledge (D5.5)

Annex XXXI: Second progress report (D5.6)

Annex XXXII: Final meeting minutes

Revised Versions

This document has been revised posterior to the submission date as established in the following table
([Revision 1] considered as deliverable at submission date):

Revision n°	Date	Partner in charge
1	02/05/07	SANTAVERDE