Encapsulation of betalain into double w/o/w emulsion

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INTRODUCTION AND OBJECTIVES

Consumers are becoming more and more aware of health issues and partly because of that, they prefer natural colorants and other additives over artificial ones. Betalains are water-soluble natural pigment compounds, but as with other natural colorants, they are usually unstable in food applications. Microencapsulation could be one way to enhance the stability of these natural pigments. We have previously produced yellow o/w emulsions containing lutein in the oil phase (Kaimainen 2012), and now we tried to use similar methods for betalains. As betalains are water-soluble, we used a double w/o/w emulsion, as most foods are aqueous environments. The aim of this study was to prepare a red double w/o/w emulsion with betalain pigment in the inner water phase for possible food coloring use. We also subjected the double emulsion to simulated lipid digestion, and to our knowledge, simulated lipid digestion of double emulsions has not been done before.

MATERIALS AND METHODS

Materials

The oat polar lipid fraction used for o/w emulsifier was extracted from oat flakes (*Avena sativa*) using a supercritical fluid process described by Aro et al. (Aro 2007). The polyglycerol polyricinoleate (PGPR) used for w/o emulsifier was PGPR 4175 received from Palsgaard (Denmark). The betalain pigment was extracted with hot water (70 °C, 30 min) from red beets (*Beta vulgaris*) bought at a local grocery store. Commercial rapeseed oil bought at a local grocery store was used for the oil phase. Salts and other reagents were of analytical grade.

Preparation of double emulsions

First we prepared the oil phase of the primary w/o emulsion by adding 2 % (w/w) of PGPR into rapeseed oil. Beet extract was added slowly with mixing at 10 000 rpm with a SilentCrusher M high-speed mixer (Heidolph, Germany) so that the water phase accounted for 30 % (w/w) of the total w/o emulsion. After the whole inner water phase was added, the emulsion was homogenized at 20 000 rpm for 5 min. The outer water phase of the double emulsion was prepared as described by Kaimainen et al. (Kaimainen 2012) for simple o/w emulsions with slight modifications. The outer water phase was a 80 mmol/L, pH 5.8 citrate-phosphate buffer with 0.5 % (w/w) oat polar lipids as emulsifier, 0.2 % (w/w) guar gum and 0.2 % (w/w) xanthan gum as stabilizers and 3.9 % glucose for adjusting the osmolarity to match that of the inner water phase. Primary w/o emulsion was slowly added to the outer water phase with mixing at 13 000 rpm, and after the whole amount (3 %, V/w) was added, the double emulsion was homogenized at 18 000 rpm for 5 min.

Measurements from the emulsions

The osmolarities of water phases were measured with a Micro-Osmometer type 13 Autocal (Roebling, Germany) and as already mentioned, glucose was used to balance the osmolarities of the two water phases. Droplet sizes were measured by laser diffraction using a Mastersizer S equipped with a 2 mW He–Ne laser of 633 nm and the 300RF lens (Malvern Instruments Ltd, UK). Emulsions were diluted with distilled water in the dispersion unit to reach an oil volume concentration near 0.01% for the circulation in the measurement cell. Droplet sizes were also investigated with a light microscope equipped with a digital camera. Encapsulation efficiency was measured by centrifuging a double emulsion sample at 3000 x G for 10 min and filtering the outer water phase through a 0.45 µm syringe filter. The absorbance at 530 nm of the filtered sample was measured with a Multiskan GO spectrophotometer (Thermo Scientific, Finland) and the value was compared with a standard curve prepared by adding calculated amounts of beet extract to the outer water phase corresponding to 5, 10, 20, 40, 60 or 100 % leakage of the inner water phase.

Simulated digestion

The double w/o/w emulsion was subjected to simulated lipid digestion to see what might happen to the double emulsion in the conditions of the small intestine. Double emulsion was mixed with bile salt buffer (130 mmol/L, pH 7.4 phosphate buffer) in proportion 1:1 and pancreatic lipase was added to the mixture. The mixture was incubated at 37 °C under magnetic stirring, and samples were taken in the beginning and after 30, 60 or 180 min for microscopic investigation and droplet size measurement.

RESULTS AND DISCUSSION

Encapsulation efficiency and droplet size

The encapsulation efficiency was 89 % (duplicates 91 % and 87 %), which is quite high. The double emulsion was pale red in color. The paleness is mostly due to the usual creamy appearance of an emulsion. Two peaks could be seen from the droplet size analysis, corresponding to primary w/o emulsion droplets and double w/o/w emulsion droplets. The smaller primary emulsion droplets accounted for 20 %

of the total droplet count, and the average size was 367 nm (range 100-900 nm) and the larger double emulsion droplets accounted for 80 % of the total droplet count with an average size of 13.5 µm (range 2-50 µm). Under microscopic investigation it seems that inside the smaller double emulsion droplets there are only very little or no primary emulsion droplets, as can be seen in figure 1.

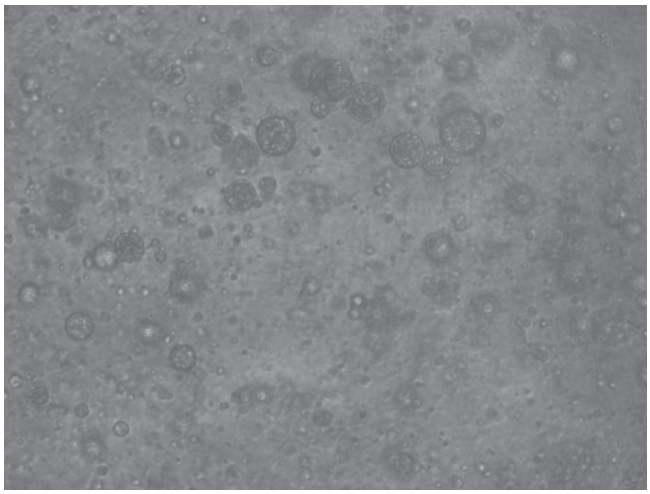

Figure 1. Microscopic image of a double emulsion

Simulated digestion

During the simulated digestion the droplet sizes changed a little as can be seen in table 1. The proportion of primary emulsion droplets decreased from 18 % to 14 % after 60 min. There might be some error in the 60 min measurement, as the droplet size of double emulsion droplets is considerably larger than again after 180 min. The volume of the digestion test was not high enough to make duplicate droplet size measurements. It should be noted that the bile salt buffer had lower osmolarity than the double emulsion, and this difference was not adjusted in the experiment.

Table 1. Changes in droplet size during simulated lipid digestion

digestion time	primary emulsion		double emulsion	
	mean (nm)	range (nm)	mean (µm)	range (µm)
0 min	354	80-900	13,7	2-40
30 min	368	100-900	17,2	2-50
60 min	337	100-900	41,7	5-140
180 min	633	100-2000	16,3	2-50

We detected some vesicle-like structures forming during the simulated digestion, as can be seen in figure 2. They formed already in the beginning of digestion, but they were larger and clearly visible after 120 min of digestion. This might be due to coalescence of the primary w/o emulsion droplets, which is also consistent with the increase in the droplet size of the primary emulsion droplets measured after 180 min.

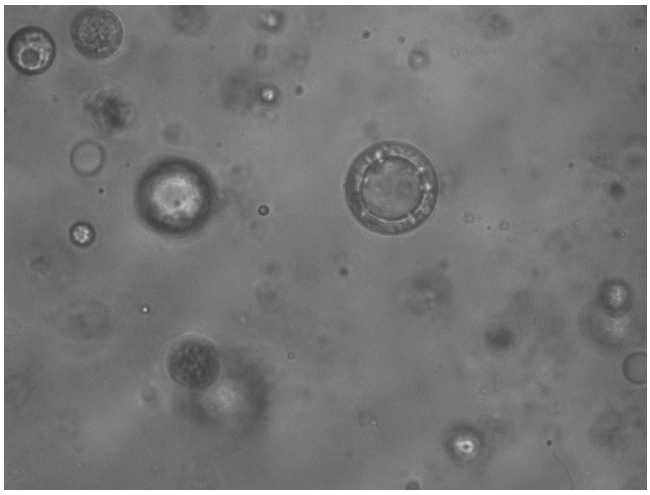

Figure 2. Microscopic image of a double emulsion after 120 min simulated digestion

CONCLUSIONS

We managed to produce a reddish double w/o/w emulsion that seemed to be stable for a couple of days. The stability of the double emulsion and the effect of encapsulation into betalain stability need to be investigated further. Also the amount of betalain in the inner water phase should be higher to produce a stronger color. The simulated lipid digestion of a double emulsion seemed to affect the droplet sizes of both the primary emulsion and the double emulsion. The experiment should be repeated with a larger sample to enable duplicate measurements and maybe more time points for droplet size measurements.

REFERENCES

- Aro H. et al. (2007) *The characterization of oat lipids produced by supercritical fluid technologies.* J. Cereal Sci. 45 116-119
- Kaimainen et al. (2012) *Polar lipid fraction from oat (*Avena sativa*): characterization and use as an o/w emulsifier.* Eur. Food Res. Technol. 235(3) 507-515

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