Journal of Pharmaceutical Investigation (2022) 52:601–609
https://doi.org/10.1007/s40005-022-00580-0

ORIGINAL ARTICLE

Development and evaluation of a digestive formulation using a microbial enzyme for treatment of dyspepsia

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Received: 2 April 2022 / Accepted: 24 May 2022 / Published online: 30 July 2022
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Abstract

Purpose A digestive enzyme is prepared as a treatment for dyspepsia by aiding in the breakdown of food, and its representative ingredient is porcine pancreatin. This study aims to develop a fast and effective digestive formulation by replacing animal pancreatin with a microbial enzyme.

Methods A non-animal digestive tablet was developed as a film-coated tablet, and its digestibility and disintegration properties were evaluated by the KP (Korean Pharmacopeia 12th edition) method.

Results Porcine pancreatin has amylase activity, protease activity, and lipase activity, and the activity scores of the three enzymes differ slightly. The microbial digestive enzyme has various characteristics depending on the source. A coated tablet containing microbial digestive enzymes, simethicone for gas removal, soluble azulene as a mucosal repair agent, and swertia as a stomachic was developed. It showed stable results for 6 months under long-term and accelerated storage conditions. The coating layer of the tablet dissolved rapidly at gastric pH, and the tablet completely disintegrated within 26 min. The amylase activity, protease activity, and lipase activity of the tablet were relatively higher than those of the commercial product at gastric pH after meals. In particular, lipase activity was higher than that of the commercial products at both gastric and small intestinal pH after meals.

Conclusion Reflecting the food intake of modern Koreans, we developed a non-animal complex digestive tablet containing microbial enzymes. The tablet disintegrated rapidly at postprandial gastric pH and showed high digestive activities in the range from gastric pH to small intestine pH after meals.

Keywords Digestive enzymes · Pancreatin · Microbial enzyme · Optimal pH · Dyspepsia

Introduction

Dyspepsia is persistent or recurrent abdominal pain or abdominal discomfort centered in the upper abdomen and includes the symptoms of post-prandial fullness, early satiety, and nausea (Talley 1991). The causes of dyspepsia include peptic ulcer disease, gastro-esophageal reflux, and functional dyspepsia. Functional dyspepsia (FD), also known as non-ulcer dyspepsia, is the most common cause of dyspeptic symptoms (Locke 1998). Globally, the prevalence of unreported dyspepsia (UD) ranges between 7 and 45% depending on the definition used and geographic location, and the prevalence of FD varies between 11 and 29.2% (Mahadeva and Goh 2006).

According to Rome IV criteria, FD is one or more of postprandial fullness, early satiety, epigastric pain, and epigastric burning without evidence of structural disease via upper endoscopy that could explain the symptoms (Tack et al. 2006). Basic pathophysiology of functional dyspeptic symptoms is unclear. FD symptoms are considered to occur due to a combination of visceral hypersensitivity, gastric motor dysfunction, and psychological factors. Strategies such as acid suppression, prokinetics, and *H. pylori* eradication have been used with some success (Chen 2013). Transient deficiency in digestive enzymes is one of the contributors to FD. A commonly used and safe

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treatment is oral enzyme supplementation therapy, and the main digestive enzymes are amylase, protease, and lipase (Swami and Shah 2017).

Recent studies have shown that FD is related to food type and eating habits. Accumulated data suggest that infection and food play important roles in some cases of FD (Talley 2016). High-fat meals slow gastric emptying and can lead to dyspepsia, while moderate-to-fast eating and irregular meals are also associated with dyspepsia (Pilichiewicz et al. 2009; Keshteli et al. 2015).

Dysfunction of the digestive system, eating habits, dietary choices, and age can cause a deficiency of digestive enzymes (Swami and Shah 2017). Aging reduces secretion of digestive enzymes, with decrease of both basal and stimulated pepsin, and secretion of pepsin decreases by approximately 40% in the elderly (Feldman et al. 1996). In a study on pancreatic exocrine secretion with age, lipase, phospholipase, and chymotrypsin decreased linearly in concentration and output with age from 30 years (Laugier et al. 1991).

Digestive enzymes such as amylase, protease, and lipase are produced and secreted by the gastrointestinal (GI) system and aid in digestion by facilitating the breakdown of larger molecules present in food such as carbohydrates, proteins, and fats and absorption of nutrients (Ianiro et al. 2016). Most commercially available enzyme supplements are pancreatic enzymes extracted from a porcine source (Ianiro et al. 2016, KP). Pancreatin is the active pharmaceutical ingredient of various digestion products (EP, USP, KP). Though no virus contamination events in pancreatin have been reported, viral contamination is a common feature of all biological products obtained from all materials of animal origin. Some pancreatin manufacturers are taking steps to eliminate such virus in pancreatin of porcine origin (Caruso et al. 2014). In addition, African swine fever (ASF) was reported in August 2018, creating supply and demand issues of porcine pancreatin (Ma et al. 2021). Recent studies have shown increased interest in replacing or mixing pancreatin with plant-based and microbial-derived enzymes (Ianiro et al. 2016; Majeed et al.2018; Ran et al. 2009).

The purpose of this study is to develop safe and effective digestive enzyme products. Pancreatin is an animal source and has the disadvantage of being unstable at gastric pH. Therefore, we intend to develop a fast-acting digestive tablet that is digested quickly in the stomach by replacing pancreatin with a microbial digestive enzyme that is stable at gastric pH.

In addition, considering the change in food intake of modern people, who have increased their fat intake by 26% over the past 10 years, we have developed a digestive tablet that enhances fat digestion compared to commercial products (Korea Health Statistics 2019). In addition to digestive enzymes, the complex digestive tablet contains antacid, stomachic, stomach mucosal restorative agent, and degassing agent that help digestion.

Materials and methods

Materials

Porcine pancreatin was purchased from Biozym (Germany), Terhormon (Italy), Sichuan Deebio (China), and Biosyn (China) as commercially available raw materials that had undergone virus inactivation. The microbial enzymes diastase protease cellulase, Prozyme, and lipase were purchased from Amano Enzyme Inc. (Japan). All other ingredients for tablet formulation were purchased as pharmaceutical grade. Other chemicals used were of HPLC or analytical grade.

Preparation of tablets

The seven compounds diastase protease cellulase (Amano Enzyme Inc., Japan), prozyme (Amano Enzyme Inc., Japan), lipase (Amano Enzyme Inc., Japan), swertia (Dongbang FTL, South Korea), magnesium oxide (Tomita Pharmaceutical Co., Ltd., Japan), simethicone (Eigenmann & Veronelli SPA, Italy), and soluble azulene (Alps Pharmaceutical Ind. Co. Ltd., Japan) were used as active pharmaceutical ingredients (APIs). Diluents, disintegrant, and lubricants were mixed with the active ingredients using a blender (L.B.Bohle LM20, Germany), compressed on a tablet machine (Killian SP300, Germany), and coated in a lab coater (O'Hara Labcoat LCM, Canada). The detailed compositions of digestive tablets are given in Table 1.

Table 1 Compositions of the new digestive product

Category	Ingredients
APIs	Diastase protease cellulase
	Prozyme
	Lipase
	Magnesium oxide
	Swertia
	Soluble azulene
	Simethicone
Diluents	Silicified microcrystalline cellulose 90
Disintegrant	Croscamellose sodium
Lubricants	Magnesium stearate
	Colloidal silicon dioxide
Coating agents	Hypromellose 2910
	Polyethylene glycol 6000
	Eudragit E PO Readymix clear 390.05
	Titanium dioxide

Stability test

Coated tablets were packed with Perlalux®-tristar ultra and aluminum foil using a blister packing machine (Sepha, EZ blisterII, UK). The stability studies were carried out at 25 ± 2 °C/60 ± 5%RH, 40 ± 2 °C /75 ± 5%RH for 6 months using a stability chamber (Vötsch VP 2000, VP1300, Germany).

Digestion test

The digestion test measures the activity of digestive enzymes on starch, protein, and fat in raw materials and products according to the KP method. pH buffers of pH 4.5, 6.0, 7.0, 8.0, and 9.0 conditions are specified in the KP.

Amylase activity is determined according to the characteristics of each enzyme. The amylase assay of pancreatin was performed through the measurement of starch saccharifying activity. Such activity can be obtained by measuring an increase of reducing activity due to hydrolysis of glucoside linkages when amylase acts on starch, and the concentration of test solution was 0.4 to 0.8 starch saccharifying activity units/mL. The amylase assay of microbial enzyme was performed through the measurement of starch dextrinizing activity. The starch dextrinizing activity can be obtained by measuring a decrease in starch coloration by iodine resulting from hydrolysis of the straight chain component in starch under amylase activity, and the concentration of test solution was 0.2 to 0.5 starch dextrinizing activity units/mL.

Protease activity of pancreatin and microbial enzyme was measured using the same method. The protein digestive activity can be obtained by colorimetric measurement using Folin' reaction of the amount of acid-soluble low-molecular-weight products and is increased due to hydrolysis of peptide linkages when protease acts on casein. The concentration of test solution was 15 to 25 protein digestive activity units/mL.

Lipase activity of pancreatin and microbial enzyme was measured using the same method. The fat digestive activity can be obtained by back titration of the amount of fatty acid produced from hydrolysis of ester linkages when lipase acts on olive oil. The concentration of test solution was 1 to 5 fat digestive activity units/mL.

Assays

The amylase, protease, and lipase assays were performed according to the digestion test in KP. Amylase was measured by starch dextrinizing activity method at pH 5.0, 37 ± 5 °C reaction conditions, and the absorbance was determined with a UV (Ultraviolet–visible) spectrophotometer (Jasco V650, Japan) at the wavelength of 660 nm. Protease was measured at pH 7.0, 37 ± 5 °C reaction conditions, and the absorbance was determined with a UV spectrophotometer (Jasco V650, Japan) at the wavelength of 660 nm. Lipase was measured at pH 7.0, 37 ± 5 °C reaction conditions and titrated using a potentiometric titrator (Metrohm 888 titrando, Switzerland).

The magnesium oxide assay was performed based on the acid-neutralizing capacity test in KP. The test determines the acid-neutralizing capacity of a medicine that reacts in the stomach. The swertia assay was performed according to powdered swertia herb in JP, and the content of swertiamarin was determined using HPLC (Agilent 1200, Santa Clara, CA, USA). Soluble azulene was tested with a modified KP assay and determined using HPLC (Agilent 1200, USA). Simethicone was tested according to USP, and the content of polydimethylsiloxane was determined with an infrared spectrophotometer (Jasco FT/IR 4700, Japan).

Disintegration test

The disintegration test was performed by the method of immediate-release preparations of the general tests of KP. It was carried out using a disintegration tester (Erweka ZT324, Germany) in pH 4.5, pH 6.8, and pH 7.0 buffer.

Results

Digestive activities of porcine pancreatin

The digestion tests of four commercially available pancreatins were performed by EP and KP methods, and the activities of the three enzymes were compared. As shown in Fig. 1, each pancreatin has amylase activity, protease activity, and lipase activity, and the activity ratios of the three enzymes are slightly different among four commercially available pancreatins.

Enzyme activity of pancreatin was pH-dependent. The amylase activity of Biozym pancreatin was measured under the conditions of pH 4.5, pH 6.0, pH 7.0, pH 8.0, and pH 9.0. Figure 2 shows that the optimal pH of amylase of pancreatin was 7.0. At pH below 6.0 and above pH 9.0, the activity was relatively low.

Characteristics and digestive activities of microbial enzymes

The microbial enzyme has various characteristics depending on microbial source.

Among microbial enzymes that meet pharmacopeia standards and can be used as active ingredients in pharmaceuticals, enzymes were selected for high digestive activity and stability at gastric and intestinal pH after meals. Three digestive enzymes with amylase, lipase, and protease activities, as listed in Table 2, were finally selected.

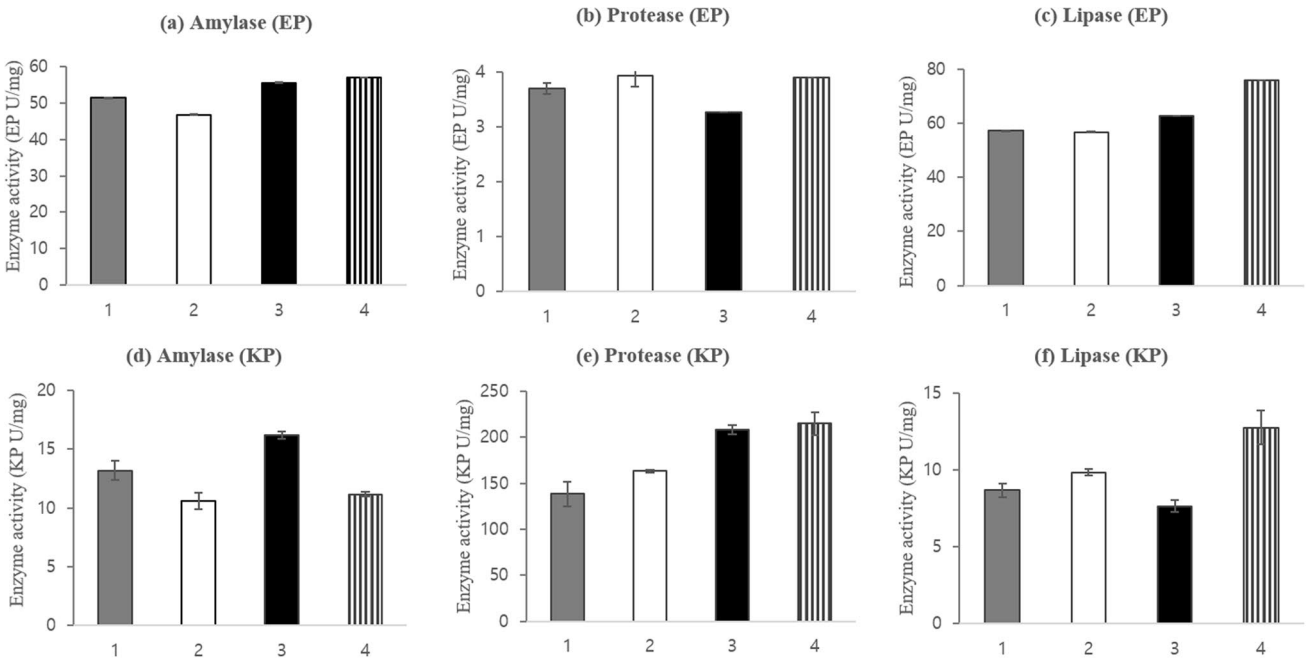

Fig. 1 Enzymetic activities of porcine pancreatin. The four enzymes were compared by EP and KP assays: **a**, **d** Amylase activity, **b**, **e** protease activity and **c**, **f** lipase acitivity pancreatins are products from the following manufacturers: (1. pancreatin from Biozym, 2. pancreatin from Sichuan Deebio, 3. pancreatin from Sichuan Biosyn, 4. pancreatin from Terhormon) Enzyme activities are means ± SD (n = 3)

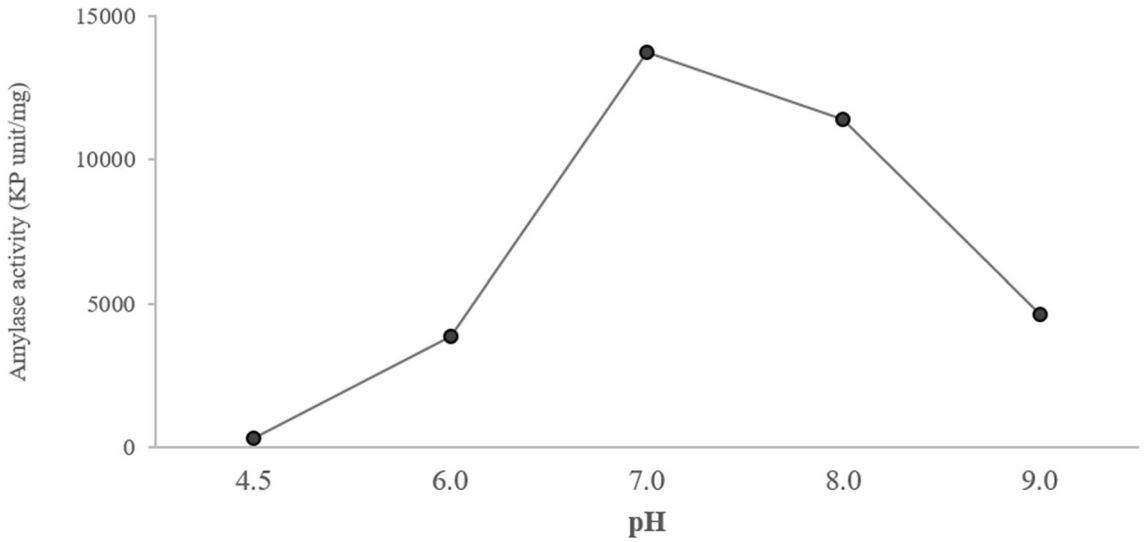

Fig. 2 Amyalse activity depending on pH of pancreatin from Biozym. Amylase activity was performed through the measurement of starch saccharifying activity under the conditions of pH 4.5, pH 6.0, pH 7.0, pH 8.0, and pH 9.0

The optimum pH data were provided by the manufacturer, and the optimum pH range for each material was in the range of pH 5.0 to pH 8.0. In particular, protease was active in two materials and in a wide pH range from pH 2.5 to pH 10.0.

In KP, the activity of each enzyme is set to not less than 90% of the indicated amount. Therefore, in order to establish a specification, the activity of the selected enzymes was measured by the KP method.

Development of a non-animal digestive product

The new digestive product Superzyme (HD-P104) was developed using non-animal ingredients. As shown in

Table 2 Characteristics of microbial enzymes

Ingredients	Microbial source	Enzyme type	Optimal pH (range showing greater than 50% activity)	Measured enzyme activity (measured pH)
Diastase·protease·cellulase	*Aspergillus* genus or *Trichoderma koningi*	Amylase	pH 5.0 (pH 3.0 ~ 6.5)	33,546 U/g (pH 5.0)
		Protease	pH 6.0 (pH 2.5 ~ 8.0)	84,885 U/g (pH 5.0)
		Cellulase	pH 5.0 (pH 3.5 ~ 5.5)	0.2 U/g (pH 4.5)
Protease	*Aspergilus melleus*	Protease	pH 8.0 (pH 6.0 ~ 10.0)	839,443 U/g (pH 7.0)
Lipase	*Rhizopus oryzae*	Lipase	pH 7.0 (pH 5.0 ~ 7.0)	164,595 U/g (pH 7.0)

Table 1, Superzyme contains microbial enzymes as digestive enzymes, antacid, stomachic, stomach mucosal restorative agent, and degassing agent. Magnesium oxide antacid was used as a stabilizer for digestive enzymes at gastric pH as well as for neutralizing stomach acid. In addition, swertia as a stomachic agent, soluble azulene as a mucosal restorative agent, and simethicone as a degassing agent were added to the Superzyme.

To increase the stability of Superzyme, the coating was applied in two layers using different coating agents. The primary coating agent, Hypromellose 2910 was used to physically separate the core tablet containing various ingredients from Eudragit E PO Readymix clear 390.05, a cationic polymer, was selected as the secondary coating agent because it forms a film at a low temperature and has superior taste masking effect and high moisture protection effect.

The active pharmaceutical ingredients were compared with the commercial product based on the one-time intake in Table 3. Superzyme contains relatively diverse categories of ingredients compared to commercial products, and all active ingredients are non-animal ingredients.

Evaluation of a non-animal digestive product

The stability of Superzyme was confirmed in the long-term conditions (25 °C /60%RH) and the accelerated conditions (40 °C /75%RH). All ingredients showed no significant difference for 6 months under the long-term storage conditions (Table 4). Lipase activity was decreased under the accelerated conditions (40 °C /75%RH), but was within product specifications (Table 5).

Since swertia is a natural product, the content of swertiamarin varies greatly depending on the cultivation environment, and the batch used for the stability test had a relatively high content (Tables 4 and 5).

Superzyme was coated with a coating agent that disintegrates at low pH for rapid decomposition in the stomach. The pH of saliva is about 7.0, the pH of the stomach after eating is 3.5–5.0, and the pH of the small intestine is 6.0–6.8. The coating layer of the tablets dissolved within 2 to 3 min at gastric pH, and the tablets completely disintegrated within 26 min. At pH 6.8, the tablets did not disintegrate at all over 2 h (Table 6). The coating layer does not disintegrate in the

Table 3 Comparison of active pharmaceutical ingredients (APIs) with commercially available digestive products

Classification[a]		APIs	Superzyme 2 tablets (mg)	Festal Plus® 2 tablets (mg)	Bearse® 1 tablet (mg)
Column III	Digestive enzyme (microbial enzyme)	Diastase Protease Cellulase	120.0	–	50.0
		Prozyme	70.0	–	–
		Lipase	60.0	–	15.0
		Pancellase	–	–	30.0
		Panprosin	–	–	20.0
		Cellulase	–	20.0	–
	Digestive enzyme (pancreatin)	Pancreatin	–	630.0	–
		Pancreatin enteric granules	–	–	78.6
	Cholagogues	Ursodeoxycholic acid	–	20.0	10.0
Column I	Antacid	Magnesium oxide	120.0	–	–
Column II	Stomachic	Swertia	16.7	–	–
Column VII	Mucosal restorative agent	Soluble azulene	1.0	–	–
Column VIII	-	Simethicone	60.0	60.0	40.0

[a]Classification was according to Korean manufacturing standard for medicine

Table 4 Stability results under the long-term storage conditions

APIs	Specification	Long term conditions (25 °C /60%RH)		
		Initial	3 months	6 months
		Mean ± SD	Mean ± SD	Mean ± SD
Amylase	Amylase activity ≥ 90.0%	137.6 ± 3.7	139.5 ± 3.5	147.2 ± 3.2
Protease	Protease activity ≥ 90.0%	139.6 ± 2.6	143.7 ± 6.8	148.4 ± 6.4
Lipase	Lipase activity ≥ 90.0%	135.6 ± 3.1	146.7 ± 3.3	135.5 ± 1.1
Magnesium oxide	Acid-neutralizing capacity ≥ 50 ml	62.2 ± 1.4	60.9 ± 0.3	61.1 ± 1.3
Swertia	Swertiamarin ≥ 90.0%	522.5 ± 24.1	528.0 ± 14.2	577.3 ± 24.3
Soluble azulene	Soluble azulene 90.0–110.0%	96.4 ± 0.9	95.9 ± 1.0	95.0 ± 1.2
Simethicone	Simethicone 85.0–115.0%	101.0 ± 0.2	101.5 ± 1.5	98.3 ± 1.2

The specifications for assays of all APIs were established in accordance to Korean manufacturing standard for medicine. All data are results for three batches, and data are shown as mean ± SD

Table 5 Stability results under the accelerated storage conditions

APIs	Specification	Accelerated conditions (40 °C/75%RH)			
		Initial	2 months	4 months	6 months
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Amylase	Amylase activity ≥ 90.0%	137.6 ± 3.7	141.3 ± 1.1	129.8 ± 3.4	147.2 ± 3.2
Protease	Protease activity ≥ 90.0%	139.6 ± 2.6	143.9 ± 2.9	135.8 ± 3.6	138.4 ± 2.1
Lipase	Lipase activity ≥ 90.0%	135.6 ± 3.1	143.0 ± 3.9	124.1 ± 5.4	123.5 ± 5.6
Magnesium oxide	Acid-neutralizing capacity ≥ 50 ml	62.2 ± 1.4	61.2 ± 1.0	–	59.2 ± 1.3
Swertia	Swertiamarin ≥ 90.0%	522.5 ± 24.1	531.8 ± 41.9	–	561.8 ± 11.7
Soluble azulene	Soluble azulene 90.0–110.0%	96.4 ± 0.9	96.5 ± 0.6	94.9 ± 1.1	93.6 ± 0.3
Simethicone	Simethicone 85.0–115.0%	101.0 ± 0.2	98.0 ± 1.2	–	96.0 ± 0.5

The specifications for assays of all APIs were established in accordance to Korean manufacturing standard for medicine. All data are results for three batches, and data are shown as mean ± SD

Table 6 Disintegration test of coated tablets

pH buffer	Disintegration time
pH 4.5 buffer	Within 26 min
pH 6.8 buffer	No disintegration
pH 7.0 buffer	No disintegration

mouth, so there is no smell of raw materials, and it disintegrates quickly in the stomach, so the disintegrated digestive fluid is expected to flow into the small intestine.

The digestive enzyme activity of the developed tablet was compared with that of a commercial product. Since the pH of the stomach after a meal is 3.5–5.0, and the pH of the upper small intestine after a meal is about 6.0, the digestion of digestive enzymes was measured at pH 4.5 and pH 6.0, similar to that of the GI tract after a meal.

The activities of all three digestive enzymes of Superzyme were relatively higher than the activities of the commercial products (Festal plus® and Bearse®) at the pH of the stomach after a meal. Especially, lipase activity was much higher than that of commercial products at both pH

values. In addition, the amylase and protease activities of the developed tablets were relatively high at pH 4.5 compared to those of commercial products.

Discussion

The pharmaceutical constituent pancreatin is a substance containing enzymes prepared from the pancreas of edible animals, mostly the hog (*Sus scrofa* Linne var. *domesticus* Gray (Fam. Suidae)), and has amylolytic, proteolytic, and lipolytic activities (EP, USP, KP). The activity and concentration of these pancreatic enzymes are determined by several factors, including animal species, sex, and age, as well as husbandry practices (Cichoke 2006). Pancreatin is labeled as a whole-number multiple of the three minimum activities, and pancreatins, which are about 8 times the USP standard, were prepared and the activities were measured. The developed pancreatin showed activities of amylase, protease, and lipase; the proportion of each activity varied depending on source; and there was a difference between the KP and EP test methods (Fig. 1).

In the standardization study of pancreatin, when pancreatin evaluated by the KP analytical method was converted to EP analytical method, amylase, protease, and lipase activities previously were multiplied by constants 12.65, 0.013, and 4.27, respectively (Shin et al. 2003). However, the constants in this study were different for each pancreatin, with average respective constants of 4.22, 0.02, and 6.65. Amylase activity in particular was different from that in previous studies.

The optimal pH for the activity of pancreatic enzymes in the human duodenum is 7–8 (Capurso et al. 2019). More specifically, pancreatic enzymes can be divided into three groups according to their function: proteolytic enzymes, amylolytic enzymes, and lipolytic enzymes. The optimal pH range of proteolytic enzymes (trypsin and chymotripsin) is 7.9–9.7, the optimal pH range of amylolytic enzymes (pancreatic amylase) is 6.7–7.2, and the optimal pH of lipolytic enzymes (principally lipase) is 8.0 (Roxas 2008; Berdutina et al. 2000). Even in the pharmacopoeia, digestion tests of pancreatin are performed at 5.0–6.8 for amylase, 8.0–8.5 for protease, and 7.0–9.0 for lipase (EP, USP, KP). In this study, the optimal pH of amylase of pancreatin was 7.0 (Fig. 2). Because pancreatin is unstable at gastric pH, it requires an enteric coating. Digestive products using pancreatin play a role in digestion in the intestine rather than the stomach.

Recently, the global supply of pancreatin has become unstable due to African swine fever (ASF) and increasing price. Although pancreatin is manufactured through a virus inactivation process, preference for non-animal raw materials is increasing in consumption of products or foods, such as halal and vegan products.

As an alternative to pancreatin, a commercially accessible microbial enzyme of the pharmacopoeia standard was reviewed. Since microbial enzymes have many characteristics depending on the source, drug substances were selected based on amylase, protease, and lipase enzymatic activities, which are active in gastric pH and at small intestinal pH after a meal (Table 2).

According to the Korea Health Statistics Report (2014, 2019), over the past 50 years, Koreans' carbohydrate intake has been decreasing and fat intake has been increasing. In particular, fat intake increased by 26% in 10 years from 40.5 g/day in 2009 to 51.1 g/day in 2019. Therefore, Superzyme was developed with higher lipase activity than commercial products to reflect food intake of modern people.

In accordance with Korean manufacturing standard for medicine, ingredients suitable for modern people's digestion were selected and developed into coated tablets. In addition to microbial digestive enzymes, antacid, stomachic, stomach mucosal restorative agent, and degassing agent were added into the new developed tablet. Antacid was used to prevent heartburn and to stabilize digestive enzymes at gastric pH. It has been reported that *Swertia japonica* extract, a stomachic agent, stimulates gastric emptying and GI motility (Kimura and Maho 2011). Soluble azulene extracted from eucalyptus was added as a mucosal repair agent, and simethicone was combined as a degassing agent (Table 3). In a clinical trial of FD patients by Holtmann et al. (2002), it was reported that 105 mg of simethicone was significantly better than placebo in improving dyspeptic symptoms. Simethicone had a faster onset of action and was superior to cisapride, a prokinetic agent, in the first 2 weeks of treatment.

It is important for a digestive drug to have a rapid effect. Therefore, a functional coating agent that is rapidly decomposed within 2 to 3 min at gastric pH after meals was used. It was expected that the coating layer would not decompose at the pH of the mouth but rapidly would decompose at the pH of the stomach. This was confirmed through a disintegration test (Table 6). In addition, it was confirmed through a digestion test that the developed tablet has digestive activities at the pH of the stomach and intestines after a meal. In addition, the digestive activity of all three enzymes at gastric pH was relatively high compared to that of popular commercial products (Fig. 3). Festal Plus® is an enteric-coated tablet that contains a high dose of pancreatin and is effective in the intestines. Bease® contains microbial enzymes and pancreatin enteric granules and is effective in the stomach and small intestines, though its digestive activities are relatively low. Superzyme, the product developed in this study, is effective in the stomach and small intestines and is expected to have a rapid effect due to its high digestion activity in the stomach.

In recent years, several clinical studies have shown that pancreatic or digestive enzyme supplements could be alternative approaches in managing dyspepsia. In a

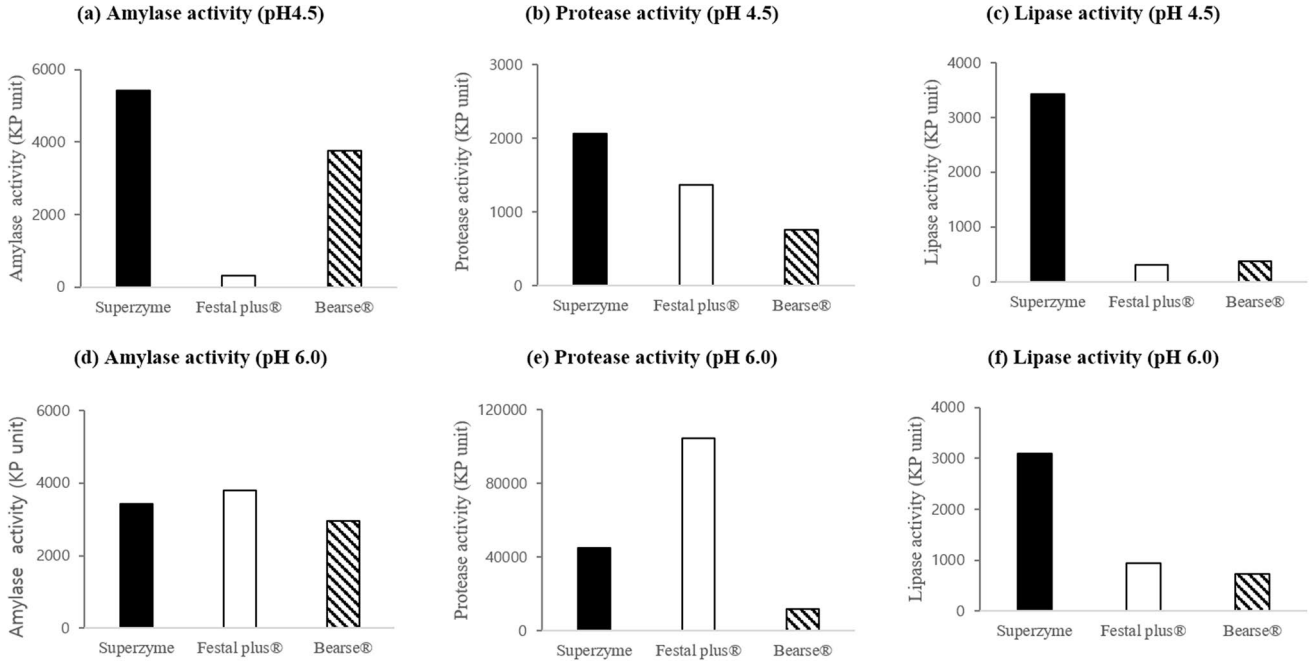

Fig. 3 Digestive enzyme activities of Superzyme and commercial products at pH 4.5 and pH 6.0. The digestive enzyme activity of the developed tablet (Superzyme) and commercial products (Festal plus® and Bearse®) were measured at the pH condition (pH 4.5) of the stomach after a meal and the pH condition (pH 6.0) of the upper small intestine after a meal

clinical trial conducted on dyspepsia patients, 2 weeks of treatment with Combizym® (Daiichi-Sankyo Europe, Munich, Germany) had a significant effect compared to the placebo (efficacy rate: Combizym 89.63% vs. placebo 21.68%, $P < 0.01$). Combizym® has improved symptoms of the upper digestive system, including anemia, bloating, belching, and epigastric burning. No patients reported adverse events during that study (Ran et al. 2009). In a clinical trial conducted on FD patients, treatment with Multienzyme Complex (MEC, DigeZyme®; bacterial and fungal origin) for 60 days had a significant effect compared to the placebo ($P < 0.001$). No patients reported adverse events during that study (Majeed et al.2018).

The types and amounts of digestive enzymes used in the clinical trials are different from those of Superzyme. Based on the input amount and conversion factor, the digestibility in the stomach is expected to be higher than that of Combizym® or DigeZyme®.

Digestive enzymes have long been used to treat indigestion as they break down food into small units and are a safe ingredient with no serious side effects reported. In particular, digestive agents alleviate indigestion by reducing the secretion of digestive enzymes for various reasons such as aging and eating habits. Superzyme is expected to relieve symptoms of indigestion by combining digestive enzymes with ingredients that promote GI motility and gas removal.

Conclusion

Reflecting the food intake of modern Koreans, we developed a non-animal complex digestive tablet containing microbial enzymes. The tablets disintegrated rapidly at postprandial gastric pH and showed high digestive activities in the pH range from gastric to small intestine after meals. The safe digestive agent helps minimize indigestion caused by decreased secretion of digestive enzymes due to various reasons such as aging and eating habits.

Acknowledgements The authors would like to acknowledge the help of Jinwoo Lee, Sukyung Heo, and Youngeun Lee of Handok R&D Center for scale up study of this research.

Declarations

Conflict of interest All authors (H.J. Park, I. Song, B.G. Moon, and H.J. Lee) declare that they have no conflicts of interest.

Statement of human and animal rights This article does not contain any studies with human or animal subjects performed by any of the authors.

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