SUMMARY
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Anthocyanins are flavonoids responsible for the colour of blueberries. These compounds are interesting due to their impact on food sensory attributes, since they may be used as natural food colorants. In addition, they offer potential health benefits because of their high antioxidant activity.

The objective of this PhD thesis was to study the extraction process of anthocyanins from fresh blueberries and to analyze the use of the extracts in food products formulation.

Firstly, the influence of the major process variables on the anthocyanin extraction from fresh blueberries was determined: temperature, pH, extraction time, type of solvent and raw material / solvent rate (RM/S). Two extraction methodologies were evaluated: solid-liquid extraction (ASLE) and extraction by fermentation (EFP). Different experimental designs were established in order to reduce the number of assays without affecting the significance of results.

These results were modelled by response surface methodology. It was observed that all the studied process variables had a significant influence on the extraction of anthocyanins from blueberries. The influence of temperature, pH and process time on anthocyanin extraction total yield (ATY) was studied in the first series of ASLE experiments. An ethanol-water solution (50:50 %v/v) was used as solvent and the relation RM/S was 1:3 kg/kg. The optimum values for the variables were: pH 2.1, temperature 36.6 ºC and extraction time 1 h (the shortest time of all assays). Under these conditions ATY was 51 %, while higher and lower process variable values resulted in a lower ATY. The ATY decreases when pH increases. This fact was probably due to the degradation of the flavylium cation, being electron deficient and highly reactive, with, formation of hemiacetal and chalcone, both unstable. ATY increases with a temperature increment up to 36.6 ºC, that could be explained by the stimulation of extraction by the temperature increase because anthocyanins solubility and diffusion coefficient are also incremented. However, extracted anthocyanins were degraded above the aforementioned temperature because the heat effect may
have caused glycoside sugar loss at position 3 of the molecule and ring opening with subsequent production of colourless chalcone. The best ATY was obtained with the minimum time extraction. This may be attributed either to a solid-liquid phases equilibrium or to the counteractive effect of anthocyanin degradation on the anthocyanin extraction rate occurrence caused by water nucleophilic attack on the flavylium cation forming colourless compounds.

During the second series of ASLE experiments, RM/S and extraction time variables were analyzed. The extraction solvent was ethanol acidified with citric acid (1%), the extraction temperature was 36 ± 1 °C and pH 3.5 ± 0.1. Under these conditions the maximum anthocyanin extraction was obtained (57%) after 2 h 12 min of extraction and using a RM/S of 1:2.8 kg/kg, while an increase or decrease of the studied variables caused ATY decrease. The ATY increase due to RM/S increment was ascribed to a higher concentration gradient between blueberry particles and the solvent thus mass transfer process was enhanced. Nevertheless, an increase of the RM/S above the optimum caused a ATY decrease, probably due to the higher amount of ethanol molecules in the medium. Ethanol, as well as water, can attack the flavylium cation thus generating more unstable anthocyanin structures. Likewise, it was observed that the longer the extraction time, the higher the ATY value obtained. In the same way, extraction decreased with longer extraction times. This fact suggests that extraction yield incremented until reaching anthocyanin concentration equilibrium between blueberry particles and solvent, and then, the yield decreased due to anthocyanin deterioration.

The two series of assays showed that the best conditions for anthocyanin extraction were: acidified ethanol with citric acid (1 %) as extractant, 1:3 kg/kg as raw material / extraction solvent rate, temperature 36 ± 1 °C and extraction time of 2 h.

In the AEF experiments, soluble solids concentration of the substrate was adjusted to 12 °Brix and fermentation was carried out using *Saccharomyces cerevisiae* strains during 72 h. In this case, temperature and pH were the studied parameters. The combination of a temperature of 28 °C
and pH 4.2 yielded the highest ATY value (49 %). An increase or decrease of any of the studied factors decreased ATY values. Two aspects were considered for this extraction methodology: factors that affect anthocyanin stability and conditions that favour the growth of *Saccharomyces cerevisiae* yeasts. Temperature and pH are very important in this aspect. Temperature increase to the optimum value enhanced yeast growth and ethanol production in the medium. As a result, anthocyanin extraction was increased as well as solubility and diffusion coefficient. Temperatures above optimum affected yeast growth and, therefore, ethanol production was reduced. In addition, chalcone production is enhanced since anthocyanin deterioration is an endothermic reaction. Regarding pH, there is an opposite effect. Lower pH values are more beneficial for the flavylium cation structure, which is the most stable anthocyanin. At pH 5 the colourless carbinol pseudobase species formation is favoured, while low pH inhibits yeast development that proliferates within 4.0-4.5 pH range. Consequently, a compromise value between factors affecting anthocyanins stability and favourable conditions for *Saccharomyces cerevisiae* yeast growth was determined. The aforementioned combination of factors was the optimum obtained in this study.

The anthocyanin extracts obtained under the conditions determined for both extraction methods (ASLE and AEF) were diluted extracts and were concentrated using a rotary evaporator. Concentrated products were defined as anthocyanin extract obtained by solid-liquid extraction (SLEP) and anthocyanin extract obtained by fermentation (EFP). No significant differences in total anthocyanin concentration were found between SLEP and EFP. However, total phenols concentration and antioxidant activity were higher in the EFP than in the SLEP.

A study of degradation of anthocyanin in SLEP and EFP extracts was performed. Firstly, extract susceptibility to heating was established by determination of destruction kinetics of anthocyanins at different temperature treatments (55, 65, 75 and 85 °C). Next, degradation kinetics during storage at two different temperatures (5 and 25 °C) was also studied. The experimental
results showed that anthocyanin degradation rate increased with temperature rise during heating as well as during storage experiments. In general, all experimental data fit adequately to first-order kinetics. Although anthocyanins obtained by solid-liquid extraction were more stable than those extracted by fermentation during temperature assays, both extracts were temperature susceptible. Therefore, results suggest that these extracts could be used for products that require low temperature and short time heat treatments. With regard to storage conditions of extracts, 5 °C should be the adequate temperature to keep degradation rate of the antioxidant pigments as low as possible.

In conclusion, the solid-liquid extraction method was the most adequate due to the simplicity and the speed of the process, the higher yield obtained and the higher stability of the anthocyanin extracted.

The extract obtained under the optimum conditions (solid-liquid extraction, ethanol extractant acidified with citric acid 1 %, 1:3 kg/kg raw material / extraction solvent, temperature 36 ± 1 °C and 2 h of extraction time) was then double concentrated. The concentrated (AE) was used for vacuum impregnation (IV) of melon cubes. Vacuum impregnation (IV) was performed in two stages: stage 1, vacuum or reduced pressure stage (IV₁), and stage 2, atmospheric pressure stage (IV₂). In the first stage three experiments were performed using different vacuum pressures: 210, 130 y 53 mbar for 10 min (t₁) at 25°C in order to evaluate the effect of reduced pressure on total anthocyanin concentration in the impregnated melon cubes. In the second stage the set of melon cubes –AE was submitted to atmospheric pressure for 10 min (t₂) at 25 °C. The response to IV was measured and expressed as volumetric fraction (X), volumetric deformation (Y), effective porosity (εₑ) and total anthocyanin concentration (TA). During the vacuum stage, a significant volume decrease was observed and the decrease was higher at 130 and 53 mbar. The negative values of the volumetric fraction suggested the exit of native liquid from inter and extra cellular spaces during expansion of the occluded gas, and this mass transfer process was greater at 130 and 53 mbar. Samples increased their
volume during the atmospheric pressure stage. Consequently, pore volume increased compared to the initial value and a significant impregnation of melon cubes with AE was observed. These values were higher as the vacuum in the impregnation chamber increased during stage 1. Regarding to the global process, a large amount of AE impregnating melon cubes was observed, and increased with the degree of degasification. In addition, effective porosity demonstrated the IV process efficacy particularly at 53 mbar. The AT concentration in AE impregnated melon cubes at 210, 130 and 53 mbar, was 3.9, 7.1 and 13.9 mg cianidyn-3-glucoside/100g melon, respectively. Therefore, AE melon cubes impregnation using IV methodology showed the best response to vacuum pressure at 53 mbar.

The results obtained in this PhD thesis suggest the feasibility of developing functional fresh foods using the vacuum impregnation method to incorporate anthocyanin extract from blueberries. Thus, the surplus of fresh blueberry could be used in the development of new food products with functional characteristics.