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Protein extraction from spinach juice using vacuum explosion and their separation by active carbon, heat, and $CaCl_2$

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Summary

The goal of the study described here is to produce a clear, uncoloured protein solution from spinach. Activated carbon, 50 °C heat and 50 °C+ $CaCl_2$ are applied to decolour the juice by removing unwanted material. The Betaprocess (vacuum explosion) is tested at different temperatures for its ability to assist in the process. It is concluded that the Betaprocess releases more protein from slowjuiced spinach, but that this protein does not end up in the final treated juice. The activated carbon method does not result in satisfactorily decolourised juice. The 50 °C and especially the 50 °C+$CaCl_2$ treatment result in clarified juice. The protein concentrations of the juice after the two latter treatments do not differ much from each other. There are indications that Betaprocess at 20 °C results in slightly higher protein concentrations in the final juice after subsequent treatments, compared to when other temperatures or no Betaprocess is applied.

Samenvatting

Het doel van de hier beschreven studie is om een heldere, ongekleurde eiwitoplossing te produceren uit spinazie. Actieve kool, 50 °C warmte, en 50 °C warmte in aanwezigheid van $CaCl_2$ worden toegepast om spinaziesap te ontkleuren en te ontdoen van ongewenst materiaal. Het Betaprocess (vacuümexplosie) wordt getest bij verschillende temperaturen om te beoordelen in hoeverre het bijdraagt aan het proces. Concluderend wordt gesteld dat het Betaprocess meer eiwit vrijmaakt uit het sap dat met een slowjuicer uit spinazie is geperst, maar dit extra eiwit komt uiteindelijk niet in het verder behandelde product terecht. De toegepaste methode met actieve kool resulteert niet in voldoende ontkleuring van het sap. De 50 °C behandeling en zeker ook de 50 °C+$CaCl_2$ behandeling resulteren in geklaard sap. De eiwitconcentraties na deze twee behandelingen verschillen niet veel van elkaar. Er zijn aanwijzingen dat Betaprocess bij 20 °C resulteert in iets hogere eiwitconcentraties in het uiteindelijke sap na de verdere behandelingen, vergeleken met de andere geteste temperaturen, of wanneer geen Betaprocess wordt gebruikt.

1 Introduction

Within the Groenblad work package of the project Kleinschalige Bioraffinage, the general goal is to produce protein by extraction from green leaves, such as sugar beet leaves. The focus was shifted to spinach leaves, so that testing applications for the protein concentrate are not hindered by novel food regulations, as would be the case for sugar beet leaves. Project partner ABC-Kroos produces a green protein concentrate from spinach leaves and the specific goal of trials performed at WUR-FBR and WUR-ACRRES in 2015 is to look for and test processes or process adjustments leading to a colourless protein concentrate. This report describes the results of trials performed at WUR-ACRRES, using the Betaprocess equipment of project partner DSD to treat spinach juice produced with a slowjuicer. The Betaprocess entails a vacuum explosion, and as temperature is known to affect both this process as well as protein precipitation by denaturation, three temperatures are tested (20 °C, 40 °C, and 65 °C) and compared to unprocessed juice. Furthermore, three downstream processes are compared: activated carbon, 50 °C heat treatment, and 50 °C heat treatment in presence of $CaCl_2$, focussing on the concentration of colourless dissolved protein from spinach by removing chlorophyll and other cell components.

1.1 Experimental set up

Spinach is ground and pressed using a slowjuicer, after which the juice is treated by vacuum explosion and filtered. The filtrate is processed further by: 1) adding activated carbon, or 2) a 50 °C heat treatment, or 3) a 50 °C heat treatment in presence of $CaCl_2$. Following these treatments, the liquid is centrifuged and the decanted supernatant is filtrated. The resulting filtrate is analysed for protein content, which is compared to the protein content of the juice before treatment (Figure 1). The supernatant is also visually evaluated.

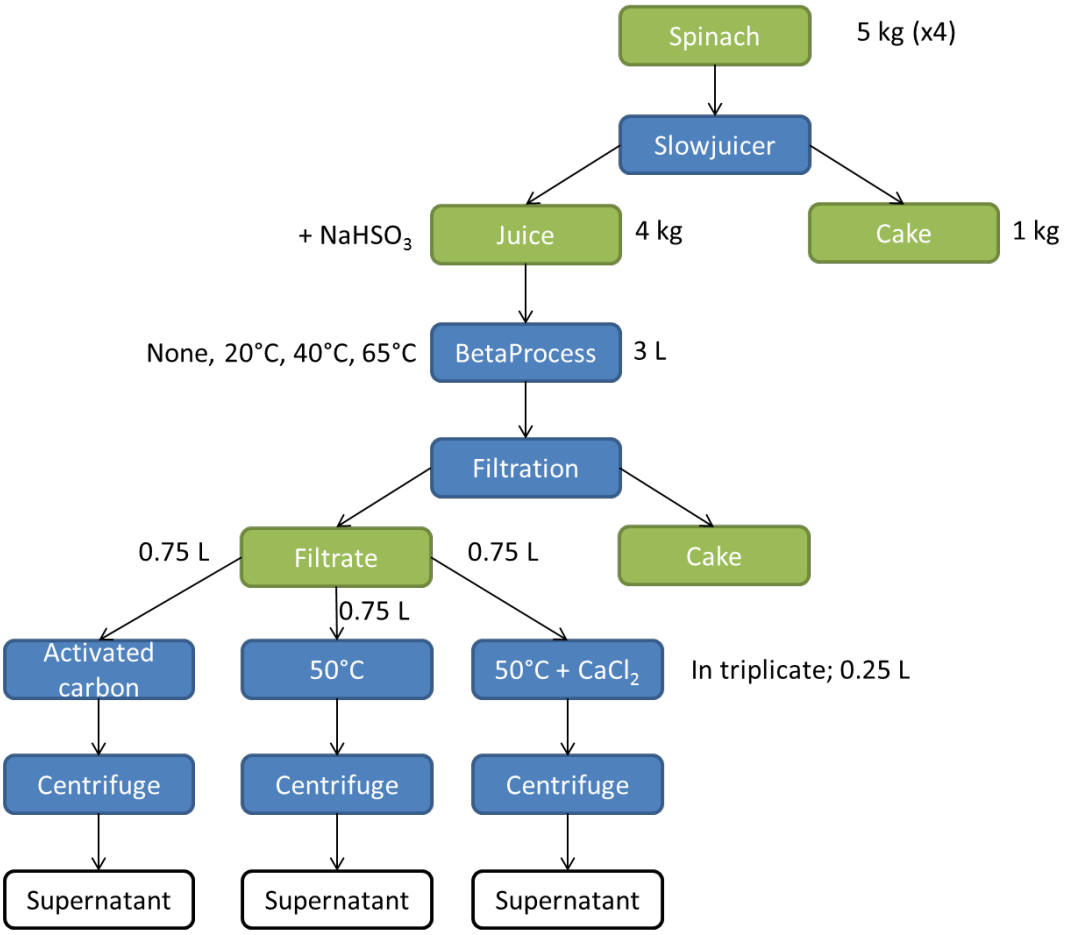


Figure 1. Experimental set up

2 Materials and methods

2.1 Spinach and juicing

For each Betaprocess treatment, 5 kg of spinach leaves (Spanish import, from MTS Broersen, Lutjebroek, the Netherlands, storage at 4 °C) was processed with a slowjuicer (Greenstar Elite Juicer GSE-5000, smallest pore sieve, maximum pressure on cake), resulting in ±4 L juice and 1 kg press cake. Sigma antifoam A (9.5 g/L) and sodium metabisulfite (40 g/kg fresh spinach leaves, equals ±47 g/L juice) were manually stirred in to the juice during processing.

2.2 Vacuum explosion and filtration

The vacuum explosion was performed in a labscale version of the Betaprocess technique: the mini-Betaprocess. It consists of a vacuum vessel set at -0.970 bar gauge (30 mbar or 3 kPa absolute), combined with an appropriately preheated airlock that allows about 1.5 L to be added to the vessel at once (Figure 2). Juice to be treated in the Betaprocess was divided in three portions, heated to the desired temperature (heat up times: 15 °C to 40 °C in ±3 min 45 sec; 15 °C to 65 °C in ±8 minutes), and processed in the mini-Betaprocess. After treatment each portion was removed from the equipment and pooled, after which the treated liquid was filtered through a 100 µm felt filter bag (FSI). The filtrate was divided into three portions, to be used for further processing.

Figure 2. Mini-Betaprocess

2.3 Protein separation and centrifugation step

The activated carbon (AC) treatment consisted of adding 2.6 g (Norrit CN1) to 260 g juice filtrate and manually shaking for ±1 minute. For the heat treatment, 260 g of filtrate was heated to 50 °C (heating time ±12 minutes) and held at this temperature for 20 minutes. For the 'heat and $CaCl_2$' treatment, 5.4 g of $CaCl_2$ was added to 260 g of filtrate when 50 °C was reached, and the total was held at 50 °C for 20 minutes. All treatments were performed in triplicate. Following the treatment, the liquid was centrifuged at 4200 ×g at 10 °C for 15 minutes, after which the supernatant was decanted and filtered (Schleicher & Schuell 595 ½ 200 mm pleated filters) for analysis.

2.4 Analyses

Sample analysis consisted of dry matter content and protein content (Kjeldahl method). Liquid samples after protein separation were freeze dried before protein analysis. pH was measured in the juice after addition of sodium meta bisulphite.

3 Results and Discussion

Fresh spinach leaves contained 79.3 ±1.15 g/kg dry matter and 24.6 ±12.1 g/kg protein. Juicing the spinach typically resulted in 81 % juice and 16 % cake (Table 1), containing 9.9 % and 16.5 % dry matter, respectively. 2 % to 3 % of the spinach mass remained as partially pressed material in the slowjuicer.

Comparison of experimental results is done based on protein concentration. The experimental conditions did not provide enough data to setup up useful mass balances.

Table 1. Mass (g) of spinach, juice, juice additives, and cake

Batch nr	1	2	3	4
Betaprocess	None	20 °C	40 °C	65 °C
Spinach (g)	9630.2		10460.8	
Juice (g)	8131.3		8931.4	
$NaHSO_3$ added to juice (g)	397.2		418.5	
Antifoam added to juice (g)	18.4		25.4	
pH after $NaHSO_3$ and antifoam addition	4.96		5.05	
Cake (g)	1690.6		1652.5	
Juice after Beta and filtration (g)	2874.7	2602.8	3008.5	3066.9

An interesting observation –not supported by measurements– is that the spinach juice resulting from the slowjuicer passed through the subsequent filtration step faster when the juice was treated with Betaprocess, compared to untreated juice.

The Betaprocess clearly has a positive influence on the amount of protein that passes the 100 µm felt filter bag (Table 2, Table 3, Table 4). After filtration, the juice that has not been Beta-processed contains 12 g/L protein, while Beta-processing increases this to +/- 20 g/L protein, regardless of the temperature of the vacuum explosion. Surprisingly, the dry matter content does not seem to be affected. The extra protein that is released does not result in more protein dissolved after the subsequent treatments. It is therefore likely that this extra protein is bound to membranes or other cell material, leading to exclusion from the liquid by the centrifugation step after the subsequent treatment, possibly assisted by precipitation during said treatment. In order to harvest this part of the protein, other methods are needed than tested here.

The effect of Betaprocess on the protein concentration of the filtrates resulting from the subsequent treatments is less clear. The activated carbon treatment seems to lead to more protein being present in the resulting liquid, but it does not result in sufficient decolourisation compared to '50 °C' and '50 °C + $CaCl_2$'. There are no clear differences in protein results from the '50 °C' and '50 °C + $CaCl_2$' treatments, but it may be suggested that the 20 °C Beta-processed results in higher protein concentration in the subsequent treatment. Clearly, the 20 °C Betaprocess treatment increases the dry matter content of the liquid after subsequent treatment, more so than Betaprocess at higher temperature, or when no Betaprocess is applied.

It should be noted that the results described here are expressed as protein concentrations. Direct comparison to the dry matter content may be misleading, as the latter is augmented by the addition of sodium bisulphite, antifoam, and $CaCl_2$. In case of further processing to a protein concentrate, a subsequent mild precipitation would be needed; e.g. by lowering the pH.

Table 2. Results of treatment with Activated Carbon

		1	2	3	4
Betaprocess		None	20 °C	40 °C	65 °C
Filtrated juice (g)		259.6	260.1	260.6	259.4
Before treatment, in filtrated juice					
	Dry matter (g/L)	91.0±0.00	93.7±2.08	92.3±0.58	90.3±0.58
	Protein (g/L)	12.3±0.60	19.9±1.15	21.2±1.85	19.7±0.51
AC added (g)		2.61±0.01	2.62±0.03	2.62±0.03	2.61±0.01
After treatment, in filtrated supernatant					
	Dry matter (g/L)	69.0±1.00	82.0±6.08	71.0±1.00	66.03±2.89
	Protein (g/L)	8.7±0.58	10.3±0.58	8±0.00	6.7±0.58

Table 3. Results of treatment with heat (50 °C)

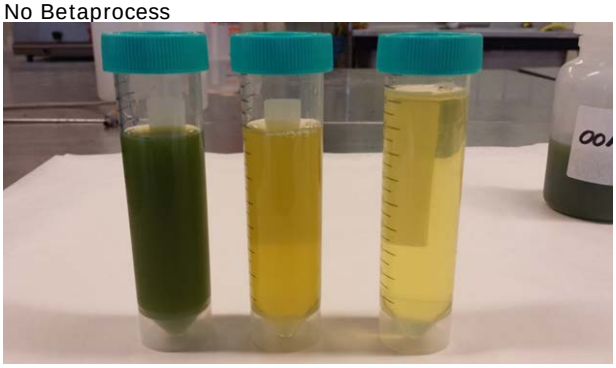
		1	2	3	4
Betaprocess		None	20 °C	40 °C	65 °C
Filtrated juice (g)		274.2	270.9	265	271.3
Before treatment, in filtrated juice					
	Dry matter (g/L)	91.0±0.00	93.7±2.08	92.3±0.58	90.3±0.58
	Protein (g/L)	12.3±0.60	19.9±1.15	21.2±1.85	19.7±0.51
After treatment, in filtrated supernatant					
	Dry matter (g/L)	61.3±0.58	72.5±3.54	59±2.00	61.7±6.03
	Protein (g/L)	7.7±0.58	8.5±0.71	7.3±0.58	7.3±0.58

Table 4. Results of treatment with heat (50 °C) and $CaCl_2$

		1	2	3	4
Betaprocess		None	20 °C	40 °C	65 °C
Filtrated juice (g)		280.0	260.8	277.2	272.0
Before treatment, in filtrated juice					
	Dry matter (g/L)	91.0±0.00	93.7±2.08	92.3±0.58	90.3±0.58
	Protein (g/L)	12.3±0.60	19.9±1.15	21.2±1.85	19.7±0.51
$CaCl_2$ added (g)		5.45±0.04	5.41±0.03	5.45±0.04	5.42±0.03
After treatment, in supernatant					
	Dry matter (g/L)	65.3±2.08	74.5±0.71	62.7±4.04	69.7±2.08
	Protein (g/L)	7.0±0.00	7.5±0.71	6.7±0.58	7.0±0.00

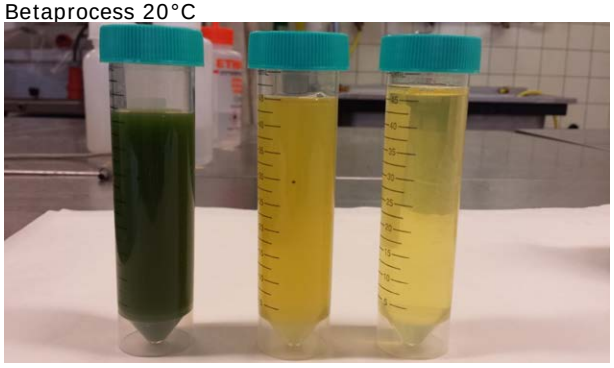
Visually, the differences in supernatants of the treatments with activated carbon, 50 °C, and 50°C + $CaCl_2$ are clear (Figure 3). The combination of heat and $CaCl_2$ results in the clearest supernatant, while activated carbon treatment only seems to have a limited effect in decolouring the liquid. Heat alone also results in a decoloured supernatant, but the effect is more pronounced when $CaCl_2$ is included. The Betaprocess treatment only seems to have a visual effect when performed at 65 °C, resulting in a supernatant after activated carbon treatment that is lighter green, compared to Betaprocess at lower temperatures or no Betaprocess at all.

No Betaprocess



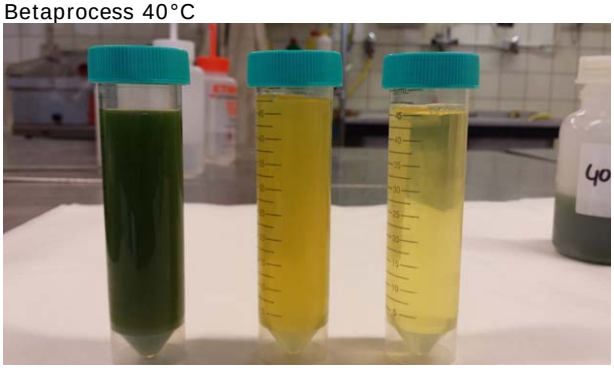
AC 50 °C 50 °C + $CaCl_2$

Betaprocess 20°C



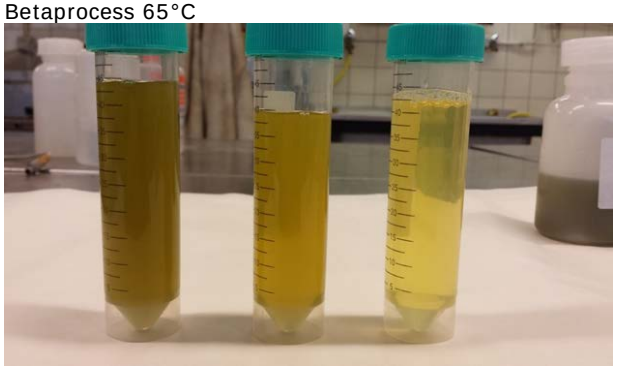
AC 50 °C 50 °C + $CaCl_2$

Betaprocess 40°C



AC 50 °C 50 °C + $CaCl_2$

Betaprocess 65°C



AC 50 °C 50 °C + $CaCl_2$

Figure 3. Supernatant of all treatments

4 Conclusions

The Betaprocess treatment positively affects the protein concentration in the juice after subsequent 100 µm filtration, regardless of the temperature at which the Betaprocess is performed. However, this additional protein released by the Betaprocess does not increase the soluble protein concentration in the final product (after treatment, centrifugation and filtration). It is likely bound to membranes or other cell material leading to exclusion from the liquid by the centrifugation step after the subsequent treatment (AC/50°C/50°C+$CaCl_2$), possibly assisted by precipitation during said treatment. Regarding protein concentration, the Activated Carbon treatment performs better than the 50°C and 50°C+$CaCl_2$ treatments, but it does not result in sufficient decolouring. Little difference seems to exist between the 50 °C and the 50 °C+$CaCl_2$ treatment; both seem to do best after a 20 °C Betaprocess, but differences with other temperatures are not clear. The 50°C+$CaCl_2$ treatment outperforms the other two treatments in resulting in a more clear end product.

5 Acknowledgments

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