Saponin-based micelles as carrier for lutein: Effect of pH and presence of glucose syrup

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INTRODUCTION AND OBJECTIVE

An own recent study has shown the possibility to incorporate a health-promoting carotenoid, lutein, in saponin-based micelles. Other ingredients can influence the sensation of natural colorants in a food product. Therefore the aim of the present study was to investigate the effect of product parameters like pH and glucose syrup content on color, absorbance, particle size and zeta potential of lutein-loaded micelles.

MATERIALS AND METHODS

A commercially available purified extract of *Quillaja saponaria Molina* with a saponin content of 75 % of the total dry matter was used in the study. Lutein was a kind gift of Chr. Hansen GmbH, Germany.

Micellar solutions have been prepared under continuous agitation for 24h at 20 °C. Excess lutein was isolated by centrifugation and removed from the micellar solution. pH was varied from pH 3 to pH 7 using citric acid monohydrate – Na_2HPO_4 buffer. Influence of carbohydrates was examined adding spray dried glucose syrup. In a first experiment a general factorial design was employed to investigate the influence of lutein, pH and glucose syrup on the particle size, zeta potential, conductivity, L*a*b values and absorption. In a second experiment the point of adding glucose syrup in the process of micellization was examined.

Size and zeta-potential of the micelles were analyzed by dynamic and electrophoretic light scattering, respectively, using a Zetasizer Nano (Malvern Instruments). Values for conductivity were accompanied during zeta potential measurements. Micelle load with lutein was estimated from a spectrophotometric assay, and the color of the micellar solution was analyzed using a chromameter CR300 (Konica Minolta Sensing Europe B.V.).

RESULTS AND DISCUSSION

In general, adding lutein provided a significant enhancement of particle size, absorption and b value as well as a significant reduction of the a value and zeta potential (Table 1).

As seen in figure 1 the particle size did not change with increasing content of glucose syrup or pH. This result is in agreement with the results of Weiss & Liao (Weiss, 2000).

Table 1: The particle size, zeta potential, absorption, a-value and b-value of non-loaded and lutein-loaded micellar solutions at pH 3 and without glucose syrup

	without lutein	with lutein
particle size [nm]	6 ± 0.1	137.6 ± 1.8
zeta potential [mV]	-8.7 ± 0.9	-25.3 ± 4.2
absorption	0.023 ± 0.001	0.129 ± 0.006
a-value	-0.55 ± 0.01	-2.99 ± 0.25
b-value	3.91 ± 0.04	13.02 ± 0.77

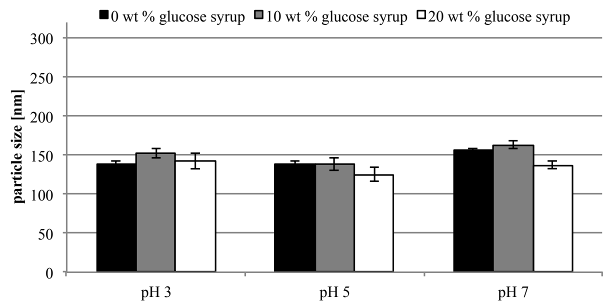

Figure 1: Influence of pH and glucose syrup content on the particle size of the micellar solution

In general, the absolute value of the zeta potential of loaded micelles decreased slightly with increasing pH (Figure 2). Changes in zeta potential at different pH arise from ionic strength and not from electric charge of functional groups. All pH values studied are higher than pK_a of carboxylic acid group within saponin molecule, so the carboxyl groups are fully charged (Yang, 2013).

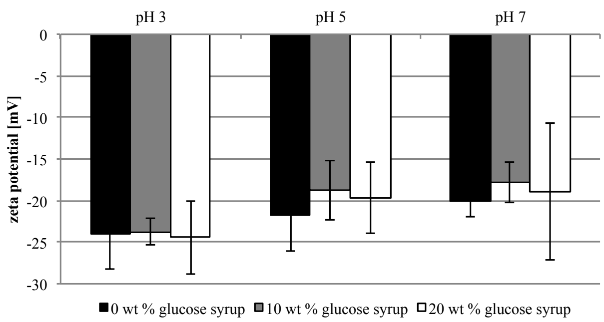

Figure 2: Influence of pH and glucose syrup content on the zeta potential of the micellar solution

As a consequence the decrease in zeta potential with decreasing pH must be ascribed to a lower conductivity (Benitez, 2006) (Table 2). Glucose syrup showed no influence on the zeta potential. Hence particle size and zeta potential are independent of studied product parameters and therefore the colloidal stability is not influenced.

Table 2: Conductivity [mS/cm] of the lutein-loaded micellar solutions depending on pH and glucose syrup content

	glucose syrup [wt %]		
	0	**10**	**20**
pH 3	5.8 ± 0.1	4.4 ± 0.0	3.3 ± 0.1
pH 5	12.5 ± 0.3	9.6 ± 0.2	7.9 ± 0.4
pH 7	19.8 ± 0.5	14.9 ± 0.3	11.3 ± 0.2

With increasing pH the a-value of lutein-loaded micelles increased independent of glucose syrup content. Only at pH 3 the content of glucose syrup had a significant influence on the a-value (Table 3).

Highest b-values and absorption were observed in samples without addition of glucose syrup at pH 3. With increasing glucose syrup content absorption and b-value decreased. A possible explanation is a higher refractive index in the presence of glucose syrup (Weiss, 2000). The b-value and absorption of loaded micelles were significantly reduced at pH 5 independent of glucose syrup content.

Table 3: The a-value, b-value and absorption of lutein-loaded micellar solutions depending on pH and glucose syrup content

		glucose syrup [wt %]		
		0	10	20
a-value	pH 3	-3.0 ± 0.3	-2.4 ± 0.0	-2.1 ± 0.1
	pH 5	-1.7 ± 0.1	-1.5 ± 0.1	-1.4 ± 0.1
	pH 7	-0.2 ± 0.1	-0.3 ± 0.1	-0.4 ± 0.1
b-value	pH 3	13.0 ± 0.8	10.7 ± 0.0	9.7 ± 0.2
	pH 5	8.9 ± 0.1	8.5 ± 0.6	8.3 ± 0.1
	pH 7	12.6 ± 0.3	11.4 ± 0.1	10.7 ± 0.4
Absorption	pH 3	0.129 ± 0.006	0.087 ± 0.002	0.076 ± 0.006
	pH 5	0.054 ± 0.004	0.048 ± 0.005	0.047 ± 0.002
	pH 7	0.102 ± 0.007	0.086 ± 0.003	0.077 ± 0.003

The decrease in particle size, absorption and b-value at pH 5 is not due to the fact that lutein conformation changes from all-trans into cis forms as pH does not influence configuration (Chen, 1995). The influence was also observed without the addition of glucose syrup, i.e. glucose syrup does not cause this effect. In addition zeta potential does not show this effect, hence changes in charge of saponin molecule can also be excluded. Further investigations using spectroscopic methods should provide more information about the structure of the micelles.

In another experiment the influence of addition of glucose syrup before or after micellization was analysed using micellar solutions at pH 3. When adding glucose syrup after micellization, particle size and zeta potential showed similar results compared to samples, in which glucose syrup was added prior to micellization. For the absorption as well as a- and b-values opposite trends for the effect of glucose syrup were observed. In contrast to the decrease in the absorption as well as a- and b-values with addition glucose syrup for micelles with glucose syrup added before micellization, the parameters keep almost constant for micelles with addition of glucose syrup after micellization (Figure 4). These results shows that there is an influence of glucose syrup on the incorporation of lutein into saponin-micelles. If a higher refractive index induce a decreasing absorption, the decrease should also occur for micelles with addition of glucose syrup after micellization (Weiss, 2000). Recent investigations have shown an increase of the CMC of saponin-micelles with increasing glucose content. Thus more saponin is necessary for lutein solubilization, and absorption of solutions where glucose syrup was added before micelle formation might be lower than absorption where it was added afterwards.

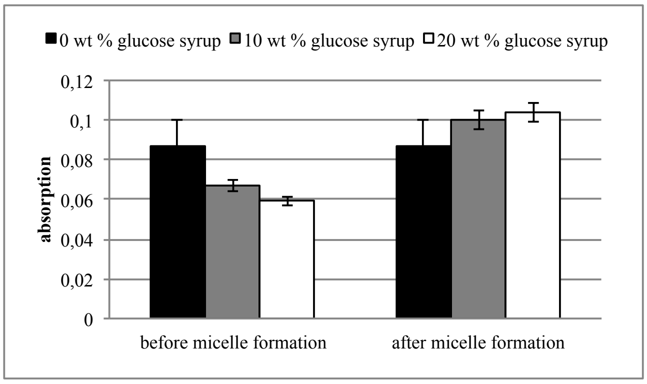

Figure 4: Effect on absorption of the micellar solution at pH 3 when glucose syrup is added before and when it is added after micelle formation

CONCLUSION

Physical properties of the micelles are independent of the system studied. In contrast, the coloring effect depends on the pH of the system, the presence of glucose syrup and on the experimental procedure. The underlying mechanism of the effect of the presence of carbohydrates needs to be elucidated. However, the effects presented in the study have an impact on product formulation and process design in commercial applications of micellar dispersions containing colorants.

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