Encapsulation of Yeast Cells as Probiotics by Tableting

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INTRODUCTION

There is an increasing demand from the pharmaceutical and food industries for new probiotic products. Some yeast strains offer probiotic benefits (Sadaa et al. 2013). Capsules and sachets containing yeast cells are currently available on the market; however, tablet dosage forms offer advantages including ease of manufacture and convenience of dosing. Direct compression is regarded as the best technique for producing tablets containing dried yeast (Al-Mohizea et al. 2007). The main challenge of tabletting a powder mixture containing yeast granules is the mechanical sensitivity of the cells (Plumpton et al. 1986).

The objective of this work was to develop a rigid yeast tablet containing adequate numbers of viable yeast cells by optimizing the formulation to allow a relatively low compaction force to be used and minimising damage to them.

MATERIALS AND METHODS

Selection of tablet excipients Tablets were produced using Microcrystalline Cellulose, (Avicel PH102) (MCC) d_{50} 166 µm, Dibasic Calcium Phosphate, (anhydrous) (DCP) d_{50} 46 µm and Magnesium Stearate as a filler, binder, and lubricant, respectively. Dried yeast granules (*S. cerevisiae*) d_{50} 416 µm provided by Lesaffre, France were included in the formulation as the active.

Tablet press Tablets were prepared by direct compression using a single punch tablet press at 30 $mm.min^{-1}$ punch speed. A 1 g pre-weighed powder mixture containing yeast granules and powder blend of different ratios was introduced into a die of 13 mm diameter. Tablets were produced in triplicates per characterisation test under a determined pressure of 105 MPa.

Mechanical strength characterisation of tablets Resulting tablets were characterised with respect to their mechanical strength using the diametric compression test. The force (N) required to cause failure of the compact was converted to determine the tensile strength (MPa).

Determining cell viability in tablets A cell enumeration experiment was conducted in order to determine the effect of compaction pressure on yeast cell viability. 1 g tablets were dispersed in 100 mL TS Buffer. 0.1 mL of suspension were diluted and placed on yeast malt agar medium plates. The plates were incubated at 25 °C for 72 hours and colonies counted.

Compaction behaviour analysis The compaction behaviour in terms of compaction pressure versus displacement of powder bed (volume reduction) was investigated. The stress versus strain curves from compaction of powders were obtained and fitted using the following Kawakita model:

$$\frac{\sigma}{\varepsilon} = \frac{1}{ab} + \frac{\sigma}{a}$$

where ε is the degree of volume reduction, which is equivalent to the uniaxial strain:

$$\varepsilon = \frac{h_i - h_p}{h_i}$$

where h_i is the initial height of the powder bed and h_p is the value at the current applied stress, σ. The parameters a and b are constants.

RESULTS AND DISCUSSION

Preformulation studies showed that for compaction pressures of 45 -187 MPa, dried yeast granules alone did not compact into rigid tablets, since the resulting tablets were unable to withstand handling indicating poor mechanical strength. Therefore, compressible excipients were included to produce rigid tablets. At a compaction force of 105 MPa with a fixed excipient formulation, different ratios of yeast and excipients in tablets resulted in varying tensile strength and viable cell number (CFU) (Fig 1).

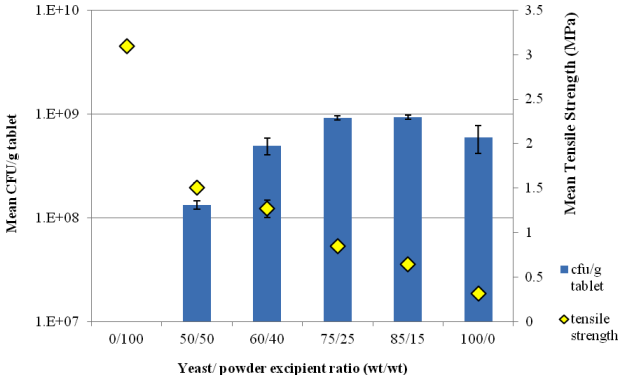

Figure 1 : The CFU and tensile strength of tablets containing varying yeast: powder blend ratio at compaction pressure 105 MPa. Mean±sd (n=3)

The reduction in tablet tensile strength with increasing

yeast quantity may be explained by the poor binding properties exhibited by the yeast granules compared to powder excipients particles. On the other hand, the positive effect of incorporating a higher quantity of yeast resulted in higher CFU per tablet post compaction.

Compression curves were fitted using the Kawakita model (Fig 2). The value of constant a is related to the initial bed voidage (Yap et al. 2008) which was found to be lowest for the formulation with greatest amount of yeast (Table 1). The difference in particle size could explain these results whereby smaller powder particles are able to fill the voids between yeast granules during the die filling process.

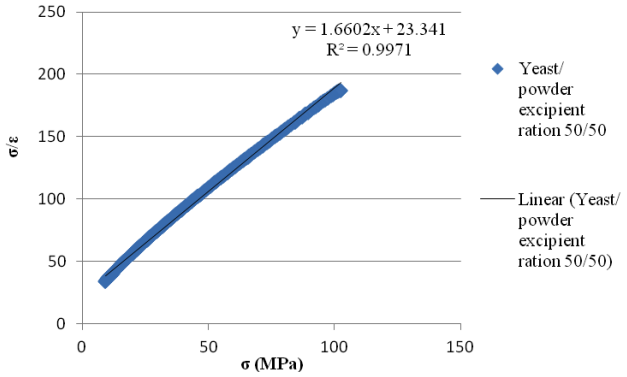

Figure 2 : A typical compression curve fitted by Kawakita model for the compaction of granular yeast and powder excipients at 50:50 (%w/w) ratio produced at compaction pressure 105 MPa.

Constant b has the dimension of the reciprocal of stress which is related to the failure stress of the single particles of the tablet feed powder. The results show that yeast granule particles require higher stresses to cause failure than powder particles.

Table 1: Kawakita parameters determined from compression of yeast granules and excipients with varying ratio at a compaction pressure 105 MPa.

Formulation Yeast: Powder	Kawakita parameter		1/b (MPa)	R^2
	a	b		
0:100	0.66	0.073	13.8	0.999
50:50	0.60	0.071	13.5	0.997
60:40	0.55	0.059	16.9	0.996
75:25	0.53	0.057	17.5	0.995
85:15	0.52	0.057	17.5	0.995

The adverse effect of the tabletting process on viable yeast cell number is further seen when tablets were produced at increasing compaction forces (Fig 3).

For a given formulation (50% yeast per tablet) as the compaction force increases, the cell viability per tablet decreases. However, positive effects are seen with tablet strength; as compaction force increases, as does the tensile strength.

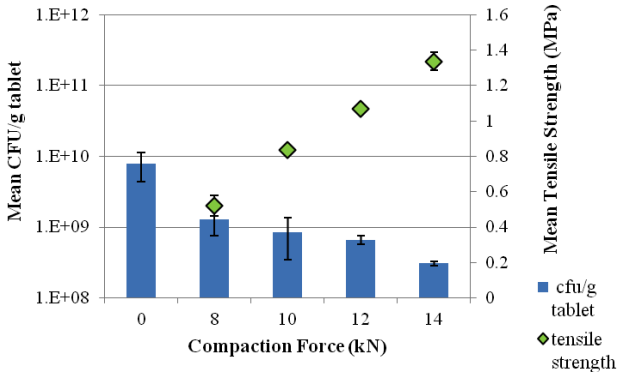

Figure 3 : Compaction of tablets containing yeast granules and powder excipient blend (50:50 ratio) at varying compaction force. Mean±sd (n=3)

CONCLUSIONS

The CFU and tensile strength of tablets made of yeast granules and powder excipients with different ratios and produced using varying compaction force have been investigated. The experimental results highlight the importance of binder and filler within a formulation and of their proportion, if rigid tablets are to be formed with minimum damage to cells. The Kawakita model used to fit the compaction data demonstrates the influence of the inclusion of yeast granules on the compaction behaviour. Future work includes understanding the mechanical properties of feed particles and particle-particle interactions, and how they are related to the compaction behaviour and tensile strength in order to produce desirable probiotic yeast tablets.

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