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*Original Article*

# The Effect of Humidity and Compactional Pressure on the Wheat Germ Lipase Activity

Mohammad Hossein Zarrintan[a*], Hak-Kim Chan[b] and Farnaz Monazjjemzadeh[a]

[a]*Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.* [b]*Department of Pharmacy, University of Sydney, Australia.*

**Abstract**

The use of proteins and peptides as human therapeutics has been increased in recent years. Lipase is a relatively homogeneous proteinaceous enzyme indicated in maldigestion. The purpose of the present study was to evaluate the effect of humidity along with compactional pressure on the enzymatic activity of wheat germ lipase.

Samples of lipase powder were kept at different relative humidity (RH) conditions (24, 40, 63 and 75 %) before compaction under various pressures (74-372 Mega pascal or Mpa). The relative enzymatic activity of the compacts was then determined by titration method using Triacetin as substrate. Density measurements were also conducted in order to describe the possible mechanism of the enzymatic activity. The results indicated densities of the compacts prepared under various compactional pressures increase as humidity rises. Based on the results, activity loss of the compacts following relative humidity increase can be related to steric hindrance caused by higher pressures. However, there was no significant difference between densities at different compactional pressures at a given humidity condition.

**Keywords:** Protein; Enzyme; Wheat Germ; Lipase; Compaction; Humidity; Activity; Density.

## Introduction

The use of proteins and peptides as human therapeutics has been increased in recent years due to: (a) discovery of novel peptides and proteins, (b) a better understanding of their mechanisms of action in vivo, (c) improvements in expression or synthesis of proteins and peptides that closely resemble human proteins and peptides, and (d) improvements in formulation or molecule-altering technologies capable of delivering polypeptides in vivo with improved pharmacokinetic and pharmacodynamic properties. In the year 2000, as many as 500 biopharmaceutical products were estimated to be in clinical trial phase. Furthermore, annual growth rates of protein products (glycoproteins, unglycosylated proteins and antibodies) likely range from 10 to 35% (1). Although more biopharmaceuticals are under development than ever before, many of them have problems typical for polypeptide therapeutics including short circulating half-life, immunogenicity, proteolytic degradation and low solubility. Several strategies have emerged to improve the properties of these biopharmaceutical preparations (1).

Castillo and colleagues investigated the activity loss of hydrolases in various organic solvents (2). Many factors affect the stability of proteins during storage among

* Corresponding author:
E-mail: zarrintanmh@yahoo.com.

which moisture and temperature are critical. Instabilities of protein pharmaceuticals, like other pharmaceutical products, are detected after prolonged storage under ordinary conditions. Therefore, in order to estimate a protein product shelf-life, formulations are exposed to elevated temperatures and/or humidity, which accelerate their instability. Many reports cited the impact of temperature and moisture on the protection of biopharmaceuticals from denaturation under thermal stress arising from drying methods (3). In 1993, Shah and Ludescher (?) concluded water is important for maintaining conformation stability and biological activity of proteins. Thus, a water layer surrounding proteins maintains folded conformations through Van der Waals' interactions, salt bridges and hydrogen bonds. Their results suggested that water acts as a plasticizer and thus the molecular mobility increases. Hence, the protein dynamically changes from a glassy, solid state to the rubbery and then liquid form (3).

Lipase is a relatively homogeneous proteinaceous enzyme indicated in maldigestion. This enzyme hydrolyses dietary triglycerides at the alpha position resulting in fatty acid formation (4). Lipase is supplied as tablets with or without other enzymes (5). Teng and Groves reported loss of urease activity and some other biologically active proteins due to compaction pressure (6). Thermal energy produced during compaction process may also affect protein activity (7). There are some other factors involved that already studied by other researchers (8, 9).

We have previously shown that compaction pressure account for loss of lipase activity to some 30%. The density did not exceed a limiting value of approximately 1.2 g $cm^{-3}$, irrespective of the applied pressure. An approximately linear relationship was seen between the relative biological activity loss and density, indicating that the observed loss of biological activity was unlikely due to applied thermal energy but mainly because of steric hindrance for molecules (10).

The purpose of this study was to evaluate the effect of humidity together with compaction pressure on the activity of wheat germ lipase.

## Experimental

### Materials

Wheat germ lipase (Cat. No. L 3001), Triacetin, Tris base, Thymolphthalein indicator and Sodium hydroxide 0.05N were obtained from Sigma. Monobasic Potassium Phosphate was supplied by Fisher.

### Methods

*1. Lipase compaction*

A hundred mg of lipase powder previously stored over a desiccator of known equilibrated relative humidity was placed in a non-lubricated stainless steel punch and die set and compacted at the compressional pressures at the ranges of 74-370 Mpa as our previous report (10). Weight and diameter of tablets produced were determined immediately after ejection. Tablets were stored in a closed container until activity measurement.

*2. Activity measurement*

Enzymatic activity was measured as described elsewhere (10). Briefly, Lipase activity was analyzed by measuring the rate of hydrolysis of a standard Triglyceride, i.e. Triacetin and titration of resulting fatty acid with alkali.

**Table 1.** Mean densities ($gr/cm^3$) from three measurements for Lipase powder stored at relative humidity (24-75% RH) and compacted with compactional pressure of 74-372 Mpa.

| Mean compaction pressure (Mpa) | Density of compacts ($gr/cm^3$) | | | |
|---|---|---|---|---|
| | 24% RH | 40% RH | 63% RH | 75% RH |
| 74.4 | 1.131 | 1.225 | 1.245 | 1.299 |
| 111.6 | 1.226 | 1.224 | 1.346 | 1.350 |
| 223.3 | 1.254 | 1.295 | 1.333 | 1.360 |
| 372.2 | 1.290 | 1.302 | 1.340 | 1.360 |

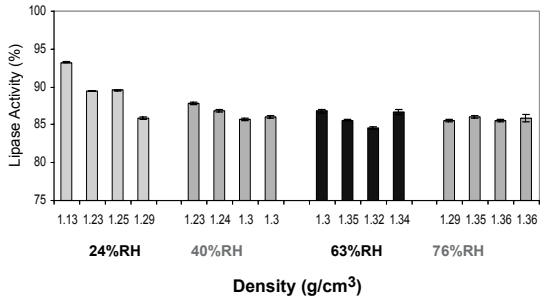


**Figure 1.** Results of relative activity of lipase compacts (Compact /powder) * 100, obtained from the compaction of lipase powder kept at different relative humidity (n=3)

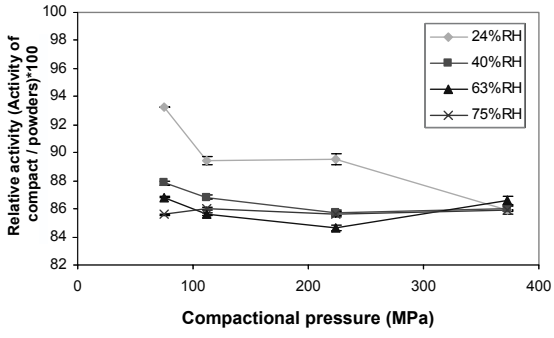


**Figure 2.** Effect of compaction pressure on Lipase activity at different relative humidity

## Results and Discussion

Densities were determined using diameter and weight of the prepared tablets. Average densities (triplicate data) for compacts of lipase samples kept at different relative humidity and produced under various pressures are shown in Table 1.

As shown in Table 1, with almost every compaction pressure, density increases as relative humidity rises. At compaction pressure of 74.4 Mpa for 24 and 75% RH, compact densities of 1.131 and 1.299 g/cm$^3$ were obtained, respectively. Porosity decrease in compacts due to high humidity that reduce interparticular distance may explain this effect as previous reported (11). However, it was evident that compact density for the relative humidity of 75% was higher in every pressure and denser compacts were produced either in higher pressures or relative humidity. This finding is in agreement with others (6).

Relative lipase activity of compacts (activity of compact/activity of powder × 100), made from samples of lipase powder kept at different relative humidity, versus compact densities are observed in Figure1.

As shown in Figure 1, lipase activity in compacts appears to be humidity dependent, the higher the humidity the lower the lipase activity at all pressures. Others (12, 13) also reported that with proteins, storage humidity prior to compaction would highly affect the compact behavior. However, there was no significant difference between densities at different compaction pressures in a given humidity.

As shown in Figure 2, relative lipase activity is associated with humidity and compaction pressure. Concomitant effects of the two factors are not easy to explain. Nevertheless, a compact with desirable lipase activity could be prepared using certain pressures and humidity.

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