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Contents

EFFECT OF OAT β-GLUCAN ON IN VITRO DIGESTION CHARACTERISTICS OF SET-TYPE YOGURT

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Abstract
The main objective of the study was to evaluate the effect of added 0.3% (w/w) oat β-glucan (OG) in set-type yogurt on its protein digestion using an in vitro gastrointestinal model. During gastric digestion phase, the amount of soluble proteins and peptides increased to 25% and 40% for control yogurt (yogurt without OG) and 0.3% OG yogurt, respectively. Buccal digestion has little effect on the structure of yogurts, while large spherical vesicles were formed for both control yogurt and 0.3% OG yogurt after gastric digestion. The presence of 0.3% OG promoted the hydrolysis of yogurt in the gastric digestion phase and caused higher antioxidant activity. Compared with that of control yogurt, the inhibition of cholesterol solubility of 0.3% OG yogurt showed no differences after buccal digestion but significantly higher after gastrointestinal digestion (21.3% for gastric and 22.7% for intestinal digestion). Overall, this study enhances the understanding of digestion characteristics of 0.3% OG-fortified set-type yogurt and provides a theoretical basis for the development of this kind of dairy products.

Keywords
oat β-glucan; yogurt; in vitro digestion; antioxidative activity; cholesterol solubility

Introduction

Yogurt is a kind of dairy product fermented by two kinds of lactic acid bacteria (Lactobacillus bulgaricus and Streptococcus thermophilus). It has received much attention from consumers due to the high nutritional value and biological benefits. In addition, it is considered to influence some regulatory systems (such as glucose and lipid metabolism), reduce blood pressure, promote insulin secretion, and maintain the body weight, etc. [1–5].
Yet, these nutritional and biological functions of yogurt are closely related to their digestion process. The bioactive peptides, existing in the amino acid sequence of protein, can be released and activated only through enzymatic hydrolysis during the digestion process. Especially, some branched-chain amino acids, which can influence several postprandial metabolic responses, are present in digested dairy products [6].
Oat β-glucan (OG) is an important soluble dietary fiber, consisting of linear chains of β-D-glucopyranosyl units linked with (1→3) and (1→4) linkages [7]. It has many biological activities, such as enhancing antioxidant activity,

reducing blood lipid, preventing cardiovascular diseases, regulating gastrointestinal environment and cholesterol level in body [8–11]. OG is also well-known for its thickening, stabilizing, emulsifying and gelling properties to maintain the stability of ingredients [12]. More importantly, it has been found that, OG has prebiotic properties and could selectively enhance activity and raise growth of probiotic bacteria (such as lactobacilli and bifidobacteria). So, OG can be used as a texturizer, fat replacer, and prebiotic in enhancing the physical characteristics and nutraceutical qualities of yogurt [13–15]. According to our previous study, the addition of 0.3% (w/w) OG could maximize the quality characteristics of set-type yogurt, and shorten the fermentation time [16]. Due to the addition of OG, the digestion characteristics (e.g., the degree of hydrolysis) of set-type yogurt and the structural and functional properties of proteins or peptides after digestion (e.g., molecular weight, charge and hydrophobicity, etc.) may be changed. However, there are relatively few studies on the effect of 0.3% OG on the *in vitro* digestion characteristics of set-type yogurt, which will limit the application of this type of yogurt.

In vitro digestion models have been designed to study the structural changes, digestibility/degradation, and digestion characteristics of food components under simulated gastrointestinal conditions [17]. Through these models, the digestion characteristics of food systems, such as plant-, dairy-, and emulsion-based foods, has been successfully studied.

So, the main objective of this study was to investigate the effect of 0.3% OG on the in vitro digestion characteristics of set-type yogurt by an in vitro gastrointestinal (GI) model. The proportion of yogurt soluble proteins and peptides after digestion was measured. The microstructural morphology and particle size of yoghurts after digestion were characterized by optical microscopy and dynamic light scattering, respectively. The antioxidant activities and inhibition of cholesterol solubilization into micelles were also evaluated.

Methods

Pure milk was purchased from Yili Industrial Group Co. Ltd (Neimenggu, China). Oat β-glucan (95% purity) were purchased from Zhongkang Food Co., (Guangzhou, China). Starters: Streptococcus thermophilus and Lactobacillus bulgaricus (Lactobacillus dechellii Bulgarian subspecies) (viable bacteria count was about 1×10^9 CFU/g) were purchased from Danisco (China) Co., Ltd, (Shanghai, China). Amylase (1000–3000 U/mg protein), pepsin from porcine stomach mucosa (1:60,000), pancreatin from porcine pancreas (8 × USP) and sodium deoxycholate, cholesterol, oleic acid, phosphatidylcholine, and bile from bovine were obtained from Sigma Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade.

Yogurt preparation

Yogurt fortified with OG (0.3%, w/w) was prepared according to our previously reported method [16]. 0.3% of OG was added to pure milk. After stirring, the milk was sterilized at 95 °C for 5 min, and then cooled to 43 °C, added the starters (containing Streptococcus thermophilus and Lactobacillus denderi Bulgarian subspecies), fermented at 43 °C for 5 h, and stored at 4 °C for 24 h.

In vitro digestion

The simulated gastrointestinal digestion study was performed according to the methods described by Minekus et al. and Asensio-Grau et al. with some minor modifications [18,19]. The gastrointestinal digestion process was conducted as follows:

a. Buccal stage: Simulated salivary fluid at pH 7.0 was added to yogurts in a ratio 1:1 (w/v) under gentle stirring using a kitchen blender for 2 min at 37 °C. Human α–amylase was added as a part of the salivary fluid to reach a desired concentration (75 U/mL) in the saliva mixture.
b. Gastric stage: After the buccal stage, simulated gastric fluid (pH 3.0) was added to tubes in a 1:1 (v/v) ratio including pepsin, reaching a desired concentration (2000 U/ml) in the gastric mixture. The pH of yogurts was adjusted to 2.0–2.5 with 2.5 M HCl. Then, the sample solutions were mixed thoroughly and incubated at 37 °C for 30 min by a shaking incubator. After the incubation, the sample solutions were centrifuged at 10,000 g for 10 min (Centrifuge 5430R, Hamburg, Germany). Further analyses were conducted for the collected supernatant.
c. Duodenal stage: After the gastric stage, simulated intestinal fluid containing 2 mL of porcine pancreatin and 1 mL of bile acid mixture (pH 6.0 or 7.0) was added in 1:1 (v/v) ratio to tubes containing the gastric chime. The pH of sample solutions was adjusted to 7.0 with 4 M NaOH. The sample solutions were incubated at 37 °C for 90 min by a shaking incubator. After the incubation, the sample solutions were centrifuged at 10,000 g for 10 min (Centrifuge 5430R, Hamburg, Germany). Further analyses were conducted for the collected supernatant.

Total soluble protein content of digested samples

Protein contents in control yogurt (yogurt without OG) and in supernatants from digested yogurts (after centrifugation) were determined with the bicinchoninic acid protein assay kit (Pierce Company). The content of soluble protein in digested yogurts was expressed as percentage (%) of total protein in undigested ones. All experiments were performed in triplicate.

Optical microscopy

The microstructure of control yogurt and digested yogurts was observed by optical microscopy (Axio Vert.A1, Carl Zeiss), according to the previous works [20]. Yogurt samples were put between glass slides and immediately observed at a magnification of 100× at room temperature. All experiments were performed in triplicate.

Particle size and size distribution

The particle sizes of control yogurt and digested yogurts were measured by dynamic light scattering using a Zetasizer Nano ZS90 (Malvern Instruments Ltd, Worcestershire, U.K.). Particle size was obtained by the Stokes-Einstein equation. The polydispersity index (PDI), representing the distribution of particle size, was also reported. Before measurement, all samples were diluted by 1:5 (v/v) with deionized water at the corresponding pH values and then equilibrated for 2 min inside the instrument at 25 °C. Data were collected over at least 20 sequential readings. All experiments were performed in triplicate.

Antioxidant activities

The 2, 2-diphenyl-1-picryhydrazyl (DPPH) assay for antioxidant activities of control yogurt and digested yogurts were determined according to the method of Unal et al. [21] with minor modifications. Briefly, 2 mL of each yogurt sample and 2 mL of 0.1 mM DPPH solution (90% methanol) were mixed and vortexed vigorously. Then, the mixtures were allowed to keep in the dark for 30 min at room temperature. Finally, solution absorbance at 517 nm was measured by an ELX800 Microplate Reader (Bio-Tek, Bedfordshire, UK). Blank samples were prepared by replacing the yogurt samples with methanol. All experiments were performed in triplicate.

The scavenging activity was determined as follows:

(1) $$\text{Scavenging Activity } (\%) = 100 \times \frac{\mathrm{A_{DPPH}} - \mathrm{A_S}}{\mathrm{A_{DPPH}}}$$

where A_S is the absorbance of the yogurt samples, and A_{DPPH} is the absorbance of the blank samples.

The 2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay for antioxidant activities of control yogurt and digested yogurts was determined according to the method proposed by Liang et al. [22]with minor modifications. ABTS radical cation was produced by reacting 7 mM ABTS with 2.45 mM potassium persulfate, stored in the dark for 15 h at room temperature. Before usage, the ABTS solution was diluted with phosphate buffered solution (pH 7.4) to get an absorbance value of 0.70 ± 0.02 at 734 nm. 0.2 mL of each yogurt sample was mixed with 3.8 mL of the prediluted ABTS solution, standing for 6 min at room temperature before measurement. All experiments were performed in triplicate.

The scavenging activity was determined as follows:

(2) $$\text{Scavenging Activity } (\%) = \left(1 - \frac{\mathrm{A_{yogurt}}}{0.700}\right) \times 100\%$$

In vitro cholesterol micelle

Cholesterol micelles were prepared following the two methods described by Kirana et al. and Ashraf et al. with some minor modifications [23,24]. An emulsion at pH 7.4, mainly containing 0.5 mM cholesterol, 10 mM sodium taurocholate, 1 mM oleic acid, 1 mM cholesterol, 132 mM NaCl, and 15 mM sodium phosphate buffer, was prepared. And then, the emulsion was treated with ultrasonic energy (400 W, 20 kHz, 20 min), and incubated at 37 °C overnight. Each yogurt sample was mixed with the emulsion and the obtained mixtures were incubated at 37 °C for 24 h. Afterwards, the mixtures were centrifuged at 8000 g for 30 min and the supernatants were

collected. Cholesterol contents in the supernatants were determined by a total cholesterol kit. All experiments were performed in triplicate.
Micelle cholesterol uptake inhibition was calculated according to the formula used by Marques et al. [25]:

$$\text{Inhibition Capacity (\%)} = (1 - C_1/C_0) \times 100\% \quad (3)$$

where C_0 is the cholesterol concentration in the micelle, and C_1 is the cholesterol concentration with peptides.

Statistical analysis
OriginPro 8.6.0 (Originlab, Northampton, MA, USA) was used for the construction of the graphs. Data were presented as means ± standard deviations of three independent experiments and analysed for significant difference by one-way analysis of variance (ANOVA) using the SPSS software, version 18.0 program (SPSS Inc., Chicago, USA).

Results and discussion
In vitro protein digestibility
Figure 1 shows the proportion of yogurt soluble proteins and peptides after the buccal, gastric and duodenal digestion phases. Overall, the amount of soluble proteins and peptides increased during digestion.

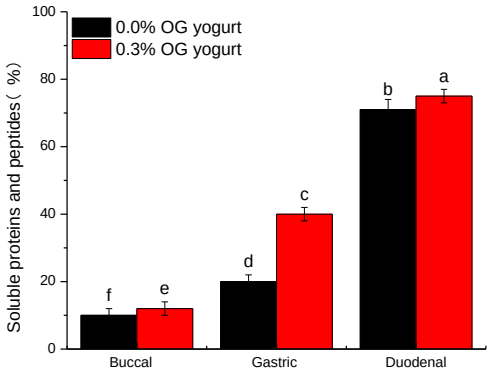

Figure 1. Soluble proteins and peptides (%) after the buccal, gastric and duodenal digestion phases. Different characters on the top of columns indicate statistically significant differences at $p < 0.05$ between different samples ($n = 3$).

The amount of soluble proteins and peptides increased slightly during buccal digestion phase. But, after the simulated gastric digestion, there was a significant ($P \leq 0.05$) increase (25% for control yogurt, 40% for 0.3% OG yogurt) of the soluble proteins. This result is in agreement with the study by Rinaldi et al. [6], who reported that due to the presence of OG, yogurts exhibited faster proteolysis, thus leading to the lower release behavior of large peptides while higher percentage of free amino acids. After the simulated duodenal digestion, the soluble proteins and peptides were slightly higher for 0.3% OG yogurt than for the control yogurt. Thus, OG addition does influence the in vitro protein bioaccessibility in yogurt, especially after the gastric step. It was reported that some polysaccharides, such as gum arabic, low-methylated pectin, and xylan, could inhibit β-lactoglobulin digestibility, due to the formation of protein-polysaccharide complexes [26]. The difference might be attributed to the different physiochemical characteristics of polysaccharides. This also may suggest that OG is more suitable for use as a functional food ingredient in enhancing the nutraceutical quality of yogurt compared to other polysaccharides.

Microstructure and particle size
To gain more structural insights, the microstructural morphologies of particles for control yogurt and 0.3% OG yogurt after buccal, gastric and duodenal digestion were observed by optical microscopy (Figure 2).
Buccal digestion has little effect on the structure of yogurts. After buccal digestion, the microstructure of control yogurt showed a clear three-dimensional protein network structure (Figure 2 (a1)). In general, during fermentation, casein aggregates form a three-dimensional network in yogurt [27]. The microstructure of 0.3%

OG yogurt also showed a denser three-dimensional network structure (Figure 2 (b1)). This could be due to the network structure formed by OG or the complexes dominated by OG-casein interactions, in good agreement with in our previous work (studied by scanning electron microscopy) [16].

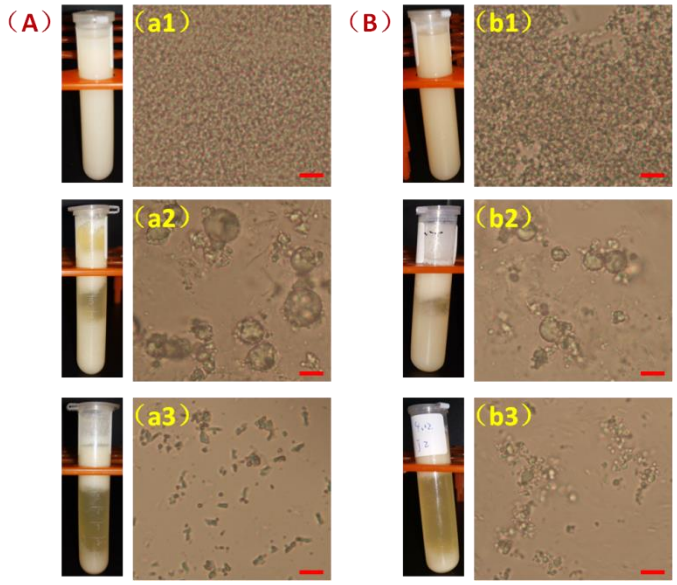

Figure 2. The microstructure of yoghurts after digestion. (A) control yogurt; (B) 0.3% OG yogurt. (a1, b1) after buccal digestion; (a2, b2) after gastric digestion; (a3, b3) after duodenal digestion.

After gastric digestion, large spherical vesicles were formed for both control yogurt and 0.3% OG yogurt (Figure 2 (a2, b2)). In general, the main role of pepsin is to enzymatically hydrolyze proteins into large peptides. These spherical vesicles should be protein aggregation caused by gastrointestinal digestion. Interestingly, the particle sizes of spherical vesicles for 0.3% OG yogurt were smaller than those for control yogurt. As far as we know, the smaller particle sizes of spherical vesicles were observed for the first time by optical microscopy. This clearly suggested that the presence of 0.3% OG caused a fast enzymatic hydrolysis, leading to a significant ($P \leq 0.05$) increase in the proportion of low-molecular-mass peptides. An earlier investigation even pointed out that after gastric digestion, intact dairy proteins remained in the control yogurt whereas less in yogurts enriched in pectin/OG, as measured by sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis [6].
After duodenal digestion, spherical vesicles disappeared, and some small flake structure occurred instead for control yogurt (Figure 2 (a3)). This indicates complete digestion of control yogurt. In fact, Arora et al. pointed that some compounds, such as short chain fatty acids, are easier to hydrolyze by lipases under the role of bile salts [28]. In comparison, there were still some spherical particles with small particle sizes in 0.3% OG yogurt, and they are related to each other (Figure 2 (b3)). Clearly, these connected spherical particles were related to the presence of OG, as OG can interact with proteins and peptides and resist to hydrolysis by lipases to some extent [29]. This may play an important role in stabilizing and reinforcing the functional properties of these peptides. In fact, OG has been used in the food, cosmetic and pharmaceutical fields to deliver bioactive compounds [30].

It can be seen from Table 1, for control yogurt, the average particle size was as high as 7.2 μm with PDI of 0.73 after buccal digestion. This indicated that particles were aggregated with each other. After the gastric digestion, its particle size was decreased to 5.3 μm, and PDI also decreased to around 0.63. After the intestinal digestion, its particle size was about 1.2 μm, with a low PDI of 0.31. This directly indicated that yogurt was completely digested.
Differently, the particle size after buccal digestion was higher for 0.3% OG yogurt than for the control yogurt. This was obviously related to the addition of OG. But, after the gastric digestion, the particle size was decreased to 3.4 μm, and PDI also decreased to around 0.53. This again indicated that 0.3% OG addition caused an increase in the proportion of peptides during digestion, as observed *via* optical microscopy. The fast protein digestion for yogurts with OG could suggest a phase separation phenomenon between OG and protein. We hypothesize that in gastric solution, digestion conditions favor the phase separation, forming a “micro-reactor” among OG, yogurt

proteins, and enzymes. Similar result was also obtained by Rinaldi [6]. In the micro-reactor, enzymes and yogurt proteins are in close contact, thus facilitating the hydrolysis, leading to small particle sizes.
After the intestinal digestion, the particle size was slightly higher (about 1.5 μm) than that of control yogurt, with a higher PDI of 0.39. This may be due to the undigested OG.

Table 1. Particle size and PDI of yoghurts after the buccal, gastric and duodenal digestion phases. Different characters on the top of columns indicate statistically significant differences at $p < 0.05$ between different samples (n = 3).

Digestion stages	**Samples**	**Average particle size (d/nm)**	**Polydispersity (PDI)**
Buccal digestion	control yogurt	7211 ± 45^{b}	0.73 ± 0.09^{b}
	0.3% OG yogurt	8327 ± 52^{a}	0.82±0.11a
Gastric digestion	control yogurt	5319 ± 122^{c}	0.63 ± 0.08^{c}
	0.3% OG yogurt	3427 ± 53^{d}	0.53 ± 0.04^{d}
Intestinal digestion	control yogurt	1253 ± 22^{f}	0.31 ± 0.02^{f}
	0.3% OG yogurt	1503 ± 42^{e}	0.39 ± 0.05^{e}

Antioxidant activities

Yogurt is an important source of food derived protein. In the digestion process, yogurt can release some functional active substances from milk protein, especially some bioactive peptides with good antioxidant properties. At present, DPPH assay and ABTS assay are often used to evaluate the antioxidant activity of functional foods. Here, the two methods were used to evaluate the antioxidant activity of set-type yogurt throughout digestion.
For the DPPH assay, as shown in Figure 3 (a), after buccal digestion, both control yogurt and 0.3% OG yogurt showed certain DPPH radical scavenging activity. Moreover, 0.3% OG yogurt had stronger antioxidant capacity, which can be attributed to the antioxidant activity of OG. OG has been reported to significantly inhibited the fat oxidation of low-fat beef patties [31].
After the gastric digestion, it was increased by 25% compared with that after buccal digestion, indicating that some active components were produced during gastric digestion. This result was similar to other report [10]. Interestingly, 0.3% OG yogurt exhibited higher DPPH scavenging ability (43%) than the control. The result clearly indicated that the presence of OG promoted the yogurt protein to produce more antioxidant components, giving enhanced antioxidant properties.
After the intestinal digestion, the DPPH radical scavenging activity of yogurts cannot be detected. In the study, DPPH was dissolved in methanol, which is suitable for the determination of hydrophilic compounds, not suitable for lipophilic compounds [32,33]. It was speculated that the lipophilic compound after the intestinal digestion may interfere with the determination. Detailed reasons need further study.
The results of ABTS assay were similar to DPPH assay for buccal and gastric digestion (Figure 3 (b)). In comparison, the ABTS radical scavenging capacity of yogurts can be detected after the intestinal digestion. It was further improved (43% for control yogurt and 59% for 0.3% OG yogurt). Clearly, the antioxidant activity of yogurt was further improved.

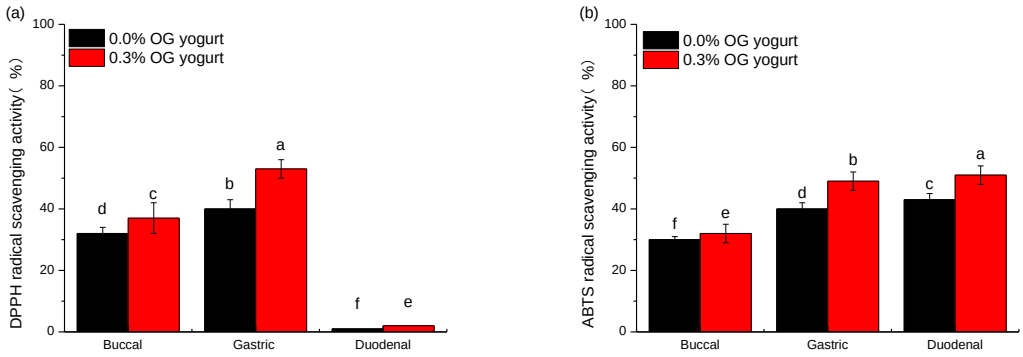

Figure 3. DPPH (a) and ABTS (b) radical scavenging capacity of yogurts after the buccal, gastric and duodenal digestion phases. Different characters on the top of columns indicate statistically significant differences at $p < 0.05$ between different samples (n = 3).

In short, through DPPH and ABTS assays, the antioxidant activity of yogurt was mainly produced during the gastric digestion phase, and 0.3% OG could further improve the antioxidant activity of yogurt by promoting the beneficial enzymatic hydrolysis. And, compared with DPPH assay, ABTS assay is more suitable for evaluating the antioxidant activity of set-type yogurt during digestion.

In vitro cholesterol micelles

In most developed countries and a few developing countries, cardiovascular diseases are considered to be the first leading cause of death and morbidity, and a major contributor to greatly reduced quality of life [34,35]. Prevalent cases of total cardiovascular diseases nearly doubled from 271 million in 1990 to 523 million in 2019, and the number of cardiovascular diseases deaths steadily increased from 12.1 million in 2019 [35]. The risk of cardiovascular diseases can be reduced 2%-3% by every 1% decrease of serum total cholesterol. Dietary cholesterol need to be digested by various enzymes under salivary and gastrointestinal conditions to form micellar solution with triglycerides, phospholipids and bile acids before it can be transported into intestinal mucosal cells [36]. So, the cholesterol lowering effect was evaluated by an in vitro cholesterol micelle model [37].

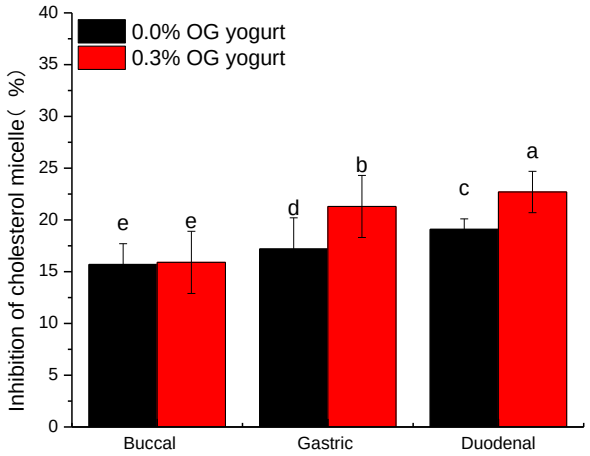

Figure 4. Percent inhibition of the micellar cholesterol solubilization of yogurts after the buccal, gastric and duodenal digestion phases. Different characters on the top of columns indicate statistically significant differences at $p < 0.05$ between different samples (n = 3).

For control yogurt, the inhibition of cholesterol solubilization into micelles gradual increased throughout digestion (15.7% for buccal digestion, 17.2% for gastric digestion, and 19.1% for intestinal digestion) (Figure 4). Clearly, this could be related to the released bioaccessible peptides and amino acids.

Interestingly, compared with that of control yogurt, the inhibition of cholesterol solubility of 0.3% OG yogurt showed no differences after buccal digestion but significantly ($P \leq 0.05$) higher after gastrointestinal digestion (21.3% for gastric digestion and 22.7% for intestinal digestion). This can be related to the presence of OG. On the one hand, OG could influence the type and conformation of amino acids present in peptides, facilitating the production of more hydrophobic amino acids. It has been reported that peptides with more hydrophobic residues can compete with cholesterol molecules through rearrangements [37]. On the other hand, OG could compete with cholesterol to enter the micelle solution and reduce the cholesterol solubility.

Yet, for OG-fortified yogurt, its property of inhibition of cholesterol solubilization into micelles may not be solely due to the two reasons above. Recent studies reveal that the gut microbiota plays a significant role in lowering cholesterol in humans [38–40]. Importantly, OG has the ability to modulate the gut microbiota in human [41,42]. So, further studies are necessary to evaluate the effect of specific interactions between digested yoghurt components and the human gut microbiota on the inhibition of cholesterol solubilization into micelles.

Impact

Worldwide, the number of chronic diseases such as hypertension, diabetes, dyslipidemia and overweight/obesity caused by unhealthy lifestyles is increasing. How to improve the health of this kind of people is the research direction of food scientists in recent years. Regular consumption of yogurt is one of the most advantageous strategies to solve these problems. This is mainly because of the functional proteins and peptides from digested yogurt. Interestingly, after gastrointestinal digestion, 0.3% OG-fortified set-type yogurt exhibited better functional properties in comparison to control yogurt. Thus, the 0.3% OG-fortified set-type yogurt can be developed as a new functional fermented dairy product to respond to consumer demand for healthier and more sustainable products.

Conclusions

In the study, the protein digestion of 0.3% OG-fortified set-type yogurt was evaluated using an in vitro gastrointestinal model. In comparison with control yogurt, the amount of soluble proteins and peptides increased throughout digestion for 0.3% OG yogurt. The presence of 0.3% OG promoted the hydrolysis of yogurt in the gastric digestion phase and caused higher antioxidant activity and higher inhibition of cholesterol solubility. Overall, this study provides a theoretical basis for the development of the 0.3% OG-fortified set-type yogurt.

Conflict of interest

There are no conflicts to declare.

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ANALYSIS OF TEFF MARKET CHAIN: EVIDENCE FROM SOUTH GONDAR ZONE, ETHIOPIA

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Highlight
The authors have identified current overview of teff market chain. The actual marketing flaws and tackling strategies for sustainable, good performing and innovated teff marketing are well organized.

Abstract
Dera is one of the potential districts in teff production. Nonetheless, there are some restraints and prospects in teff commercialization still unaccustomed. This study initiates to examine the teff market chain in the Dera district. It concentrates to synthesize the behavior, transparency, and effectiveness of teff market expending data from different sources. It involved teff producers, wholesalers, retailers, rural assemblers, cooperatives, urban assemblers and processors. The data investigation engaged expressive inferential statics & SCP model. Concerning marketing, the result indicates that producers supplied to the market via rural retailers, wholesalers and directly to consumers 57.7% of teff produced in 2017 cropping season. There are eleven marketing channels in transferring 2268 Quintals of teff from farmers to different intermediaries until reaching end buyers. The market structure of teff is weakly oligopolistic, implying that the market is not competitive. The producers' share of margin for the teff market accounts 68.96% associated with a 31.04% total gross margin. The marketing channel with more teff supplied can have a high or low marketing margin depending on the presence of value-adding market agents in the track. It implies that the channel with more teff supplied is not necessarily the channel in which the highest market margin. The marketing agents in the study area incur primary transaction costs like packing, loading and unloading, storage, transportation, communication and other personal costs. Therefore, improved bargaining power, access to accurate market information and infrastructural development are essential for a better performing teff market.

Keywords
structure; conduct; performance; market Margin; concentration ratio.

Introduction

Teff is an indigenous cereal crop to Ethiopia. Some scholars affirm that it was derived from the Arabic word tahf, a name given to a similar wild plant used by Semites of south Arabia during the time of food insecurity [1]. Based on linguistic, historic, geographic and botanical notes, teff is assumed to have originated in northeastern Africa. The current area of cultivation is probably not the initial one of domestication; domestication probably occurred in the western area of Ethiopia, where agriculture is precarious and semi-nomadic [2], [3]. Teff is among Ethiopia's

prominent returns generating cash crops grown. It is highly essential in terms of either coverage or worth of yield [4]. It comprised the principal portion of production region (28.5%) in 2013, tailed by maize (20.3%) and the second production quantity. It covers 24.02% of land under cultivation by cereals (first among all cultivated crops in terms of land coverage) and contributes 17.57% to grain production, second next to maize in terms of total grain production [5].

According to Amentae [6], there is potential for teff to be the second gift of Ethiopia to the world after coffee. Quality is the leading aspect of every food product market usually assessed by origin. Teff color is also a demanding discrepancy most people focused in Ethiopian teff market. *Magna* (super white), white, Sergegna (mixed), and red teff considered the famous teff colors by farmers, traders and end-users [7]. Moreover, physical presence, shells, smell, roughness, and dietary superiority were prevalent factors, Minten et al. [8] also judge teff's quality.

The market participation decision of teff farmers is influenced significantly and positively by the perception of farmers on the lagged market price of teff, family size, the land allocated for teff production, ownership of transport equipment, and agroecology farmers. Family size, agroecology, distance to the nearest market, farm size, tropical livestock unit (TLU), the income obtained from other farming and off-farm activity and farmers' perception towards farm gate price affect the intensity of teff market participation. Among the factors significantly and hardly affecting the power of teff market surplus are age, family size, number of livestock owned, agroecology, distance to the nearest market, and perception of farm gate price whereas, farm size allocated for teff production and income from off-farm and other farming activities influences positively. Teff farmers decision to augment further value affected negatively by their time of life. Antagonistically, market proximity, agricultural familiarity, schooling position, extension service and credit access affect definitely [9].

The livelihood system of the residents of South Gondar districts is both crop and livestock production. Agriculture in the Dera district is mainly dependent on rainfall, though various surface and groundwater resources are available to maximize water utilization for Agriculture. The main crops cultivated by farmers in the district are teff, finger millet, maize, sorghum and rice in *Woinadega* (midland) areas; and *Dega* (highland) areas of the district, barely, wheat and teff are grown. Barely, finger millet & maize are mainly used for household consumption while teff, oilseeds and horticultural crops are marketed, making up an essential source of cash income for farmers [10].

Dera district stands 1st in teff production from the south Gondar zone [11]. Almost all the 29 rural kebeles are potential teff producers. However, there are significant constraints concerning agricultural production in the study area. These include high fertilizer price, loss of soil fertility, shortage of land, use of low yielding poor local variety and crop pest (because of continuous sole cropping of the same ground repeatedly). Besides, teff producer's marketing problems are underestimated price setup by a wholesaler (Selling agricultural products at low prices), selling farm outputs in the harvest time for loan repayment, lack of government intervention and weakness of cooperatives [12].

Farmers need immediate income to compensate for fertilizer, seed, and children's stationery fees in the study area. Moreover, the lack of storage options sometimes forces farmers to sell crops at harvest time when the price is low. At present, only some intermediaries further process teff produce to powder and Injera. The nutrient-packed small grains of teff does not acquired the prospect of being used as an industrial crop [13].

Evidence acquired from DDAO [14] illustrated how teff yield predominantly bruised by pre-and post-harvest losses. Shattering is a reason for momentous loss of produce in teff; better to harvest the crop on time. On the other hand, during threshing, considerable yield losses are incurred. Since the thresh perform on the ground, the quality of the teff grain is adversely affected as the grains mix with the soil, sand and other foreign matter, which ultimately affects the market value of teff. These problems, in sum, deteriorate the surplus of teff to be value-added and supplied to the market that improves the livelihood of farmers and profitability of each teff market chain actor [12].

Researchers have done studies of the cereal sector, looked at cereal market chains [15], [16]; however, their study mainly concentrated on the role that intermediaries play in the build-up of market prices [17], [18]. Their result misses the organized illustrations of basic marketing transparency, behavior, efficiency and sustainable promotion of innovated marketing with comparative studies. Gabre-Madhin [15] found that brokers are vital in the functioning of the cereal markets in Ethiopia. They deliver many services (especially on search and aggregation functions), and farmers might or might not select to use them based on the type of services they provide. Indeed, the study did not consider the negative aspects of brokers. A stretched chain irrespective of visible values is nor worthy. Thus, in this study the real advantages of these actors have been described.

Several authors had also found a relatively unsophisticated teff marketing chain in their study. There are no interlinked transactions with buyers of the produce. The role of credit is minor. Most of the transactions are cash transactions [4], [7], [8] Significant efforts in checking quality and quantity come to mind at each transaction along the chain since the absence of organized grading and standardization.

Mirie et al.[19] brought deprived perforformance of teff market chain. However, researchers were cramped to inspect an insufficient numbers of evaluating criteras due to inadequate size of sample (1.6% of the total population) limited chain actors and limited data. Thus, leaving the gaps in the litrature that this study proposes to fill. Unlike previous market chain studies, we increase the sample size and comprise additional chain actors like processers. We also include Degree of market transparency and term of payment to analyze structure and conduct of teff market.

Teff production is a potential for many farmers for additional income [11], despite the crop not being given the adequate policy attention. Adequate information concerning the analysis of the teff market chain that guides the proper participation of smallholder farmers and other actors in the teff market chain is still missing. The produce flows from producers to the final consumers stretching long chains without creating value additions, which is against the fair benefit of producers. Even though farmers produce the teff grain well, they do not bother about quality, standard, improved variety and post-harvest handling. They follow the traditional production, harvesting, collection, and handling. There have not been well-established linkages among traders and processors.
Therefore, this study intends to identify teff market chain actors and their roles. It also investigates the teff market concentration, behavior and profitability that gives an enhanced revelation and possible upgrading strategies from the basis of smallholder farmers advantage.

Methods

- The Research Area

Dera is amongst districts in *the* south Gondar zone in Northwest Ethiopia. It is bordered to the south by the Abay River, which separates it from the East Gojjam; to the west, it is bordered by Lake Tana, to the north by Fogera, to the northeast by East Estie; and to the east by West Estie. The *district* covers 158,948 ha, of which 35% is plain, 20% is mountainous, 18% is gorges, and 27% is undulating. The altitude of the district ranges from 1,560 m to 2,600 m above sea level, while the annual average rainfall is 1,250 mm. Regarding agroecology, 85% is *Woinadega(Midland)* while 15% is *Dega* (highland) [14]. There are 32 *kebeles* in the *district,* of which 29 are rural and three are town *kebeles*. The district's total population is 279,845, of which 142,851 are male and 136,994 are female. The number of households in the district is 69,961, 58,767 are male-headed, and 11,194 are female-headed [14].

The district experiences annual rainfall ranging from 1000– 1,500mm, which puts it among the relative moisture sufficient district in the country. It has one long rainy season, "*kirem*t", which lasts from June to September. The main crops cultivated by farmers in the district are teff, finger millet, maize, sorghum and rice in *Woinadega* (midland), and Barely wheat and teff are grown in Dega (highland) areas. Households also grow crops like Irish potato, onions, tomato and sugarcane, and fruit, such as mango, orange and spice, and chili pepper. Oil Seeds, such as oats, flax and nigger seed, are also cultivated using irrigation during the dry season. Barely, finger millet and maize are mainly household consumption items while teff, oilseeds and horticulture crops are marketed, making up an essential source of cash income for farmers [10]. Figure 1 shows the geographical location of the research area.

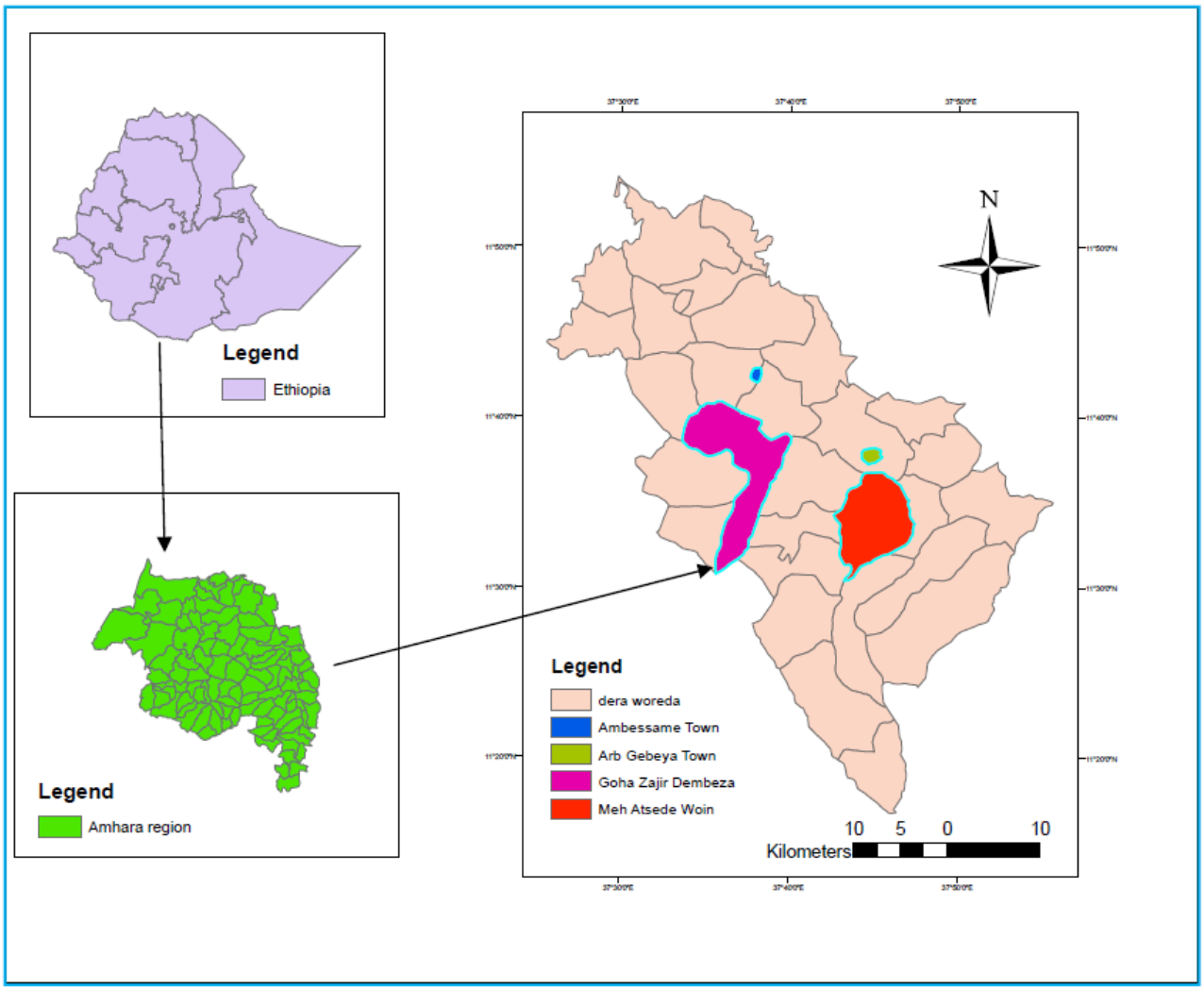

Figure 1. Location of the Research Area. *Source: [14].*

- Data Types Sources and Method of Collection

The research deployed primary and secondary data sources which can be quantitative or qualitative. Household surveys, focus group discussions, key informants and personal observations were sources of the preliminary data. In contrast, district agriculture office, trade and industry, Amhara regional agricultural research institute, and CSA were sourcing the secondary data. Quantitative data were information about quantities, information that could be measured and written in numbers, including household socioeconomic variables like age, family size, and economic factors. Qualitative data was information about qualities that could not be measured, focusing on the respondents' expressions and feelings.

- Sample Size and Sampling Technique

This study employed a three-stage sampling procedure to select respondents. In the first stage, Dera is chosen among 13 districts of the South Gondar Zone. The underlying principle for the choice was ranking in crop production. Dera stands first in teff production compared with the rest of the districts. In the second stage, discussing with the agriculture and natural resources development office, two *kebeles* carefully chosen from the Dera district tracing their teff yield and volume of marketing supply. In the third stage, using probability proportional to size (PPS), the number of respondents were selected from each sample *kebele* sample frame using a simple random sampling technique.

Accordingly, using a formula developed by [20], 171 teff producers were identified as $n = \frac{N}{1+N(e)^2}$ where:

n = size of the research sample
N = size of the entire population (overall number of households in the selected *kebeles*)
e = precision level.

There were 2136 and 2797 households in Meha-Atsedeweyine and Goha kebeles, respectively. In determining the required sample size, we use a 95% confidence level, with a 0.5 degree of variability and a 7.5% level of precision. The number of male-headed and female-headed households was 1867 and 269 and 2462 and 335 in Meha-Atsedeweyine and Goha *kebeles*.
Respondent farmers

(1) $$n = \frac{4933}{1+4933(0.075)^2} = 171$$

The number of traders and extra intermediaries fixed purposively by using the list of each actor of identified *kebeles*. The targets were licensed traders as it was challenging to identify the unlicensed ones. Thus, their number depended upon the availability of the licensed ones. When they were few, all of them were capable of selection. Based on this, three wholesalers, 11 rural retailers, seven urban retailers, one rural assembler, one cooperative, ten urban assemblers and three processors (small *Injera* shops) were selected purposively.

- Methods of Data Analysis

We used descriptive and inferential statistics and econometric analysis for data analysis. Both techniques were employed using Microsoft office excel 2016, SPSS 26 and STATA (Statistical & Qualitative Data Analysis Software) 15 statistical software packages.

- Descriptive Statistics

This method explained and interpreted the data obtained from sampled households and traders' socioeconomic characteristics. It employed tables, figures and graphics. Tables describe mean, minimum, maximum, frequency, percentages, and standard deviations. Moreover, we calculated Concentration ratios, market shares and market margins to describe the teff market structure, conduct and performance. Appropriate statistical tests such as t-test (for continuous variables) and (Chi-square test) for discrete variables were employed to compare and test the mean or proportion difference between selected characteristics.

- Structure Conduct Performance Analysis

The model was actively used to illustrate the basic overview of market structure conduct and performance [21]. Several authors employed this model to evaluate the vegetables, teff and pepper respectively [19], [22], [23].

Structure: In analyzing the market structure of teff buyers/sellers, concentration ratio, degree of marketing transparency, and entry and exit barriers were applied. Concentration Ratio can be illustrated by the formula:

(2) $$C = \sum_{i=1}^{r} S_i$$

Where *Si* = the percentage market shares of *the* i^{th} firm and *r* = the upper ranked firms that the ratio calculated

(3) $$S_i = \frac{P_i}{\sum P_i}$$

Where S_i is teff market share, P_i - the quantity of teff held by buyer *i* and $\sum P_i$ - Overall volume of teff held.

This method is very important in showing the major share of products and actors influencing the market. However, this method cannot quantify market power directly. A concentration ratio of 50% or above for the first big four firms in the trade point toward a strong oligopoly. Simultaneously, a 33%-50% point out a frail oligopoly, while the rest smaller amount represent a competitive business [24].
Conduct: Environments illustrating the marketing behavior of farmers and other traders believed to direct the conduct of market. Nevertheless, a commonly accepted measures to investigate the conduct of market, the behavior of the teff market is examined by producer farmers and traders way of getting price data, price-setup, buying and reselling approaches [25].
Price is the prime element responsible for producers' choice to whom and which market to distribute their goods. The main determinants of price as a single buyer, single seller, local assemblers or through negotiation of seller and buyer are analyzed. Factors affecting the price setup like supply and demand conditions, informal price restraints like cut-*price (Waga Koreta* locally) are synthesized. The basis for price differentiation and the impact of the physical location of the market on prices and marketing arrangements are also addressed to analyze price setting. In ascertaining the obtaining and marketing system, the home of product, the presence of accredited and unknown trading actors which disturb the bargaining power, the nature of the buying/selling in the market, the type of channel chosen, and the incidence of unethical trading practices were seriously analyzed. The term of payment is investigated whether an immediate transaction in the spot market, futures market or contract farming form of relations of sellers and buyers) was also assessed. A timely payment can play an indispensable role in improving the social relations of the traders and the producer society and vice versa.

Performance: Analysis market margin executed to estimate the profitability of the teff market. It is an interesting measure of market performance [26]. It is the value of the difference price between farmers and consumers [27]. The producers' share is commonly calculated as the ratio of farmers' price to end-users (retail) price [28]. Mathematically, producers' share articulated as:

(4) $$Ps = \frac{P_x}{P_r} = 1 - \frac{MM}{P_r}$$

Where:

PS = Producers' share
P_X = Farmers' price of teff
Pr = consumer price of teff
MM = marketing margin.

The total marketing margin was calculated using the following formula:
Marketing margin**:**

(5) $$TGMM = \frac{End\ Buyer\ Price - Farmer's\ Price}{End\ Buyer\ Price} * 100$$

The producer's margin or share in the consumer price $\mathrm{GMM_P}$ is calculated as:

(6) $$GMMp = \frac{Price\ Paid\ by\ End\ Buyer - Gross\ Marketing\ Margin}{Price\ Paid\ by\ End\ Buyers} * 100$$

The consumer price share of market intermediaries is calculated as:

(7) $$GMM = \frac{SP-BP}{EBP} * 100$$

Where:

GMM = Gross Marketing Margin (%)
SP = Selling price at each level
BP = Buying price
EBP = End buyer price

(8) $$NMM = \frac{Gross\ Margin-Marketing\ Cost}{Price\ Paid\ By\ End\ Buyer} * 100$$

Where NMM: is Net Marketing Margin
For the research, GMM was deliberately used instead of NMM, since it was challenging to approximate the implicit costs suffered during the teff deal.

Results and discussion

- Socioeconomic Characteristics of Teff Traders

Sex, age, and educational status of household heads describe the demographic characteristics of traders. The result specified that the age of the respondents was 37.94 years old on average dictating they were young. Urban retailers were young, 25 years old on average, while urban assemblers (53) were premature. The study also inference that most traders were males (77.7%). Most sample traders account for rural retailers (30.6%), followed by urban assemblers (27.8%) as depicted in table 1.

Table 1. Demographic characteristics of teff traders. *Source: Survey results of authors' research.*

Type of trader	**Sex of trader**			**Age of Trader**			
	Male	Female	Total	Min	Mean	Max	St. Dev
Wholesaler	2 (7.1)	1 (12.5)	3 (8.3)	35	38	41	3
Rural retailer	7 (25)	4 (50)	11 (30.6)	35	38.45	50	5.87
Urban retailer	6 (21.4)	1 (12.5)	7 (19.4)	28	38.86	53	10.14
Rural assembler	1 (3.6)	0	1 (2.8)	50	50	50	0
Urban assembler	10 (35.7)	0	10 (27.8)	25	34.5	48	7.38
Cooperative	1 (3.6)	0	1 (2.8)	44	44	44	0
Processors	1 (3.6)	2 (25)	3 (8.3)	34	37.33	40	3.06
Total	28(77.8)	8 (22.2)	36 (100)	25	37.94	53	7.383

Note: values in parenthesis represent percentage.

The formal educational status of sample teff traders ranges from zero to higher education as some urban assemblers and retailers were graduated from university. In contrast, some others can read and write using their knowledge of religious education. The average educational level of the sample traders was primary education (7.84). Based on the categorization of schooling, 27.8% of the dealers followed primary education, whereas only the 9% joined diploma and university. Urban assemblers (29.4%) pursued formal education finishing secondary and preparatory

school, followed by rural retailers (23.5%). However, traders were participating in teff trade simply by looking at their elder families and parents' particularly few retailers, as presented in table 2.

Table 2. Educational Status of teff traders. *Source: Survey results of authors' research*

Type of trader	**Educational status of trader**					**Total**
	Informal Education	Grade 1-4	Grade 5-8	Grade 9-12	Higher education	
Wholesaler	0	1 (33.3)	0	2 (11.8)	0	3 (8.3)
Rural retailer	2 (66.7)	1 (33.3)	4 (40)	4 (23.5)	0	11 (30.6)
Urban retailer	1 (33.3)	0	1 (10)	3 (17.6)	2 (66.7)	7 (19.4)
Rural assembler	0	0	0	1 (5.9)	0	1 (2.8)
Urban assembler	0	1 (33.3)	3 (30)	5 (29.4)	1 (33.3)	10 (27.8)
Cooperative	0	0	0	1 (5.9)	0	1 (2.8)
Processors	0	0	2 (20)	1 (5.9)	0	3 (8.3)
Total	3 (8.3)	3 (8.3)	10 (27.8)	17 (47.2)	3 (8.3)	36 (100)

Note: values in parenthesis represent percentage.

- Teff Marketing Participants, their Roles and Linkages

Different marketing actors in each teff marketing channel participated in buying and selling teff starting from producers to final consumers as described below.

Producers**:** This group of people is involved in the manufacture and distribution of teff on the market. They transport teff to the nearest marketplaces using either head or back loading or donkeys, covering a distance of 111.32 minutes on average. They sell the products to assemblers (rural and urban assemblers), cooperatives, retailers, wholesalers and consumers. Table 3 shows the distribution of actors concerning the volume of teff transacted to different marketing agents. Accordingly, wholesalers, rural retailers and consumers were the major buyers from producers.

Table 3. Proportion of teff transacted by teff producers based on the market outlets/Agents.
Source: Survey results of authors' research

Market outlet/agents	**Percentage share**
Rural assembler	5.46
Urban assembler	13.84
Wholesaler	25.01
Rural retailer	23.95
Consumer	28.77
Cooperative	3.00

Rural assemblers/Farmer traders: These groups of marketing agents play an essential role in connecting the teff producers in the study area with actors in different stages of the teff marketing channel as they have immediate contact with farmers who supply teff to the market. They buy teff from the local market and resell it for urban assemblers, retailers and wholesalers in local and district markets for a profit. According to discussions made with them, these traders are usually part-time traders, and most of them are young and with small family sizes who themselves are producers. Key informants also said that actors collect teff from different small markets using their local network and knowledge and sell it back at the similar or other markets/day and benefit from providing the products for actors in the next stage the market channel.

Urban assemblers: These players play an important role in the market chain, and most of them in the research region are men. They serve the chain by buying, storing and selling the teff from farmers and village collectors to retailers, wholesalers and consumers in the district and rural markets. Their capital is not much high since most of them collect teff and sell to wholesalers and retailers on the same day, but they are more full-time traders than village collectors. Sometimes, they add value to the product through weaving, storage, and separating adulterated teff and the like. These assemblers also supply their teff for hotels & restaurants and consumers when needed, sometimes as a supplement.

Brokers: These players are commission-based agents who operate on behalf of other market participants. They do not possess ownership of the product. Mainly, they play a facilitation role in creating fertile ground (time and place) for buyers and sellers together. However, there is no broker in the study area for a purpose; instead, they bring transport services like trucks and Isuzu and sometimes disseminate market price information to marketing agents. They are crucial in getting teff from the district market to the regional market. They receive money from each trader based on the quantity of quintals (1 birr per quintal) (i.e., around Birr 45 every one Isuzu).

Wholesalers are teff marketing chain market actors who buy teff in larger quantities than the rest of the market actors and resale the items to urban retailing dealers and, in some cases, to customers. These wholesalers are found in the district capital market and purchase teff from farmers, urban assemblers, and sometimes from retailers. Wholesalers buy teff from retailers since retailers cannot sell in the district market because consumers can purchase teff directly from farmers as producers surround the market. Hence, teff purchased from different sources is stored in one place (warehouse) mixed to meet the teff grain uniformity. Then, the stored teff is supplied to the high demand regional markets (Bahir Dar, Woreta and Dessie) on urban retailers.

Retailers in the teff marketing chain are those actors who perform the final marketing function by connecting consumers with other teff traders in most cases and, in some cases, producer farmers. These retailers are full-time traders and operate in rural and urban markets.

Rural retailers: Market participants link other market chain actors to end-users put their destination in the district town. They buy teff from farmers in the market and selling and purchasing center on the days other than the market. According to discussions with district trade and marketing development personnel, the biggest problem is that retailers sell teff to wholesalers inversely. It isn't easy to get many buyers as most district consumers purchase from producers as several producer farmers surround the rural market. They also sold teff to urban retailers and consumers.

Urban Retailers: These retailers sell teff to end-users (consumers and processors). They are located in the regional market and buy the product from wholesalers, urban assemblers, or rural retailers. Urban retailers are characterized by owning or renting shopping centers where buying and reselling functions occur.

Processor: These marketing agents play an essential role in marketing and processing. Processors include small *Injera* shops, cafes, restaurants, and hotels that make value additions to teff grain as they milled and backed to soft *Injera*. They buy teff from rural and urban retailers and process and sell *Injera* to consumers.

Cooperatives: Cooperatives are farmers' associations that supply agricultural inputs to farmers and buy their output at harvest. They sell the product they purchased to different traders, hostels and humanitarian associations and get income. The members of the cooperatives get benefit from their cooperative as a dividend.

Consumers: In the teff's market channel consumers are the end-users [17]. They mostly buy teff from different marketing channel actors such as producers, retailers, processors, and urban assemblers. These market actors participate in the marketing chain by purchasing either raw teff grain or processed teff *Injera* directly for their consumption or indirect supply to café and restaurants, hotels, hostels and humanitarians. Teff consumers included producer farmers (partial users of their produces), rural and urban dwellers (buying from producers, wholesalers or

- Teff Marketing Channels

The teff market channel displays the numerous marketing actors, their interactions, the alternatives open to these actors in terms of purchasing or selling teff, and the expected proportion of teff transacted over the different passages. There are eleven different types of teff marketing channels. During the primary season (Meher), total production and the amount of teff marketed were 3931quintals (Qt) and 2268Qt, respectively, in the sample *kebeles* of Dera district. Rural consumers play a significant role in buying teff from farmers and about 71% of the produce was sold in different outlets. The 25.01%, 23.95% and 13.84% of farmers' products acquired by wholesalers, rural retailers and of urban assemblers, correspondingly.

The remaining 5.46% and 3% of farmers' produce transacted through rural assemblers and cooperatives. Figure 2 shows different marketing routes used in the transaction of teff from their point of production to the end-users (consumers) in the study area. The virtual channels involved in the movement of teff in the study area are listed as follows.

Channel I: producer-rural assembler-wholesaler-urban retailer-consumers (123.82Qt).

This channel sells its teff to consumers through rural assemblers, wholesalers, and then urban assemblers. A total of 123.82Qt teff was exchanged in this channel which stands 8th in terms of quantity of teff sold. It comprises 5.5% of teff marketed in the whole chain.

Channel II: producer-wholesaler-urban retailer-processor-consumers (250.09Qt):

This channel is the second most crucial channel next to the shortest producer to consumers channel in terms of volume of teff marketed. It is carried out via wholesalers, urban retailers, and most value-adding actors (processors). This channel accounts for 11.03% of the total transaction in the market chain that transfers 250.09Qt.

Channel III: producer-wholesaler-processor-consumer (233.91Qt).

In this channel, wholesalers directly buy teff from producers and sell to processors before the product reaches the final end-users via processors. It comprises 233.91Qt of teff transacted in the study area in the survey period that accounts for 10.31% of the quantity marketed in the chain. Considering the volume of teff transacted, it is the third most crucial channel compared to other teff market channels.

Channel IV: producer-urban assembler-wholesaler-consumers (120.55Qt).

This channel is the channel producers sell the teff they produce to urban assemblers that supply to wholesalers before reaching the consumers. It transfers 120.55Qt that comprises 5.32% of total teff transacted in the whole teff market chain, which is the ninth important channel in terms of the total volume of teff marketed.

Channel V: producer-urban assembler-wholesaler-urban retailer-processor-consumer (95.06Qt).

This channel incorporates urban assemblers, wholesalers, urban retailers and processors as intermediaries to transit the producer's teff to the consumers. However, this is the second channel next to channel VI in transferring the lowest amount of teff from producers to consumers, accounting for 4.19% of the total volume of teff marketed in the study area in the survey period. It stands 10th in transacting teff among other teff marketing channels.

Channel VI: producer-urban assembler-urban retailer-consumers (79.61Qt).

This channel stands last in terms of the volume of teff transacted from all channels which pass urban assemblers and urban retailers one after the other, starting from producers and ending in consumers. It transfers 79.61Qt of teff, accounting for 3.51% of the overall quantity of teff sold. The little book could be due to the distance of the nearest output market, and the time urban assemblers buy teff was in the morning mainly.

Channel VII: producer-rural retailer-wholesaler-urban retailer-consumers (219.82Qt).

In this channel producers sell their teff produced to rural retailers, which in turn supplied to wholesalers, and wholesalers sell to urban retailers after giving some better value additions like the place and time values before enriching teff to consumers. This route transacted 219.82Qt of teff, accounting for 9.69% of the total amount and ranking fourth in terms of size.

Channel VIII: producer- rural retailer-processor-consumers (139.33Qt).
In this channel, producers sell their teff produced to rural assemblers, which in turn sell to processors, and after the row grain is processed, it is reached to ultimate consumers. In this channel, 139.33 Qt of teff is supplied, comprising 6.14% of the overall amount of teff supplied to the market. It is the sixth most crucial teff market channel in quantity sold.

Channel IX: producer-rural retailer-consumer (215.30Qt)
This channel is where producers sell their teff to rural retailers in which rural retailers' transit to final consumers. It transfers 215.3Qt of teff, and it is the fifth most crucial channel considering volume that accounts for 9.49% of the entire produce.

Channel X: producer-cooperatives-urban retailer-consumers (138.01Qt)
The channel producers sell their teff products to consumers via cooperatives and urban retailers' one after the other. In this channel, 138.01 Qt of teff is supplied, comprising 6.09% of the total volume of teff marketed. It is the seventh most important teff market channel in quantity marketed.

Channel XI: Producer-consumer (652.5Qt)
It is the channel producers sell their teff directly to consumers. The result in figure 2 indicates that 652.5Qt teff was transacted in this channel, accounting for 28.77% of the total volume of teff transacted in the chain. An enormous volume of teff transacted in this channel. The information from critical informants also shows that teff producer farmers found surrounding the market and supply more to consumers. However, this does not mean the nearest output market is in the shortest distance but a relatively better market distance than other market centers. Besides, it's clear evidence of teff Injera's popularity among city dwellers.

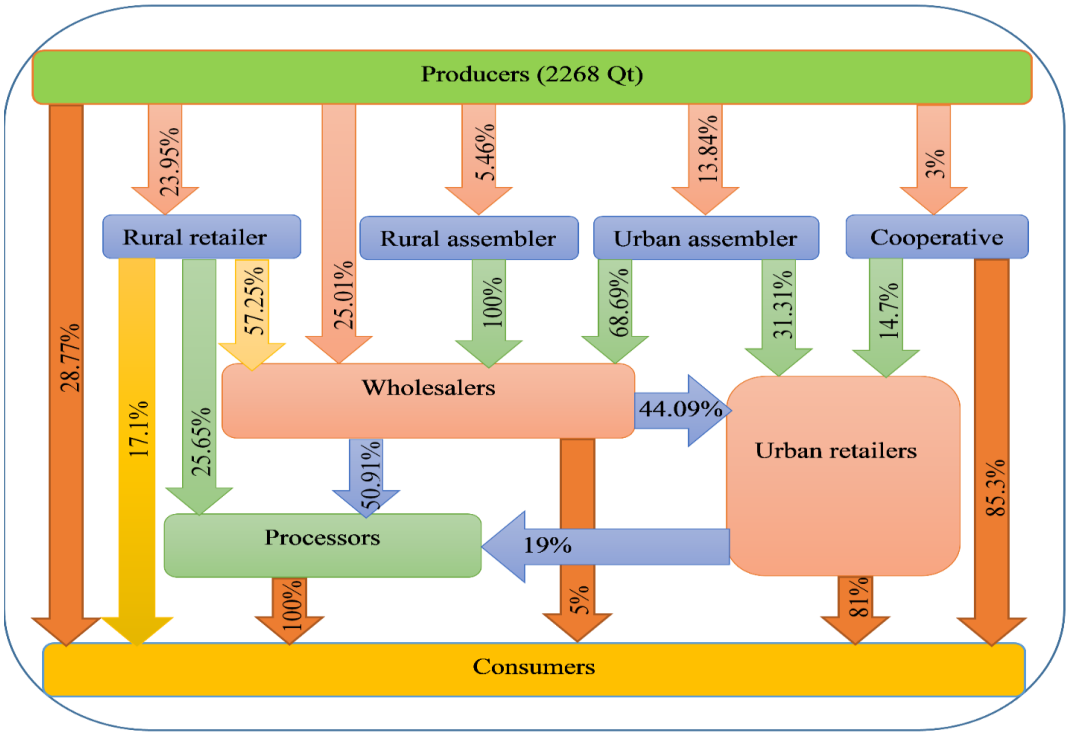

Figure 2. Teff marketing channel. *Source: Survey results of authors' research.*

- Teff Market Structure, Behavior, and Performance

Structure of Teff Market

The structure of a teff market refers to firms' size and market share, relative ease of free entry and exit from the marketing chain, degree of product differentiation, and market transparency among the marketing participants within a given subsector [29]. To assess the market structure of teff in sampled markets, researchers looked at concentration ratio, prospective entry and exit obstacles, level of product diversity and access to teff market data.

Concentration Ratio: According to Kohls et al. [24], market concentration is the portion of industry sales made by the most prominent firms, which dictates imperfect competition. The most common way of expressing the concentration ratio is using the four largest firms (CR4). The concentration ratio of teff merchants was estimated. Table 4 shows that 11.1 percent of the four largest traders account for 35.46 percent of total teff purchase, indicating a weak oligopoly market structure. The result showed a better competition in the teff market compared to other authors [19], [30], who found a strong oligopoly teff and potato market respectively. The market structure for teff is not a competitive that shows teff traders were relatively less concentrated in the area.

Table 4. Concentration ratio of traders in sampled markets. *Source: Survey results of authors' research*

No of Trader (A)	Cumulative Frequency (B)	% of traders ($C = \frac{A}{\text{No.of Trader}} * 100$)	Cumulative % of trader (D=B/36*100)	Quantity Purchased (in No)(E)	Total Quantity Purchased (F=A*E)	% Share of Purchase (G=F/2268)	Cum. of % Purchase
1	1	2.78	2.78	265	265	11.69	11.69
1	2	2.78	5.56	212	212	9.35	21.04
1	3	2.78	8.33	199	199	8.81	29.86
2	5	5.56	13.89	127	254	11.23	41.09
1	6	2.78	16.67	121	121	5.34	46.42
1	7	2.78	19.44	116	116	5.14	51.57
1	8	2.78	22.22	97	97	4.32	55.88
1	9	2.78	25	95	95	4.21	60.09
1	10	2.78	27.78	94	94	4.16	64.25
1	11	2.78	30.56	71	71	3.16	67.42
1	12	2.78	33.33	71	71	3.15	70.56
1	13	2.78	36.11	58	58	2.56	73.13
1	14	2.78	38.89	54	54	2.40	75.53
1	15	2.78	41.67	53	53	2.34	77.87
1	16	2.78	44.44	50	50	2.20	80.07
1	17	2.78	47.22	42	42	1.87	81.95
2	19	5.56	52.78	37	74	3.24	85.18
1	20	2.78	55.56	33	33	1.48	86.67
1	21	2.78	58.33	27	27	1.18	87.85
4	25	11.11	69.44	26.5	106	4.68	92.53
1	26	2.78	72.22	25	25	1.09	93.62
1	27	2.78	75	21	21	0.94	94.55
1	28	2.78	77.78	18	18	0.82	95.37
1	29	2.78	80.56	17	17	0.76	96.13
2	31	5.56	86.11	16	32	0.14	97.54
1	32	2.78	88.89	13	13	0.58	98.12
1	33	2.78	91.67	13	13	0.57	98.69
2	35	5.56	97.22	10	20	0.89	99.59
1	36	2.78	100	9	9	0.40	100
		100			2268		

Degree of Marketing Transparency: One of the characteristics of a perfectly competitive market structure is availability and easy dissemination of market information. The presence of important information about market price, supply and demand, and traders' satisfaction with this information play a critical role in any market structure. According to information gained from the Dera district trade and marketing development office, there is an organized price information center summarizing the weekly price of crops. But traders have no culture of using this information by looking forward price board rather sell and buy their product at the current market price. It is a fact that traders in the study area are not aware of the modernized price information center.
The findings of the survey show that 53% and 47% of traders get market information through (personal observation & from other teff traders) and telephone respectively though about 44% of the traders were not satisfied by the knowledge and the reason behind that was the flexibility of teff price even within a day. Among sampled traders, 65% of them replied that teff market price is volatile within the same market center and market day and indicated this as a big problem in receiving information on cost, supply and demand for teff in the study area. It demonstrates how opaque the market is.

- Conduct of Teff Market

Market conduct refers to the marketing actors' exchange practice and pricing behavior to adjust the marketing atmosphere viable to sell and buy [25]. It is vital to examine the influence of the existing market structure and the negotiation capacity of market participants. In this study, the teff market conduct has been analyzed using information like selling and buying behaviors and the price-setting strategy of sampled traders.

Producers and Traders Price Setting Strategy: The way a market price is set up is a crucial factor in making sellers and buyers agree on any market price. According to the findings of this survey, 41% of the households traded teff at agreed market price. In comparison, 26% and 33% of households sold their teff on the price set by buyers and with bargaining, respectively. The chi-square test, on the other hand, reveals an abundant variation at a significance level of less than 1%. It confirms that few households bargain on the market price while others take home to sell on another market day (67%). The result also depicts that 53% of the sampled traders set teff price by the market while 47% of buyers and 25% of sellers replied that they agreed upon negotiation. This figure is aligned with the founding of Legese [31] on Teff. In Aribgebya, 28.6% of sampled traders accepted market prices, while 42.9% set market prices by themselves. This result is consistent to Geremewe [30] though the extent varies. Table 5 describes the pricing strategy of producers and traders.

Table 5. Pricing strategy in teff market in the study area*. Source: Survey results of authors' research*

Variable	**Categories**	**Name of market**				**Total**	**χ2 Value**
		Anbessame	Aribgebya	Woreta	Bahirdar		
Traders buying price set up	Negotiation b/n the seller and me	4(36.4)	7 (50)	3 (60)	1 (50)	15 (46.9)	0.897
	by the market	7(63.6)	7 (50)	2 (40)	1 (50)	17 (53.1)	
Traders selling price Set up	by the market	8(72.7)	4 (28.6)	4 (80)	1 (50)	17 (53.1)	9.064
	by purchasers	1 (9.1)	6 (42.9)	0	0	7 (21.9)	
	Negotiation b/n the buyer and me	2 (18.2)	1 (28.6)	1 (20)	1 (50)	8 (25)	
Producers selling prices set up	Buyers	9 (9.3)	36 (48.7)			45 (26.3)	33.66***
	Set by market	48 (49.5)	22 (29.7)			70 (40.9)	
	Negotiation	40 (41.2)	16 (21.6)			56 (32.8)	

Note: (Values in parenthesis are percentages, (***) significance level at less than 1%

The number of marketplaces visited per week by the traders in the sale of teff ranged from one to three. As shown in table 6, retailers (56% of traders) were better at visiting many markets to sell their teff. However, the variation was insignificant, depicting that most traders go to least one product market.

Table 6. Number of markets visited by traders per week. *Source: Survey results of authors' research*

Type of trader	**Number of marketplaces the trader visited**			**Total**	**χ2 Value**
	1	2	3		
Wholesaler	2	0	1	3	23.54***
Rural retailer	5	5	1	11	
Urban retailer	0	1	6	7	
Rural assembler	1	0	0	1	
Urban assembler	8	2	0	10	

(***) shows significance level at less than 1%

Term of Payment: Concerning the payment strategy, the household survey result depicted that 80.7% of sample producers sold their teff in cash on the spot market, whereas 7.6% reported that they received the money on other days after the transaction. The other part of producers (11.7%) has replied that they take their money after some hours of exchange within the same marketing day as indicated in table 7. These are producers who sell their teff to assemblers since some of the assemblers collect teff from producers and submit to wholesalers and gain the money from wholesalers who in turn paid to producers. Term of payment for traders and processors was hand to hand in cash at the time of transaction.

Table 7. Payment time in teff marketing. *Source: Survey results of authors' research*

Payment Categories	**Description**	
	Frequency	Per cent
As soon as the transaction takes place	138	80.7
After some hours of the transaction	20	11.7
On other days after-sale	13	7.6

- Performance of the Teff market

Marketing Costs of Traders: The marketing cost of teff trade for various marketing actors is calculated and presented in Table 8. The highest average marketing cost of the trader category is registered by the cost of storage loss (24.38 birr/quintal), followed by the price of truck rent (17.28 birr/quintal). It supports the reality that most traders are victims of lack of own trucks for transporting teff and the storage loss is also apparent because of solid mouse ordinary in every store. The storage loss is also due to diseases by red teff worm lowering the quality of teff as fluctuating markets restrict traders to store teff for a more extended period. Cost of sack, loading and unloading and communication prices are valuable as they have a significant contribution to the marketing cost involved in teff trade. Urban retailers and wholesalers incur the highest marketing cost in teff trading business, accounting for 87.84 and 79.30 birrs per quintal, respectively, following processors (350). The average transaction cost of teff from the production site to the end-users is 113.49 birr per quintal. The higher the marketing cost by actors in marketing channels, the lower the relative competence of the marketing channel in the market chain as actors with higher prices are unviable to resist in the track.

The teff market chain actors' average selling price and market margins were 1427.14 birr and 12.45 birr per quintal. Processors, on the other hand, had the greatest market income and annual earnings share of 51.57% and 76.05%, respectively. It is the fact that processors add more value than other chain actors in the supply of teff to consumers.

Table 8. Marketing costs and benefit shares of Traders (Qt). *Source: Survey results of authors' research*

Cost items (Mean birr per Qt)	**Type of Trader**							**Total**
	Wholesaler	Rural retailer	Urban retailer	Rural assembler	Urban assembler	Processor	Cooperative	
Sack	10	8.91 (1.044)	9.71 (0.756)	10	9.2 (1.03)	9.33	10	9.31 (0.96)
Packing	2	2	2	2	2	1.33	2	2
Loading &unloading	10	10.18 (0.603)	10	10	10	0	10	10.06 (0.35)
Storage	0	0	0.29 (0.756)	0	0.45 (0.96)	0	0	0.2 (0.65)
Storage loss	30 (5)	25.36 (2.27)	31.14 (5.08)	20	17.30 (4.03)	20	18	24.38 (6.66)
Cost of cart	0.67 (1.16)	1.64 (0.809)	0.86 (1.07)	0	1.2 (1.03)	0	0	1.19 (0.99)
Truck	15 (13.23)	11.82 (11.46)	23.57 (11.07)	23	19 (10.49)	0	25	17.28 (11.46)
Communication	4.84 (0.35)	3.66 (1.1)	3.85 (0.43)	1.96	2.04 (0.43)	128.33	2.05	3.25 (1.18)
Personal expense	6.79 (0.45)	6.29 (0.77)	6.42 (0.86)	3.92	4.51 (0.95)	191.67	2.83	5.74 (1.24)
Total Cost	79.30	69.86	87.84	70.88	65.69	350	70.88	113.49
Average selling price	1284	1253	1376	1200	1217	2400	1260	1427.14
Average marketing Margin	5.26	8.61	6.69	5.81	5.66	44.94	10.17	12.45
% share of margin	6.04	9.88	7.68	6.67	6.49	51.57	11.67	100
Average Profit margin	18.7	63.47	40.07	9.12	21.31	704.25	69.12	132.29
% share of profit	2.02	6.85	4.33	0.99	2.3	76.05	7.46	100

Note: Values in the parenthesis represent standard deviations.

Marketing Margin: Every point of the sales channels takes a portion of the total weighted average selling price, which is known as the marketing margin [32]. The margin should cover the costs of moving the product from one phase to the next while also creating a clear benefit to the marketing actors involved. Table 9 summarizes the marketing margins received by each teff market actor in different channels. The overall gross marketing margin, or the total consumer price remaining for each actor, for Channels 5 and 9 was the largest and lowest, respectively. The producers' gross market margin is most elevated in channel nine (89.27%), indicating the best teff market channel to participate, leaving the shortest producer-consumer track (channel XI), while the lowest producer share

of margin is in channel V (46.33%) which is not recommended to enter as their benefit decreases. Producers also have a considerable margin in channels IV, I, VII, X and VI. The share of producer pricing grows in a small number in the route since value-adding marketing players stretching the chain are not there. Even though the number of intermediaries in channels III, IV, VI and VIII is similar, the producer margin is different. In channels III and VIII, the presence of processors (add higher value, incur high cost and get a most prominent share of consumers price) strictly lowers the producers' benefit. On the other hand, the channel with more teff supplied does not necessarily bring the largest share of producer market margin. Channel II stands first in marketing the enormous amount of teff despite channel nine, which was the fourth in dealing many quantities of teff bring highest producer share of margin without considering channel XI (producer sold to consumer directly). Hence, producers should be aware of marketing value-added teff in the shortest market channel if the number of intermediaries increases in the chain, the share of producers' price from end-buyers price becomes smaller.

Next to producers, the uppermost total profit is maintained by processors in the eighth channel (47.71%), followed by the third channel, while the best and worst net marketing margin is in the eighth channel by processors and seventh channel by wholesalers, respectively.

Table 9. Teff Marketing margins of various marketing channels. *Source:* Survey result*s of authors' research*

Market Margin	**Marketing channels**									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Total Gross Margin	18.60	52.38	52.38	12.85	53.67	19.19	18.60	53.33	10.76	18.60
Gross Market Margin of Rural Assemblers	5.81									
Gross Market Margin of Urban Assemblers				6.11	3.25	7.63				
Gross Market Margin of Rural Retailers							9.45	5.63	10.76	
Gross Market Margin of Urban Retailers	6.39	3.67			3.67	11.56	6.39			
Gross Market Margin of Wholesalers	6.39	6.04	5.54	6.74	4.08		2.76			8.43
Gross Market Margin of Processors		42.67	46.83		42.67			47.71		
Gross Market Margin of Cooperatives										10.17
Gross Market Margin of Producers	81.40	47.62	47.62	87.15	46.33	80.81	81.40	46.67	89.27	81.40
Net Marketing Margin of Rural Assemblers	0.66									
Net Marketing Margin of Urban Assemblers				0.96	0.51	2.86				
Net Marketing Margin of Rural Retailers							4.37	2.71	5.19	
Net Marketing Margin of Urban Retailers	0.01	0.01			0.01	5.17	0.01			12.22
Net Marketing Margin of Wholesalers	0.63	2.74	2.24	0.53	0.78		-3.00			
Net Marketing Margin of Processors		28.08	32.25		28.08			33.13		
Net Marketing Margin of Cooperatives										5.02

Wholesalers get the lowest benefit in the seventh channel of table 9, having only 2.76% of consumers' price. It is because of the inverted and illegal flow of teff from retailer to wholesaler. The impure, shelled, not graded, low-quality teff was sold to wholesalers at a relatively high price. Local retailers have no access to retail in the district market since local consumers were better to purchase teff from producers rather than retailers. Then wholesalers are obligated to sell this low-quality teff to urban retailers mixing with the better quality at a lower price made them incur a loss.

The study's findings revealed that the chain's total gross marketing margin was 31.04%, associated with 68.96% of producers' share of margin. The total Gross marketing margin of teff is low compared to that of chickpea (45.8%) and relatively better than that of hot pepper (29%), according to Tefera [33] and Tesfaw et al. [23] respectively.

Marketing Profit: Marketing profit is the difference in selling and purchasing price, including other transaction costs by each market participant and market channel. It is gained by transferring a product in different channels using marketing intermediaries. As shown in Table 10, the marketing profit gained by processors in the eighth channel is attractive. The lowest market profit in the seventh channel by wholesalers is not good looking as discussed above in the marketing margin part. Urban assemblers and urban retailers receive the best marketing profit in the fourth and tenth channel by having (39.31and168.16 birr per quintal) selling teff directly to wholesalers and consumers. Profits of urban retailers were low except in the tenth channel compared to other marketing actors mostly (0.1 birr/quintal), and this indicates that urban retailers purchase teff usually from wholesalers and rural retailers in which the price variation is tiny (just for only retailing).

Wholesalers uphold their exciting profit in the second channel (65.7 birr/quintal) comparing other media they participate in since they purchase marketable quality teff from producer farmers directly by themselves. In general, traders in the research area found the teff market channel to be fascinating, whereas the seventh channel was unsatisfactory and unadvisable. This result is in line with that of Hailegiorgis et al. [34] who found that traders can get better margin when they purchase the product directly from farmers to add values and supply to end users.

Currently, there are some visible limitations in the teff market chain which needs to be attempted by applying important strategies.

Limitations

- High transaction cost and inverted flow of products
- Price fluctuations
- Low performance
- Traditional marketing system

Strategies

- Promoting digital and online marketing as part innovation.
- Getting Value addition technologies and awareness creation.
- Contract farming for farmers sustainable price guarantee and traders bulk purchase.
- Auditing and inspection for some corrupted brokers that hinder competitiveness of the teff market.

Table 10. Teff marketing traders profit in different marketing channels. *Source: Survey results of authors' research*

Marketing actors		**Marketing channels**									
		I	II	III	IV	V	VI	VII	VIII	IX	X
Rural assembler	Purchase price	1120									
	Market cost	70.88									
	Selling price	1200									
	Market profit	9.12									
Urban Assembler	Purchase price				1112	1112	1112				
	Market cost				65.69	65.69	65.69				
	Selling price				1190	1190	1217				
	Market profit				12.31	12.31	39.31				
Rural Retailer	Purchase price							1120	1120	1120	
	Market cost							69.86	69.86	69.86	
	Selling price							1250	1255	1255	
	Market profit							60.14	65.14	65.14	
Urban retailer	Purchase price	1288	1288			1288	1217	1288			1120
	Market cost	87.84	87.84			87.84	87.84	87.84			87.84
	Selling price	1376	1376			1376	1376	1376			1376
	Market profit	0.16	0.16			0.16	71.16	0.16			168.16
Wholesaler	Purchase price	1200	1143	1143	1190	1190		1250			
	Market cost	79.30	79.30	79.30	79.30	79.30		79.30			
	Selling price	1288	1288	1276	1276	1288		1288			
	Market profit	8.7	65.7	53.7	6.7	18.7		-41.3			
Processor	Purchase price		1376	1376		1376			1255		
	Market cost		350	350		350			350		
	Selling price		2400	2400		2400			2400		
	Market profit		674	774		674			795		
Cooperative	Purchase price										1120
	Market cost										70.88
	Selling price										1260
	Market profit										69.12

Impact

The findings of this study are crucial for the local market sustainable development as it infers the fruitful aspects of building efficient teff market. It is also critical for the scientific community in sharing international experiences of market chain analysis considering essential methodologies.

The type of market in the area is weakly oligopolistic in which few individual traders control the flow of teff. This requires government agencies to work hard in creating a competitive and efficient market which includes creating a conducive environment for new entrants by shortening the stretched license procedure and dealing it in one place as it lowers the transport and other unofficial costs. To have a conductive teff market, the bargaining power of producers must be enhanced by supplying quality products with agreed-upon prices with wholesalers.

The highest average marketing cost of the trader was due to the traditional storage followed by infrastructure, typically road construction, even to have a better renting price of trucks and Isuzu. Hence, giving traders opportunities to have a modernized storage area/shed/shed/ at least in a group is better. The inverted flow of teff

from retailer to wholesaler must be stopped, which is illegal and irrelevant. The trade and industry office had a better train and advised rural retailers who collect and mix adulterated and quality teff to pass to wholesalers.

Conclusions

The market received 57.7% of the total teff produced in the research area, which was allocated from farmers to producers. The most important marketing actors of the teff market chain were producers, rural retailers, urban retailers, rural assemblers and urban assemblers, processors (small *Injera* shop, café and restaurant) and cooperatives. About eleven marketing channels were identified in transferring 2268 Qt teff from producers to final consumers, of which four are dominant. Among them, rural retailers, wholesalers and consumers held the higher share in purchasing teff from the farmer and transferring to end buyers.

The concentration ratio in the Anbessame market for teff was 35.46%, indicating a weakly oligopolistic market which makes it less competitive and ineffective. It is coupled with the critical entry barriers of the teff market, such as more prolonged procedural license, lack of adequate capital, high tax rate, and price fluctuation. The problem of getting timely and relevant market information was found to be a severe problem which in turn was reflected in price setting and mode of payment, making the market conduct that is skewed to one of the market actors, especially to the traders. In this regard, teff's market behavior reveals that producers' selling prices were decided by the market (41%), through seller-buyer bargaining (33%), and simply by consumers (26%). However, few households negotiate on selling price as the major turn it back to sell on another market day at better price (67%). The maximum number of markets visited by traders was three days a week and was by urban traders typically.

The total gross market margin for teff was 31.04%, with 68.96% of the margin going to the producers. This margin varies by channel, with the biggest overall gross marketing margins in channels II, III, V, and VIII, respectively, accounting for 52.38%, 52.38%, 53.67%, and 53.33%. The result shows that the producer share itself varies along channels, with the highest producer share in channel IX (89.24%). Processors have the largest net marketing margin (33.13), while wholesalers have the lowest (-3.0) due to the inverted and unlawful flow of teff from retailer to wholesaler. Moreover, the channel with more teff supplied is not necessarily the channel in which the highest market margin is recorded. The highest market margin depends on the presence of value-adding market actors in the channel.

Conflict of Interest

There is no conflict of interest to declare.

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ENGINEERED ATENOLOL-GLYCOCONJUGATES TO TARGET H9C2 CARDIOMYOCYTE CELL LINES

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Abstract
Background: One of the most important fields of biomedical engineering study nowadays is targeted drug delivery to specific cells. A drug's therapeutic efficacy can be improved and optimised by tightly targeting it to a pathophysiologically essential tissue architecture. The goal of this research is to develop saccharide conjugates for the targeted delivery of Atenolol, a -blocker.
Methods: Galactose (monosaccharide), pectin (polysaccharide), and chitosan were chosen as the saccharides (polysaccharide). By grafting Atenolol with the modified saccharides, the conjugates were created. Spectroscopic and thermal studies were used to describe the chemically changed saccharides conjugates. H9c2 cell lines were used to conduct drug release research and cellular uptake studies. To investigate cytotoxicity, a brine shrimp lethality test was done.
Results: The outcomes exhibit that Atenolol-modified saccharide conjugates can productively convey the medication to the target.
Conclusion: It can be inferred that the improvement of saccharide-drug conjugates can be a compelling methodology for targeting cardiovascular medication.

Keywords
glycoconjugates; atenolol; targeting

Introduction

The capacity to target a medicine to specific cells can boost its therapeutic efficacy dramatically. Adequate drug dosages delivered to specific areas increase therapeutic outcomes wherever they are needed and hence reduce side effects, potentially resulting in a large reduction in side effects [1–3]. The drug targeting concept, according to Martinez, is frequently related with the utilisation of carrier systems, which can possibly deliver medicines, imaging agents, or therapeutic genes selectively to the site of action.

Natural-source oligosaccharide and polysaccharide polymers are non-toxic, biocompatible, and biodegradable. Other biopolymers, such as lipids and proteins, are less thermally stable than polysaccharides [4,5]. According to Sabyasachi [6] integrating the therapeutic agent within a chemically modified polymeric matrix may help to protect the physiologically active component from degradation, improve absorption, control drug release, improve therapeutic efficacy, and reduce administration frequency. Chemical grafting is a method of connecting one or more species of blocks to the main chain as a side chain, resulting in macromolecular copolymers with different

physicochemical features. The number, length, and molecular structure of the side chains identify the newly created copolymer [7].

Cardiovascular illnesses such as arrhythmia and hypertension are serious public health concerns since they affect a vast number of people around the world. Because of their affinity for sympathetic receptors found in many organs, cardiovascular medicines such as sympathetic antagonists have a variety of side effects. As a result, medications like -blockers must be targeted for cardio-selective activity. Passive or active targeting can be used to deliver these medications to cardiac tissues with precision [8].
Because of anatomical and physiological variations, drug administration to the cardiovascular system differs from drug delivery to other systems [2]. Carbohydrate transporters like GLUT4 (Human Solute carrier family-2) are abundant in cardiac cells and are responsible for glucose transport across the cell membrane. The conjugation of cardiovascular medicines with oligosaccharides will be an appealing strategy for manipulating medication pharmacokinetics [9].
The goal of this project is to alter saccharides and construct saccharide conjugates for targeted administration of Atenolol, the -blockers, in order to improve therapy efficacy while reducing side effects.

Materials and Methods

Materials.

- Chemicals

Wockhardt Limited, Aurangabad, donated the drug atenolol (ATN). Loba Chemicals, Mumbai, offered galactose, pectin, and chitosan. Molychem, Mumbai, provided oxalic acid and thionyl chloride. H9c2 adherent rat heart cells were obtained from the National Center for Cell Sciences (NCCS, Pune, India). Himedia lab provided Dulbecco's Modified Eagle's Medium (DMEM), L-glutamine, antibiotics (streptomycin-penicillin solution), foetal bovine serum (FBS), Trypsin-EDTA, Phosphate buffered saline solution (PBS), Hank's Balanced Salt Solution (HBSS), Tris-base, and Triton-X 100, and Qualigens supplied methanol (HPLC grade). Merck Pvt Ltd provided the silica gel aluminium plates 60F254. HPLC column (4.6 mm x 150 mm, 5 m ODS-3, 100 mL) Phenomenex provided the ProdigyTM C18. Tissue culture flask (75 cm^2) with 96 well polystyrene tissue culture plate with flat bottomwere purchased from Tarsons Pvt. Ltd.

- Instruments

Spectrophotometric examination was performed utilising a matched pair of 10-mm quartz cells on a Systronics 2201, India UV-Visible spectrophotometer with a spectral bandwidth of 2 nm and wavelength precision of 0.3 nm. Nicolet-iS10, USA FTIR was used for the FT-IR investigation. On a Differential Scanning Calorimeter-Mettler-Toledo, Switzerland, thermal behaviours were investigated using a nitrogen flow rate of 40 ml/min and a heating rate of 10°C/min from 25 to 300°C. The NMR spectrometer used for the conjugate analysis was a Bruker AV III 400 MHz from Switzerland. The Central Instrumentation Facility, Punyashlok Ahilyadevi Holkar Solapur University, Solapur, Maharashtra, conducted FTIR, DSC, 1H NMR, and 13C NMR research. HPLC analysis was performed on a Jasco MD-2010 multiwavelength detector from Japan, which was equipped at Bharati Vidyapeeth College of Pharmacy in Kolhapur, Maharashtra, Borwin® Version 1.5 software was used. Biocyte Institute of Research and Development in Sangli, Maharashtra, did the cell line investigation.

Synthesis of the Atenolol conjugates by cross-linking the modified saccharides.

The goal of this study was to chemically change the saccharides and conjugate Atenolol for cardiovascular targeting. Using oleoyl chloride, a monosaccharide, galactose (G), and two polysaccharides, pectin (P) and chitosan (C), were chemically changed to produce esters, Galactose oleate (G1), Pectin oleate (P1), and Chitosan oleate (C1). In a three-step approach, chemical modification of saccharides was followed by conjugation of Atenolol with the changed saccharide. AG1, AP1, and AC1 were assigned to the various conjugates.
Step 1: Modified saccharides synthesis (MS).
For the alteration of the Saccharide, the Schotten-Baumann reaction [10] was used.
Over the course of 2 hours, 10 ml of acid chloride in ethanol (20% w/v) was gradually added to the ethanolic solution while stirring. The reaction product was collected, washed, and dried in a hot air oven at 37°C.
Step 2: ATN-MS conjugates synthesis.

The method used to synthesise drug-saccharide conjugates was somewhat modified from that described previously [11]. Over the course of two hours, 10 ml of acid chloride in ethanol (20% w/v) was gradually added to an ethanolic solution of 2 g saccharide and 1 g medicine, stirring constantly. The reaction result was collected, cleaned, and dried at 37°C in a hot air oven.

Physicochemical characterization of MS.

Melting point, partition coefficient, swelling factor, and ester value were used to characterise chemically changed saccharides. TLC, FTIR, and DSC studies were used to confirm the modification reaction.

Characterization of ATN-MS conjugates.

Physicochemical characteristics of the produced conjugates were studied. Melting point and TLC were used as key parameters, followed by FTIR, NMR, and DSC investigation on equipment listed in the Materials section to confirm the reaction.

Drug release analysis

- H9c2 cells preparation

H9c2 adherent rat heart cells were cultured in DMEM with 20 M L-glutamine, 0.45 percent glucose, and 10% v/v heat-inactivated FBS. To prevent microbiological contamination during maintenance in a humidified CO2 incubator, gentamicin sulphate (50 g/ml), penicillin (100 IU/ml), streptomycin (10 g/ml), and amphotericin B (25 ng/ml) were introduced (New Brunswick, Eppendorf). To eliminate any remaining media, cells in a confluent layer were rinsed with 10 ml PBS (pH 7.4). To obtain a homogeneous solution, 0.25 percent w/v Trypsin-EDTA was added to detach the cells from confluency. The cells were mixed in a 1:1 ratio with 0.4 percent w/v Trypan blue solution and counted using a hemocytometer to yield 10^4 cells.

- Cellular uptake study

The above H9c2 cells were cultivated and incubated in a humidified incubator to attain 2×10^5 cells for the cellular uptake investigation [12–14]. The cells were rinsed with 200L HBSS and incubated for equilibrium after incubation. In each well, atenolol-saccharide conjugates (50 g/ml), Atenolol (100 g/ml), and sterile water were added, and they were incubated for 12 hours. The cells were then removed, rinsed in ice-cold PBS (pH 7.4), and incubated for 30 minutes with 0.5 percent v/v Triton-X 100. To prepare samples for HPLC analysis, cells were filled with a 90:10 methanol:water mobile phase and centrifuged (REMI, Mumbai, India). The supernatant layer was diluted and spiked for HPLC analysis to evaluate cellular absorption in H9c2 rat heart cells after centrifugation.

- Chromatographic conditions

The concentration of atenolol in H9c2 rat cardiac cells was determined using the RP-HPLC technique. For chromatographic analysis and data gathering, an HPLC system (Jasco MD-2010, multiwavelength detector) with Borwin Version 1.5 software, Prodigy™ C18 column as stationary phase, and methanol: water (90:10) as mobile phase was employed. A flow rate of 1 ml/min was set, and 20 l samples were injected. The peak's intensity was recorded at 223 nm.

- Toxicity analysis of conjugates

To assess the cytotoxicity of produced conjugates, a brine shrimp lethality test was performed. Brine shrimps (Artemia salina) were hatched in sterile artificial seawater (made with sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) in a 1L conical vessel under continual aeration for 48 hours. After hatching, active nauplii free of eggshells were removed and used for the assay in a brighter part of the hatching chamber. Each vial containing 4.5 ml of brine solution contained ten nauplii drawn through a glass capillary. As a control group, a group of nauplii was left untreated. In the test group, 0.5 ml of test samples (10, 50, 150 g/ml) were mixed with 4.5 ml of brine solution and kept at room temperature for 24 hours under light, with surviving larvae counted [15–17]. The institutional animal ethics council does not have to approve the experimental model chosen for toxicity investigations.

- Determination of lethal dose

By comparing the mean surviving larvae of the test and control tubes, the % lethality was calculated. The best-fit line plotting concentration versus percentage lethality yielded LC50 values. To determine LC50 values, the data were analysed using a Finney computer programme (Probit analysis).

Results and Discussion

The method published by the Schotten Baumann reaction was used to modify saccharides using acid chloride. As a result, galactose oleate, pectin oleate, and chitosan oleate, three distinct modified sachharides saccharides, were produced.

Characterization of modified saccharides (MS).

Characteristics of modified saccharides are depicted in Table 1.

Table 1.Characteristics of synthesized MS**.**

Code	% Yield	Melting point (˚C)	Partition Coefficient	Swelling % ± SD	Ester value	R_f
G	-	178-180	1.28	124±0.44	-	0.2
G1	78.21	153-155	4.74	114±4.97	61.71	0.63
P	-	166-168	2.2	420±3.23	-	0.14
P1	92.60	146-148	6.45	316±0.84	282.97	0.48
C	-	89-91	1.95	204±2.29	-	0.25
C1	72.33	73-78	5.88	186±4.65	206.57	0.55

Characterization of ATN-MS conjugates.

The synthesized conjugates were characterized for various physicochemical parameters, including percentage yield, melting point, and Rf value by thin-layer chromatography followed by FTIR, DSC, 1H NMR, and 13C NMR analysis on instruments as per specified in the Materials section. Confirmation of synthesized compounds was done by the results of the analysis.

Conjugate AG1: Color: colorless, yield: 85%, m.p.: 155-160°C, Rf: 0.74, IR: aldehyde at 1709 cm−1, NH (amide) at 2923 cm^{-1}, NH (amine) at 3288 cm^{-1}, OH at 3384 cm^{-1}, CO (aromatic) 1247 cm^{-1}, DSC: Tg 159°C, NMR: 1H NMR (400 MHZ; DMSO) 0.785-1.471 ppm (CH_3/CH_2), 2.12 ppm (CH_2), 3.071-4.591 ppm (CH_2), 4.966-5.032 ppm (NCH/OCH/C=C-H/CH), 6.098-6.178 ppm (hydroxyl proton of sugar), 6.770-7.138 ppm (aromatic protons), 7.8 ppm (NH2).

Conjugate AP1: Color: colorless, yield: 70%, m.p.: 124-128°C, Rf: 0.42, IR: aldehyde at 1739 cm−1, NH (amide) at 2923 cm^{-1}, NH (amine) at 3288 cm^{-1}, OH at 2853 cm^{-1}, CO (aromatic) 1222 cm^{-1}, DSC: Tg 130°C, NMR: 1H NMR (400 MHZ; DMSO) 0.8-1.247 ppm (CH_3), 2.30-4.895 ppm (CH_2), 4.860-5.045 ppm (NCH/OCH/C=C-H/CH), 5.859 ppm (hydroxyl proton of sugar), 6.880-7.164 ppm (aromatic protons), 9.535 ppm (NH2).

Conjugate AC1: Color: pale yellow, yield: 76%, m.p.: 88-92°C, Rf : 0.61, IR: aldehyde peak at 1629 cm^{-1}, NH (amide) at 2925 cm^{-1}, NH (amine) at 3274 cm^{-1}, OH at 3345 cm^{-1}, CO (aromatic) 1241 cm^{-1}, DSC: Tg 96°C, NMR: 1H NMR (400 MHZ; DMSO) 0.833-1.499 ppm (CH_3), 2.494-3.320 ppm (CH_2), 3.555 ppm-4.20 (hydroxyl proton of sugar/OCH/OCH_2/NCH), 6.826-7.172 ppm (aromatic protons), 8.178 ppm (NH_2).

- Toxicity assay

The cytotoxicity of manufactured Atenolol conjugation was investigated using a brine shrimp lethality bioassay [16,17]. By comparing the mean surviving larvae of the test and control tubes, the % lethality was calculated. The best-fit line plotting concentration versus percentage lethality yielded LC50 values. The three Atenolol conjugates showed no appreciable toxicity, with substantially higher LC50 values indicating an excellent safety profile. Table 2 summarises the toxicity results.

Table 2.Results of Toxicity Study of ATN-MS Conjugates.

Drugs	Conc. of compound µg/ml	Total no. shrimps used/tube	Shrimp survived			Total No. of Shrimp Survived	Percentage mortality	LC_{50} (µg)	(95% confidence interval)
			T1	T2	T3				
AG1	50		8	9	8	25	13.79	323.41 ± 10.00	305.23-339.58
	100		8	8	8	24	13.21		
	150		7	7	7	21	27.58		
AP1	50	10	8	8	7	23	20.68	340.81 ± 10.50	323.76-357.85
	100		7	8	8	23	16.91		
	150		7	6	7	20	31.03		
AC1	50	10	8	7	7	22	20.68	334.81 ± 10.50	318.06-351.55
	100		8	8	8	24	16.84		
	150		6	7	7	20	31.03		

- Cellular uptake study

H9c2 rat heart cells were subjected to RP-HPLC analysis to determine the quantity of Atenolol in Atenolol-saccharide conjugates [14,15]. The concentration of Atenolol present in the cells is determined by the strength of the peak observed at a certain retention time. Atenolol and its saccharide conjugates were introduced to the cells, incubated, rinsed, then carefully removed from the adherent cells with cold PBS. Furthermore, chromatographic procedures ensured that medications from chemically disturbed cells could be analysed.In view of results in Figure 1, Atenolol concentration in H9c2 rat heart cells was observed to contain 25 to 40 µg/ml after being loaded with 50 µg/ml of Atenolol and Atenolol saccharide conjugates. The reason behind the accumulation of the drug in cells was supposed to bind drug saccharide conjugates at receptors, as evidenced by docking analysis. Further, improvement in intracellular drug transport was also claimed due to bypassing the drug transport system so as to favor a dynamic balance between intra and extracellular concentration of drug during an extended period of incubation. Figure 2 shows the percentage of drug uptake in H9c2 cells after 12 h incubation was showed in the range of 40% to 53%. The chemical conjugation of Atenolol and various saccharide units influenced drug permeation across cells and ensured drug stability across cells. The individual HPLC chromatograms of Atenolol and Atenolol-saccharide conjugates are after treatment of H9c2 rat heart cells are mentioned in Figure 3.

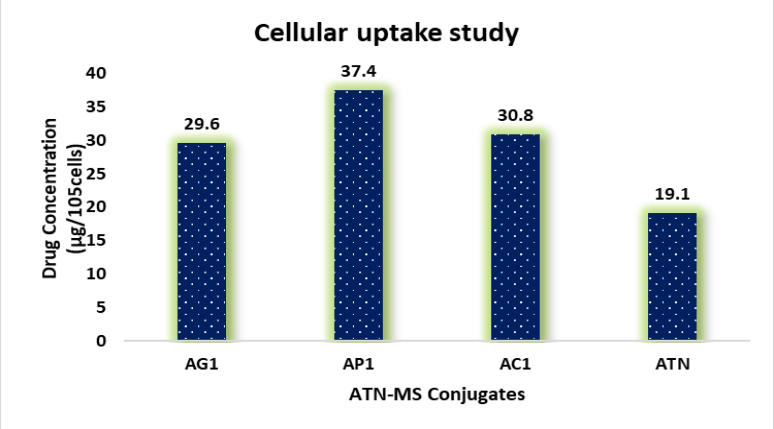

Figure 1. Cellular uptake of ATN-MS conjugates in H9c2 rat heart cells.

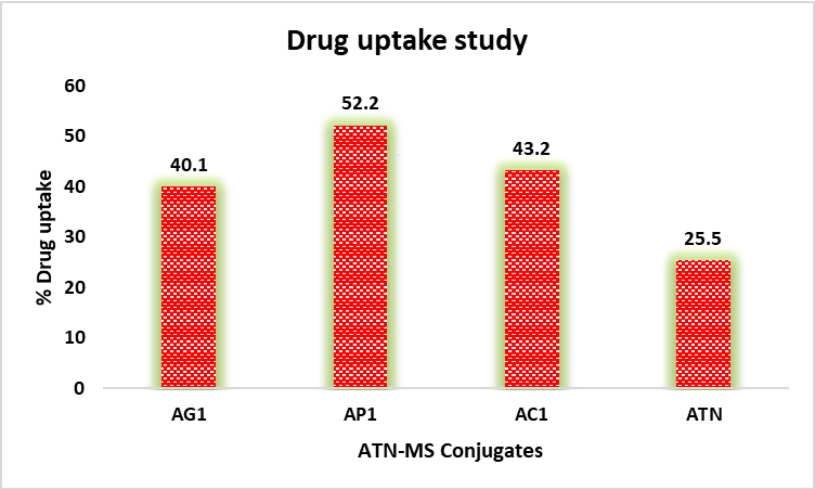

Figure 2. Drug uptake of ATN-MS conjugates in H9c2 rat heart cells.

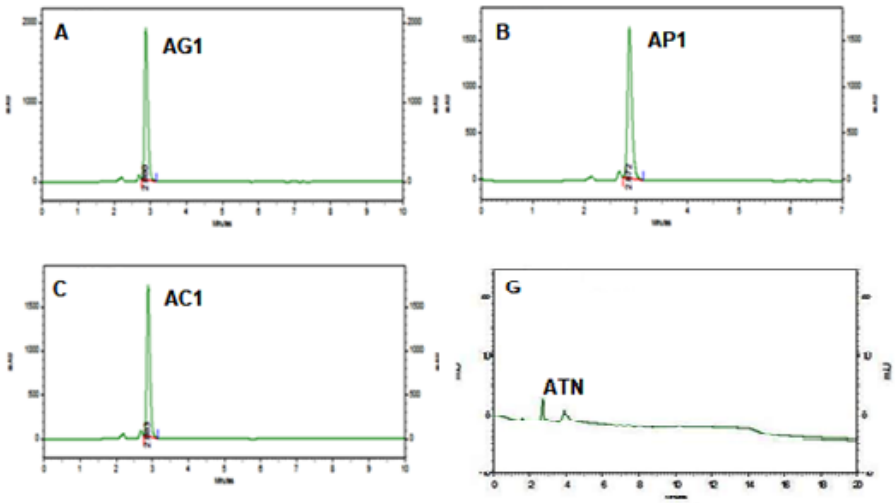

Figure 3. HPLC chromatogram of ATN-MS conjugates A) AG1, B) AP1, C) AC1 and D) and D) ATN in H9c2 rat heart cells.

Impact

Delivering therapeutically adequate doses of the drug directly to the site of action not only improves the efficacy, it also prevents high doses reaching other organs thereby significantly reducing cytotoxic effects of the drugs on other organs. Targeted delivery has the potential to revolutionize current treatments and improve the clinical outcome in cardiovascular patients. This approach can reduce the frequency of dosing, thereby improving the patient compliance and reducing the cost of therapy. This could be an important socio-economic contribution of the study for the society.

Conclusions

Various chemically modified saccharides have been effectively conjugated to Atenolol in order to improve drug availability to cardiac cells for better treatment of cardiovascular diseases and a reduction in adverse effects. The modified saccharides had a higher lipophilicity and, curiously, better swelling properties. This discovery opens

up the possibility of using modified saccharides as an excipient in extended-release medication formulations. FTIR and DSC were used to characterise the chemically changed saccharides with the cardiovascular medication. The conjugates that have been synthesised have been shown to be stable. The medication can be successfully targeted for selective cardiac administration, according to the results of cell line tests.The toxicity research demonstrates that the produced chemicals are safe to use. As a result, conjugating Atenolol with a biodegradable and biocompatible polymeric system to increase drug delivery selectivity for the treatment of cardiovascular disorders appears to be a potential way to improve drug delivery selectivity.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

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INVESTIGATING THE EFFECT OF INSOLUBLE ADDITIVES TYPE ON THE DRAG REDUCTION PERFORMANCE IN A CRUDE OIL TURBULENT FLOW SYSTEM

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Highlight
Enhancing the flow of crude oil transported at turbulent flow mode using different types of insoluble rigid additives to replace traditional soluble viscoelastic additives

Abstract
In the present work, the effect of three insoluble additives densities on reducing the drag of crude oil was investigated. The objective of the present work is to evaluate the effect of the insoluble additive's densities on their drag reduction efficiency in hydrocarbon flow medium. Three powders with different densities are chosen, namely carbon powder, glass powder, and copper powder, with a density of 1710 kg/m^3, 2550 kg/m^3, and 8950 kg/m^3, respectively. The turbulence flow environment was created in a custom-made rotating disc apparatus with a maximum rotation speed of 300 rpm. To evaluate the effect of the powder density, the particle's size was chosen to be 100 µm. All the solutions were tested at the exact operating conditions with a rotation speed ranging between 200 to 2200 rpm. The experimental results showed a clear effect of the powder density on the drag reduction performance. The glass powders showed the highest drag reduction effect, while the copper and carbon powders were lower. The effect of the degree of turbulence on the drag reduction performance of the powders was clear, where the interaction between the powders and the turbulence structures (eddies) governed the turbulence-suppression efficiency of the additives.

Keywords
drag reduction; turbulence; insoluble additives; crude oil; powders.

Introduction
The introduction of minute quantities of soluble additives (polymers, surfactants, or their complexes) to the main turbulent flow systems was proven to have a noticeable impact on reducing the pumping power dissipation [1–3]. Such an approach has attracted the attention of enormous researchers worldwide since the first discovery by Tom et al. in the late forties of the past century [4]. Soluble additive viscoelastic properties are believed to be the main reason behind the flow enhancement (drag reduction) effect in turbulent flow systems, where several mechanisms were proposed to explain this phenomenon [5–8]. Large numbers of researchers investigated polymeric drag-reducing agents (DRAs) with different operating conditions and flow mediums due to their high molecular weights that provide new viscoelastic properties to the turbulent flow systems. The presence of such high molecular weight polymers will enable "turbulence suppression" by preventing the eddies from completing their shapes or by mobilizing the laminar sub-layer on the pipe's wall, which reduces the friction with the pipe wall [9].
On the other hand, applying polymeric drag-reducing agents had several drawbacks that limited its use in several essential industries like crude oil transportation [10–12]. Polymers additive's resistance to mechanical degradation is considered a significant drawback where long-chained polymeric molecules tend to degrade when exposed to high-shearing areas in elbows, valves, and pumps.

Such degradation is irreversible and can lead to a permanent loss in the polymer's drag reduction ability. Usually, the only way to sustain the drag reduction performance after degradation is by re-injecting fresh polymeric additives to the mainstream, increasing the operating costs. Another drawback is the impact of the long-chained polymeric additives on the properties of the transported liquid itself. Any dramatic changes in the apparent physical properties are irreversible (due to the solubility of the polymeric additives in the flowing media), and that will change its market value [13].

Surface Active Agents (surfactants) are another type of DRAs used to overcome some of the polymeric DRA's drawbacks. Surfactants are polar molecules with low molecular weight compared to polymers [14–16]. These short polar molecules migrate to the interface of two immiscible liquids when they get into direct contact and reduce the interfacial tension. In single-phase flow systems, the surfactant molecules will start aggregating and forming what is called "micelles" that act as one entity to interact with the turbulence structures (eddies) and reduce the drag in pipelines. The micelle's resistance to high-shear forces is low, and they usually break when exposed to high-shearing zones during transportation. After breaking, the polar nature of the surfactant molecules will drive them to re-join to form micelles again (regain their shape), which means regaining their drag reduction abilities. Despite this important characteristic, the low molecular weight of the surfactant's molecules or even micelles, compared to polymeric DRAs, will produce limited drag reduction performance [17–19]. To match the long-chained polymers' high drag reduction performance, high concentrations of surfactant additives are needed, and that will affect the apparent physical properties of the transported liquids.

Several researchers have investigated insoluble DRAs to replace soluble additives in pipelines carrying liquids in turbulent flow mode. Different additives were introduced and tested, such as silica [20], agricultural wastes [21,22], pulps [23–25] and even some metals [26,27]. Insoluble additives were proven on many occasions to be effective flow enhancers and, what is more, important not affect the apparent physical properties of the transported liquids. Particle diameter, addition concentration, liquid flow rate, and pipe dimensions are the significant parameters usually investigated when testing any new soluble DRA. The effect of the additive's density on the drag reduction performance of insoluble additives was not critically investigated before. The additive's density effect will reflect the impact of one of the critical parameters in determining the suitability of the additives to act as a DRA, the additive density.

In the present work, three different commercial insoluble additives, namely glass powder, carbon powder, and copper powder, will be used as DRAs. The drag reduction test will be conducted using crude oil as the flowing media and rotating disk apparatus as a flow testing method to simulate the turbulent flow behavior in pipelines. The effect of the diameter and type of the particles will be investigated and compared to evaluate their impact on the flow enhancement.

Methods

Materials

Four commercial powders will be used in the present work: carbon powder, glass powder, and copper powder with a density of 1710 kg/m^3, 2550 kg/m^3, and 8950 kg/m^3, respectively. All the powders were prepared by crushing and grinding commercial glass bottles (for the glass powder), commercial charcoal (for the carbon powder), and commercial copper beads (for the copper powder) using an automated ball mill (RTSCH model PM 200). The required particles size to be investigated in the present work was 100 µm. The resulting powder was screened using an automated screen shaker to separate the particles within the desired size.

The addition concentrations for each type of powders were 100, 300, 500, 800, and 1100 weight parts per million (wppm). The concentration range was chosen to investigate the broadest possible additive concentration range and their effect on the drag reduction performance.

Crude oil was used as the testing medium. The crude oil properties are mentioned in Table 1. The purpose of using crude oil as the carrying medium was to evaluate the effect of insoluble additive's drag reduction performance in hydrocarbon medium transported in turbulent flow mode.

Table 1. Properties of crude oil.

Viscosity (cSt)	6.1
Density (kg/m^3)	846.0
API	41.1
Pour Point (^{o}F)	61.0

Rotating Disk Apparatus

A rotating disk apparatus (RDA) was designed and fabricated to simulate the turbulent flow in the pipelines. Figures 1 and 2 show the schematic diagram of the RDA and a photo of the apparatus, respectively. As shown in Figure 1, the RDA consists of a servo motor with a maximum rotation speed of 3000 rpm, mounted on an aluminum structure and connected to a torque sensor by a shaft. The torque sensor is connected directly to a disk made of aluminum with a diameter of 7 cm by a cylindrical shaft. The disk is located in the middle of a 6.5 L stainless steel container that will carry the tested solutions. The container is supported with a stainless-steel lid with one hole for the rotating disk shaft and another for the thermocouple used for the crude oil temperature measurement.

The servo motor, thermocouple, and torque sensor are connected to a SCADA system connected to a desktop computer for torque and temperature measurements and motor speed control.

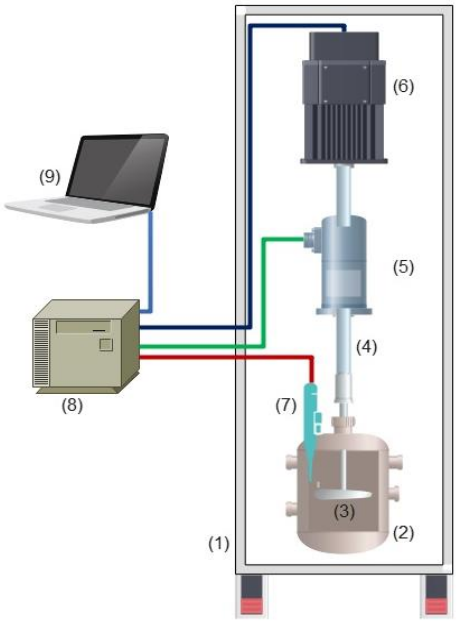


Figure 1**.** Schematic diagram of the rotating disk apparatus: (1) Outside frame, (2) Container, (3) Aluminum disk, (4) Connecting shift, (5) Torque sensor, (6) Servo motor, (7) Digital thermocouple, (8) Controlling system, and (9) PC with SCADA system.

Figure 2. A picture of the RDA system in the lab.

Testing Procedures and Calculations

- Solutions Preparation

Each solution is prepared by mixing the desired weight of the powder in crude oil. The concentration is measured in weight parts per million (wppm) which is calculated as in equation (1):

(1) $$wppm = \frac{w_s}{w_o}$$

where:
w_s is the weight of the solid powder (gm)
w_o is the weight of the crude oil (gm)

The solutions are adequately mixed using an overhead mixer before their introduction to the RDA container.

- Solutions Testing

After pouring the solutions into the testing tank, the tank will be firmly closed using the stainless-steel lid after placing the rotating disk exactly at the center of the tank. The servo motor will operate at different rotation speeds ranging from 200 to 2200 rpm. Choosing this range is to avoid heating the tested solutions that can change the crude oil viscosity. The solution temperature was monitored at each test and was kept within. The torque readings were taken for 20 seconds (1 reading/ second). Each rotation speed test was repeated at least three times to compare and minimize the experiment readings error below 0.7%. The Reynold Number (Re) was determined using the rotational speed as in equation (2):

(2) $$Re = \frac{\rho \omega r^2}{\mu}$$

where:
ρ is the fluid density (kg/m^3),
ω is the angular velocity,
r is the disk radius (m),
μ is the viscosity of the solution (Pa·s).

The drag reduction percentage was determined using the formula as in equation 3:

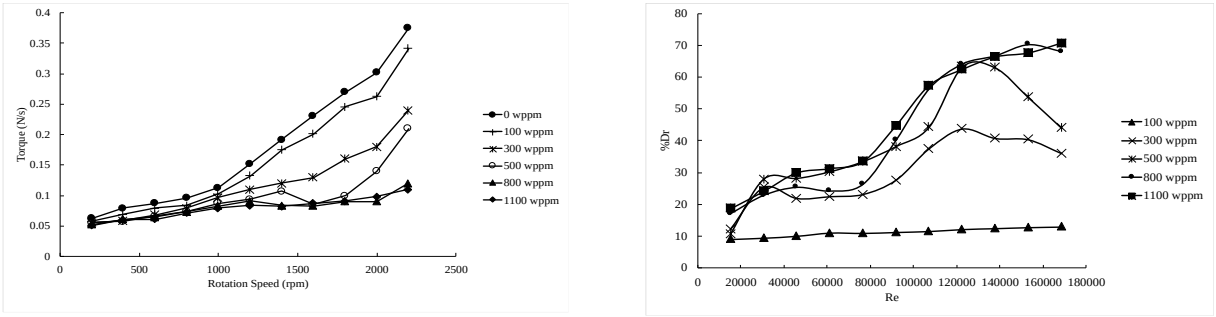
(3) $$\%DR = \frac{To - Ts}{To} x100$$

where:
T_o is the torque reading of pure crude oil (N/m)
T_s is the torque reading of the solutions (N/m)

Results and discussion

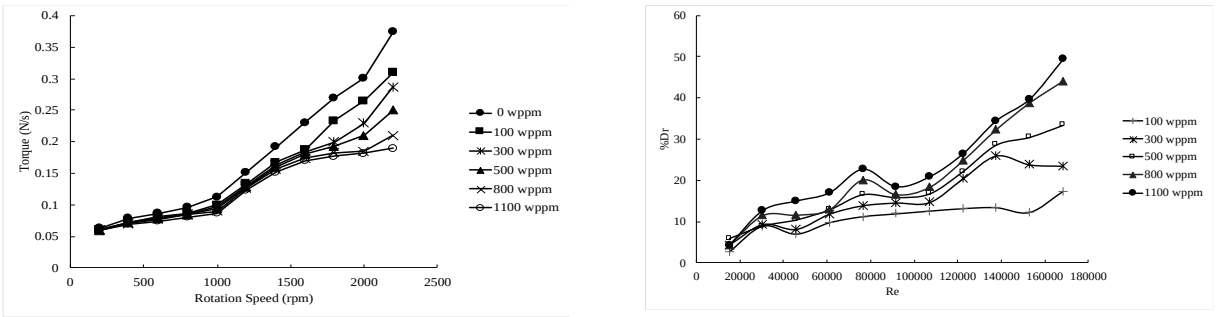
Figure 3 shows the torque readings of the glass powder-water solution tested at different rotation speeds ranging between 200 to 2100 and at different powder addition concentrations ranging between 100 to 1100 wppm. Figure 3 (a) demonstrates the effect of the degree of turbulence on the drag reduction performance of the proposed insoluble additives (glass powder). Two significant responses to the degree of turbulence increment can be observed where the differences between the pure crude oil torque values and the investigated solutions from 200 to almost 1000 rpm were very low. On the other hand, increasing the degree of turbulence (increasing the rotation speed beyond 1000 rpm) started to show more segregated lines with a distinguished drag reduction effect where the torque values of all the solutions were lower than that of the pure crude oil. The lowest torque values were observed with the 800 and 1100 wppm solutions with very close drag reduction performance for both. The reduction in the torque values means reducing the solution's resistance to high shear

forces applied by the RDA, which means a flow enhancement effect. Increasing the degree of turbulence means increasing the interaction area between the suspended solids and the flow medium, which means increasing the turbulence spectrum under the influence of the turbulence suppression effect. Figure 3 (b) shows the drag reduction performance of the glass powder solutions. Interestingly, the 100 wppm solution performance was the lowest compared with the other solutions with an almost constant %Dr of almost 10%. Increasing the concentration of the additive increased the %Dr with clear responses to the increase of the degree of turbulence represented by the Reynolds number (Re). For the 300 wppm and 500 wppm, the %Dr increased with the increase in the Re until reaching the maximum drag reduction point at Re = 122543. Further increase in the degree of turbulence resulted in a decline in the drag reduction performance, and that was expected since the relationship between the degree of turbulence the %Dr is not always linear, and it depends on the interaction between the additives and the turbulence structures formed during the flow (eddies). The decline in the %Dr curve means that the degree of turbulence has overcome the effect of the interfering additives (within the investigated concentrations), resulting in reducing their effect. Increasing the addition concentrations to 800 and 1100 wppm resulted in achieving the maximum %Dr point (70.6%) at the maximum Re investigated (168497), and that confirms the fact that the maximum performance is directly related to the additive's concentration. Increasing the additive concentration means increasing the number of additive particles interacting with the turbulence structures (eddies) responsible for power losses. The degree of interaction is governed by the number of particles and the degree of turbulence simultaneously, suggesting that the maximum %Dr point is the optimum degree of turbulence where the additives act as turbulence suppressors. Figure 4 shows the experimental results using the copper powder solutions at the same operating conditions as the glass powder solutions. It is interesting to see how the solution behavior (Figure 4 a) changed when the powder density from 2500 kg/m^3 to 8950 kg/m^3 where the lines started separating at much higher rotation speeds. In other words, the powder with higher density needed a higher degree of turbulence to be able to fully function as a drag-reducing agent with a distinguished effect of additives concentration. Increasing the powder density means increasing the additive's total weight, increasing the shear forces needed to create a suitable turbulence environment with optimum solid-liquid interaction. Figure 4 b shows that the maximum %Dr point was not reached within the investigated rotation speed range due to the high-density powder used.



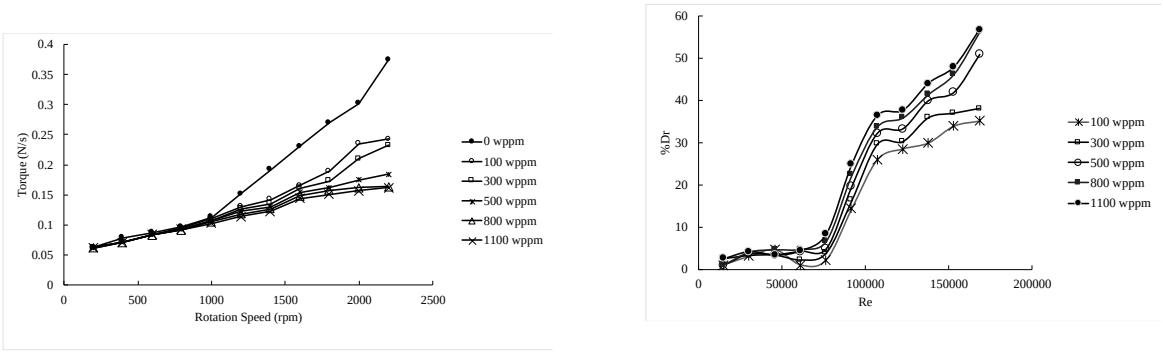
(a) (b)

Figure 3. (a) effect of glass powder concentration on the torque values at different rotation speeds, (b) effect of Reynolds number on the %Dr at different powder concentrations.



(a) (b)

Figure 4. (a) effect of copper powder concentration on the torque values at different rotation speeds, (b) effect of Reynolds number on the %Dr at different powder concentrations.



(a) (b)

Figure 5. (a) effect of carbon powder concentration on the torque values at different rotation speeds, (b) effect of Reynolds number on the %Dr at different powder concentrations

Figure 5 shows the drag reduction performance of the carbon powder with a density of 1710 kg/m^3. It is interesting to see how reducing the density of the powder affects its overall drag reduction performance where a clear drag reduction onset point was spotted at Re = 76589 while that was not observed with other powders. Even though the maximum %DR was less than the other powders, the optimum performance was distinguishable with a unified degree of turbulence that led to a clear drag reduction onset point.

The effect of the degree of turbulence on the drag reduction performance of soluble and insoluble additives was extensively investigated by large numbers of researchers [28–31]. The investigated suspended solid solutions responses to Reynolds number increment are in many cases identical to the reported behaviors of different types of additives at different flow mediums where a drag reduction onset point is detected where the additives start to interact with the turbulence flow structures effectively. Further increase in the degree of turbulence (Re) will enable observing the effects of the investigated parameters such as the additive's concentrations, additives molecular weight, soluble additive types, and conduits dimensions. In the case of suspended solids, the same phenomena apply since the suggested controlling mechanism emphasizes the additives-liquid interaction, which was observed in the present work. The effect of Re (degree of turbulence) on the %Dr observed in the present work was also observed by Suzuki et al., (2006) [18] who used trimethylolethane (TME) clathrate–hydrate slurry in water. Their experimental results show that the drag reduction onset point started when the Reynolds number was almost 10,000.0 and was more distinguishable at the higher Re range. This behavior was also observed by Akindoyo & Abdulbari [32], who investigated carbon nanotubes powder as drag reducing agent with water flowing in turbulent flow mode in pipelines. The results showed that at low additive's concentrations, the relationship between the additives drag reduction performance and degree of turbulence is not always linear where a decline in the %Dr was observed after reaching the maximum %Dr when the Re exceeds 140,000 (Figures 3 b and 4 b). To explain such behavior, it is important to bring another factor into this discussion: the additive's concentration. Generally, it is believed that increasing the additives concentrations (soluble and insoluble additives) will increase the %Dr within certain limitations. In other words, increasing the additive's concentrations will enable the suppression of a broader spectrum of turbulence at the investigated degree of turbulence reaching the maximum %Dr point where the interaction of the specific additive is almost optimum. Further increment in the degree of turbulence will result in unbalancing the mentioned optimum point by introducing extra shear forces that will overcome the effect of additives presence in the turbulent flow medium, resulting in reducing the %Dr [33]. Such a phenomenon was observed by Kazi et.al. [23] and Gharehkhani et.al. [25] when they investigated different grades of pulp and Kenaf fibers as drag-reducing agents. Their experimental results showed that the relationship between the fiber concertation, size, nature, and the degree of turbulence controls the drag reduction performance, and the effect of the addition concentrations of these fibers is directly related to the turbulent flow medium. The complex relationship between the solid additive's concentrations and the degree of turbulence was also highlighted by Akindoyo and Abdulbari [32] when testing carbon nanotubes as drag-reducing agents in pipelines. Their experimental findings confirmed the nonlinear relation between the additive's concentration and the drag reduction performance at lower concentration ranges while the maximum %Dr was achieved at the highest nanoparticles concentration.

Figure 6 shows the effect of the powder density on the %Dr for all the investigated solutions at the diameter and concentration of the same particles. The glass powder with the density of 2550 kg/m^3 showed the best and most stable drag reduction performance when compared with the other two powders that have a lower density (carbon powder, 1710 kg/m^3) and the powder with the higher density (copper powder, 8950 kg/m^3). The only

case where the cast iron showed higher drag reduction performance was at the lowest concentration (100 wppm). This is expected since the generated degree of turbulence with the presence of minute quantities of the powder (100 wppm) will dominate with maximum turbulence-powder interaction. This will reach the optimum drag reduction flow environment at low concentrations with the less dense investigated powder (Figure 6 a).

Increasing the concentrations of the investigated powders resulted in a very interesting behavior where the carbon and copper powders exchanged domination when the Re increased. The copper powder drag reduction performance was higher at low Re ranging between 15317 to 91907. On the other hand, the carbon powder drag reduction performance was higher at higher Re values. Such behavior demonstrates the relationship between the particle's concentrations, density, and degree of turbulence. Increasing the Re means increasing the degree of turbulence, which includes increasing the number of eddies and their swirl-motion intensities. The interaction of these eddies with the powders will suppress the overall degree of turbulence due to the new apparent physical properties introduced to the main flow by introducing the additives. At the low Re range, the degree of turbulence allows the interaction of high-density copper powders, which will result in suppressing more weak turbulence structures than the carbon powder due to the high-density differences. Increasing the Re will increase the turbulence intensity that might create clusters of accumulated copper powders due to the centrifugal force while the carbon powder will continue to suspend effectively in the solution with stable drag reduction performance.

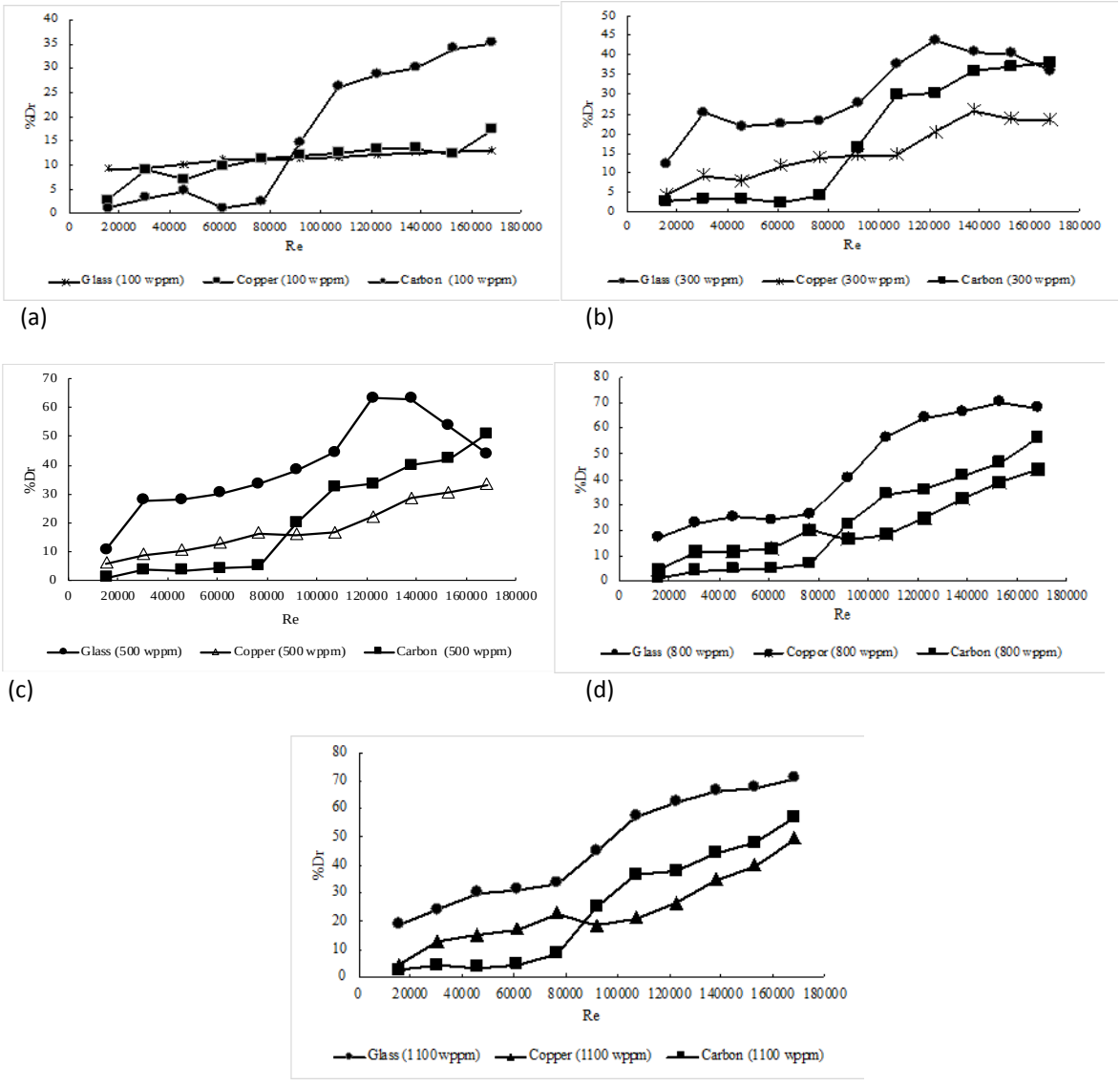


Figure 6. Comparing the drag reduction performance of the three insoluble additives at different concentrations: (a) 100 wppm, (b) 300 wppm, (c) 500 wppm, (d) 800 wppm and (e) 1100 wppm.

Impact

The present work addresses an important fundamental and commercial problem which is the stability of soluble drag-reducing agents on the overall drag reduction process. The work proved that the insoluble additives have a significant potential to replace known and commercially applied soluble additives, like polymers, with excellent flow enhancement efficiency. Adopting such an approach will eliminate the mechanical stability drawback associated with soluble additives and will introduce low-cost and effective insoluble drag-reducing agents that have no direct impact on the transported liquid's apparent physical properties, high resistance to shear forces, and are reusable. These additives can be effectively used in pipelines carrying crude oils or refinery products at turbulent flow mode.

Conclusions

The present work investigates the effect of three different insoluble additives, namely, carbon powder, glass powder, and copper powder, on the drag reduction performance using rotating disk apparatuses and crude oil as the flowing medium. The work aimed to examine the effect of the powder density on the overall drag reduction efficiency, and that was achieved by eliminating the effect of particles size where all the investigated powders were 100 µm. The experimental results showed that the glass powder with the density of 2550 kg/m^3 showed the highest and most stable drag reduction performance among the other two powders (copper, 8950 kg/m^3, and carbon 1710 kg/m^3). It is believed that the interaction between three dominating factors controlling the drag reduction performance is essential in identifying the powder with the optimum drag reduction efficiency. The high-density copper %Dr was higher than that of the carbon at a certain degree of turbulence (low Re), while the carbon powder showed a higher %Dr at high Re. This exchange of drag reduction performance domination is believed to be due to the exchange of the degree of interaction between the suspended solid particles and the turbulence structures and the effect of the particle's density on both. The relationship between the particles densities and their drag reduction performance was not linear (within the investigated conditions) where this kind of complex interaction between several factors acting at the same system is interesting needs to be examined further with a wider range of powders and the inclusion of the particles size as one of the influencing factors to generalize the understanding of this phenomenon

Conflict of interest

There are no conflicts to declare

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OPTIMIZATION OF PROCESS PARAMETERS IN TURNING OF MAGNESIUM AZ91D ALLOY FOR BETTER SURFACE FINISH USING GENETIC ALGORITHM

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Highlight
Optimization of machining parameters using genetic algorithm.

Abstract
This research examined at the optimum cutting parameters for producing minimum surface roughness and maximum Material Removal Rate (MRR) when turning magnesium alloy AZ91D. Cutting speed (m/min), feed (mm/rev), and cut depth (mm) have all been considered in the experimental study. To find the best cutting parameters, Taguchi's technique and Response Surface Methodology (RSM), an evolutionary optimization techniques Genetic Algorithm (GA) and Non-dominated Sorting Genetic Algorithm-II (NSGA-II) were employed. GA gives better results of 34.04% lesser surface roughness and 15.2% higher MRR values when compared with Taguchi method. The most optimal values of surface roughness and MRR is received in multi objective optimization NSGA-II were 0.7341 µm and 9460 mm^3/min for the cutting parameters cutting speed at 140.73m/min, feed rate at 0.06mm/min and 0.99mm depth of cut. Multi objective NSGA-II optimization provides several non-dominated points on Pareto Front model that can be utilized as decision making for choice among objectives.

Keywords
genetic algorithm; magnesium alloy; turning; optimization; pareto front; RSM.

Introduction

Magnesium alloys are advanced and lighter materials used extensively in industries like automobile, aerospace, engine block casing etc. They possess excellent strength to weight ratio which is a significant factor in modern industrial products [1]. In machining of magnesium alloy surface roughness and MRR have become significant factors in terms of quality and economy characteristics. As a result, experimental methods must be used to optimize machining parameters for newer materials [2]. Numerous optimization techniques which include Fuzzy Logic, Taguchi optimization, Ant colony optimization, and simulated annealing gives solutions for various optimization problems. Noticeably, genetic algorithm optimization is a modern method and it additionally determined to be better in arriving at optimized solution for complicated real worldwide problems [3,4]. Many academics and businesses are currently interested in improving manufacturing strategies in order to save costs, improve quality [5], and gain from increased efficiency.

Boostani et al. [6] have investigated AISI 304 austenitic stainless steel in order to improve cutting quality and productivity while lowering power consumption. Surface roughness was assessed in a short period of time using a surface roughness tester; this could be influenced by machine vibration, tool and material type, and coolant supply. Cutting parameter optimization focuses primarily on cutting force, surface quality, and processing cost, according to Su's study [7]. It also claims that the impact of cutting parameters on energy

consumption is neglected in the multi-objective cutting operation of cutting parameters using the RSM approach. Kavimani et al. [8] have investigated the influence of machining parameters on wire electrical discharge machining performance in magnesium composite, they have reported that the MRR and surface roughness are significant towards parameter involvement. Kuntolu et al. [9] investigated the modelling of cutting parameters and tool geometry for multi-criteria optimization of surface roughness and vibration using response surface methodology. They found that feed rate is the most important contributing factor to surface roughness, and that the interaction of cutting speed and tool coating has a minor impact.

Lamentably, in multi variable trouble characteristic most computational techniques for complicated machining systems require significant computational resources to assess each parameter. No method presently consequences inside the same levels of efficiency for all process. The prevailing work interests to apply the surface roughness values and MRR as multi objective capabilities, as an efficient approach for identifying the exceptional parameters for traits, via GA and NSGA-II. GA is a technique seeking out optimum combinations and solutions over the traditional optimization strategies. The stairs usually followed in GA are reproduction, crossover and mutation. There are various algorithms and techniques of solving multi-objective optimization hassle exist [10]. Therefore, multi-objective optimization is pondered as a utility of single-objective optimization for conduct a couple of goals. NSGA gives the higher maximum extraordinary solution for each objective function in terms of solutions [11]. NSGA-II based multi-objective optimization for MRR, and Surface roughness has been completed on this machining for magnesium alloy. Multi objective optimization GA (MOGA) toolbox of MATLAB has been utilized in this paper.

Materials and methods

Taguchi approach and RSM

The design of experiments has been taken using Taguchi methodology, it is an effective method in producing robust design. It provides a simple and methodical qualitative optimum design at a minimal cost. It has a collection of orthogonal arrays which can be used to study into the effectiveness of different process parameters. Genechi proposed the signal-to-noise (S/N) ratio, which took both means and variability into account and the influence of process characteristics on the performance measure is indicated by S/N ratios in ANOVA [12,13].

In Taguchi approach three levels of performance characteristics are followed in the analysis of S/N ratio that is “Smaller the better”, “Larger the Better” and “Nominal the Better”. Based on the criteria, different S/N ratios can be selected. The main objective of the analysis of variance is to evaluate the cutting parameters that significantly affect surface quality characteristics. Effective parameter is determined by F-test value and compared with standard F- table value. RSM [14] is a statistical method for modeling and analysis, which deals different variables and responses. RSM is used to develop a model for suitable approximation for relationship between the response variables and independent variables as followed by Equation (1).

$$\eta = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_i \sum_j \beta_{ij} X_i X_j \quad (1)$$

GA approach- Single objective optimization

GA is an evolutionary optimization approach for solving problems involving the application of evolutionary biology principles [15]. Genetic inheritance, natural selection, mutation, and crossover are all biologically inspired approaches used by GAs. GAs are used to model the process of biological evolution and Darwin's hypothesis of survival of the fittest. A set of ability solutions or chromosomes within the character of bit strings that are randomly picked are used to solve an optimization with GA. A population is made up of the full set of these chromosomes. The chromosomes evolve for the duration of numerous iterations or generations. New generations (offspring) are generated making use of the crossover and mutation method. Crossover is the process of separating two chromosomes and then combining one-half of each pair with the other. A single chromosomal bit is spun during a mutation. The chromosomes are then assessed against a set of fitness requirements, with the best ones being kept and the rest being destroyed. This approach is repeated until one chromosome exhibits high-quality fitness and is deemed the best answer to the challenge [16,17].

Multi-Objective Optimization with NSGA-II

The Non-dominated Sorting GA (NSGA) became proposed through Srinivas and Deb [18], and is based on several layers of classifications of the individuals and it is prominent algorithm for multi-objective optimization. Before selection is carried out, the population is ranked on the premise of domination the usage of Pareto the front.

NSGA become developed for this study to reap optimum cutting parameters in terms of population, cross over, mutation and crowding distance parameters applied [19]. Figure 1 depicts the flow chart for the implementation of the NSGA-II algorithm.

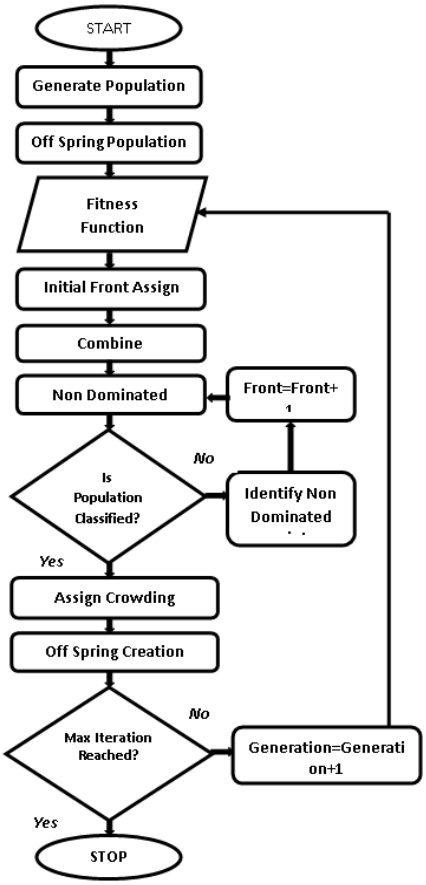

Figure 1. NSGA-II Process Flow Chart.

Experimental Design

The chemical composition of Magnesium AZ91D alloy is shown in Table 1 and the turning experiment was carried out in size of 50 mm diameter and 70 mm length workpiece. In this turning operation CVD coated carbide tool insert of triangular shape was used and the standard code of cutting tool TNMG 16 04 08 was chosen. The CNC turn master was chosen for cutting operation. Cutting parameters Cutting Speed *v* (m/min), Feed rate *f* (mm/rev) and depth of cut *d* (mm) were considered in this machining process and its levels are presented in Table 2. The machining of magnesium alloy was carried out in dry condition. The surface roughness measurement was conducted with Mitutyo Surftest 211. The turning experiment was carried out in 50 mm diameter and 70 mm length of 5 work pieces and shown in Figure 2. The roughness values were taken on the work piece circumference in three different places and the average value is presented in Table 3. The MRR (*Q)* is an important parameter in industrial economy and quality factor. In this work the MRR calculated by using standard Equation 2. MRR is calculated in each level of machining process and the data are presented in Table 3.

(2) $$Q = v \times f \times d \ (mm^3/min)$$

Table 1. Chemical Composition of Magnesium AZ91D Alloy.

Element	Al	Mn	Zn	Si	Fe	Cu	Ni	Mg
Weight %	8 7-10.5	0.15-0.4	0.35-1.0	0.3	0.05	0.15	0.01	Balance

Table 2. Cutting parameters and their levels.

Factor	Unit	Level-1 (Low)	Level-2 (Medium)	Level-3 (High)
v	m/min	80	110	150
f	mm/rev	0.05	0.10	0.15
d	mm	0.5	0.75	1.0

The Taguchi orthogonal array L27 was chosen for machining experiments. The signal to noise ratios for surface roughness Ra and MRR obtained using Taguchi's approach are shown in Table 3. A lower surface roughness value is usually desirable in metal cutting. The S/N ratio was determined using the smaller-is-better methodology for the aforementioned responses. Regardless of the category of performance criteria, the higher the S/N ratio, the better the performance. The highest value of the S/N ratio indicates the optimum value for each level of process parameter.

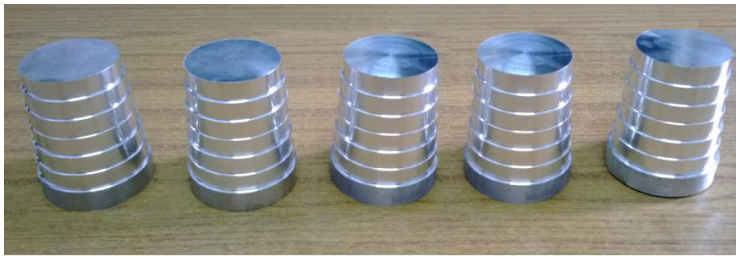

Figure 2. Machined Work pieces.

Results and Discussion

Taguchi approach- Main effect analysis

To investigate the effects of cutting parameters on surface roughness and MRR, Minitab® is used to construct a primary effect plot for various S/N ratios. The main effect graph shows a visualization of the main response values of the S/N ratio at each level of factor. Cutting speed has an impact on surface roughness, as shown in Figure 3. Increasing cutting speed reduces surface roughness in level 2 and then increases. The most essential factor is the feed rate; as the feed rate increases, the surface roughness increases to level 2, and then decreases as the depth of cut increases.

Figure 4 depicts the effects of factors on MRR values. It indicates that MRR increases as cutting speed, feed rate, and depth of cut increase. Taguchi methodology implies optimum cutting parameters of 110m/min cutting speed (level 2), 0.05mm/rev feed rate (level 1), 1mm depth of cut (level 3) to attain minimum surface roughness of 0.63µm and 150m/min cutting speed (level 3), 0.15mm feed rate (level 3), 1mm depth of cut (level 3) to reach maximum MRR of 19550 mm^3/min and shown in Table 4.

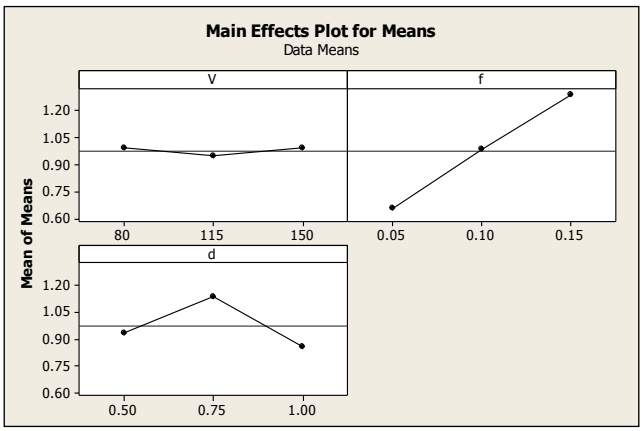

Figure 3. Main effect plot for surface roughness.

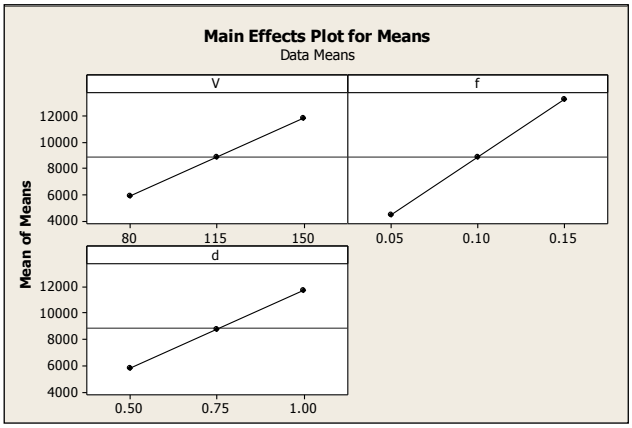

Figure 4. Main Effect Plot for MRR.

Table 3. Experimental Data with S/N ratio.

Trail No	Cutting Speed (m/min) v	Feed Rate (mm/rev) f	Depth of Cut (mm) d	Ra (µm) Ra	MRR (mm3/min) Q	Surface roughness S/N Ratio	MRR S/N Ratio
1	80	0.05	0.5	0.45	1963	6.93575	65.85619
2	80	0.05	0.75	0.88	2944	1.110347	69.37802
3	80	0.05	1	0.64	3925	3.876401	71.87679
4	80	0.1	0.5	0.86	3925	1.310031	71.87679
5	80	0.1	0.75	1.25	5888	-1.9382	75.39862
6	80	0.1	1	0.79	7850	2.047458	77.89739
7	80	0.15	0.5	1.45	5888	-3.22736	75.39862
8	80	0.15	0.75	1.48	8831	-3.40523	78.92044
9	80	0.15	1	1.12	11775	-0.98436	81.41922
10	115	0.05	0.5	0.57	2944	4.882503	69.37802
11	115	0.05	0.75	0.67	4416	3.478504	72.89984
12	115	0.05	1	0.62	5888	4.152166	75.39862
13	115	0.1	0.5	0.85	5888	1.411621	75.39862
14	115	0.1	0.75	1.23	8831	-1.7981	78.92044
15	115	0.1	1	0.82	11775	1.723723	81.41922
16	115	0.15	0.5	1.35	8831	-2.60668	78.92044
17	115	0.15	0.75	1.40	13247	-2.92256	82.44227
18	115	0.15	1	0.99	17663	0.087296	84.94104
19	150	0.05	0.5	0.63	3925	4.013189	71.87679
20	150	0.05	0.75	0.77	5888	2.270185	75.39862
21	150	0.05	1	0.70	7850	3.098039	77.89739
22	150	0.1	0.5	0.81	7850	1.8303	77.89739
23	150	0.1	0.75	1.17	11775	-1.36372	81.41922
24	150	0.1	1	1.06	15700	-0.50612	83.91799
25	150	0.15	0.5	1.42	11775	-3.04577	81.41922
26	150	0.15	0.75	1.37	17663	-2.73441	84.94104
27	150	0.15	1	0.99	19550	0.087296	87.43982

Table 4. Response table for data means

Level	Surface Roughness (Ra)			MRR(Q)		
	v	f	d	v	f	d
1	0.9911	0.6589	0.9322	5888	4416	5888
2	0.9444	0.9822	1.1356	8831	8831	8831
3	0.9911	1.2856	0.8589	11775	13247	11775
Rank	3	1	2	2.5	1	2.5

ANOVA and RSM

The findings of the analysis of variance for surface roughness and MRR are shown in Tables 5 and 6 respectively. Surface roughness and MRR are both influenced by the feed rate than by cutting depth and cutting speed. RSM is used to develop the correlation between cutting parameters and responses in terms of second order equations. The Equations (3) and (4) are the quadratic response surface model impacting surface roughness and MRR with turning factors v, f, and d.

Table 5. ANOVA for Surface Roughness

Source	DF	SS	MS	F
v	2	0.01307	0.00653	0.38
f	2	1.76780	0.88390	51.45
d	2	0.36980	0.18490	10.76
Error	20	0.34360	0.01718	
Total	2	2.49427		

Table 6. ANOVA for MRR

Source	DF	SS	MS	F
v	2	155981953	77990977	23.82
f	2	350959395	175479697	53.60
d	2	155981953	77990977	23.82
Error	20	65473906	3273695	
Total	2	728397207		

(3) $$Ra=-1.95365-0.00757143\times v+16.4071\times f+6.45714\times d+0.000038095\times v^2-4\times f^2-3.84000\times d^2\ 0.0190476\times v\times f+0.000952381\times v\times d-9.53333\times f\times d$$

(4) $$Q=9672.32-84.10\times v-96723\times f-12896\times d+841.071\times v\times f+112.143\times v\times d+117750\times f\times d$$

Single objective genetic algorithm

50 population size, 1 crossover rate, 0.1 uniform distribution mutation rate, bit number 16 for each variable, and 1500 iterations were used in this investigation. For single objective optimization, the optimal cutting parameters for surface roughness and MRR were calculated using RSM Equations (3) and (4). Cutting speeds of 80 to 200 m/min, feed rates of 0.05 to 0.2 mm, and depth cuts of 0.5 to 2 mm were used for the optimization study. The optimal value of cutting parameters resulted in the minimizing of surface roughness and maximum MRR through GA optimization as shown in Figure 5. From this optimization the results for surface roughness and MRR are shown in Table 7. Single objective optimization study carried out separately on surface roughness and MRR yields optimal parameter values as 0.4789(µm) and 23059 (mm^3/min) respectively. For optimizing both objective, multi-objective optimization technique is preferable.

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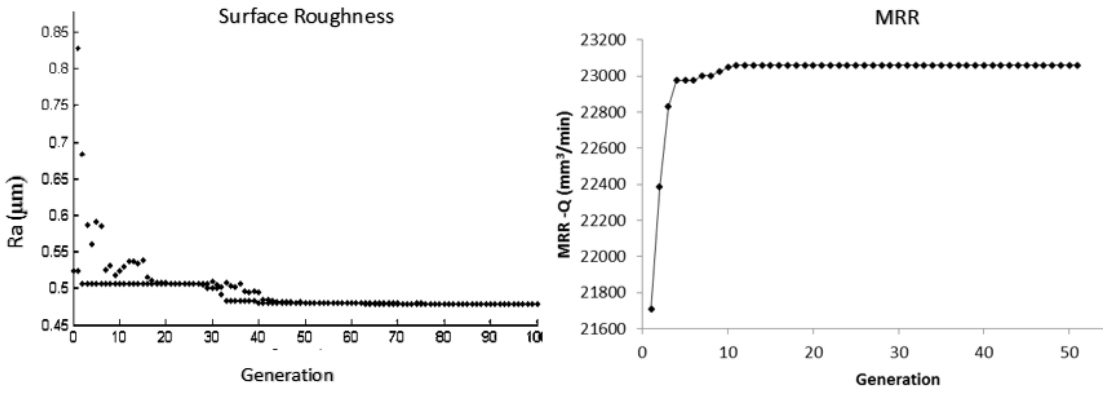

Figure 5. GA Single objective approach.

Table 7. Optimized cutting parameter in single objective GA algorithm.

Response	**v (m/min)**	**f (mm/min)**	**d (mm)**	**Optimized Value**
Ra	127	0.05	0.5	0.4789(µm)
MRR	148	0.15	1	23059 (mm^3/min)

Non-dominated Sorting Genetic Algorithm-II

In this multi objective optimization study, population size of 50, crossover rate of 1.0, and 0.1 mutation rate were utilized for 2500 iterations. Solve XL and MATLAB mathematical software's are used in this research to intensify NSGA-II and Pareto front analysis and the most efficient cutting parameters has been selected to attain minimal surface roughness with maximum metal removal rate. Figure 6 represents Pareto Front analysis, which indicate that while increase of MRR tends to increase the surface roughness of material. In graph, curve A to B shows lesser values of MRR with the increase of surface roughness when compared with curve C to D. MRR value achieved at points B and D are 8000 mm^3/min and 23000 mm^3/min respectively. Through NSGA-II optimal values of surface roughness and MRR are attained between point B and C as shown in Table 8. The most optimal value achieved in curve B to C is 0.7341 µm surface roughness and 9460 mm^3/min MRR for the cutting parameters of 140.73m/min cutting speed, 0.06 mm/min feed rate and 0.99 mm depth of cut.

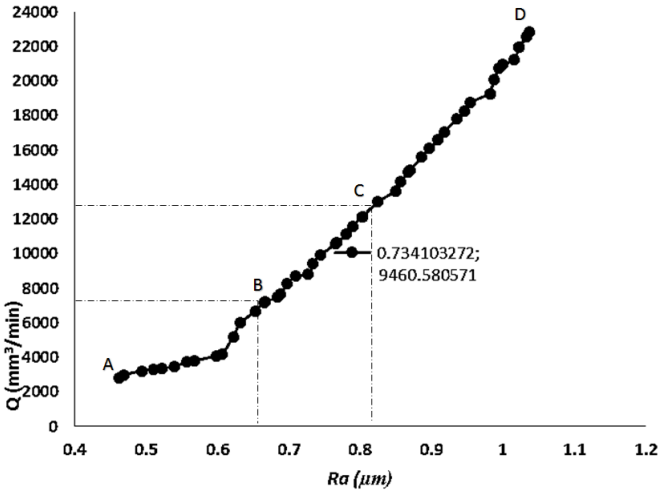

Figure 6. Pareto Front Model.

Table 8. B to C Region Values in Pareto Front Model

S. No	v	f	d	Ra	Q
1	119.12	0.0625	0.99976	0.68822	7691.3957
2	136.29	0.0565	0.99988	0.69830	8267.28214
3	131.30	0.0628	1	0.71002	8713.91085
4	147.24	0.0548	0.99878	0.72770	8829.47595
5	140.74	0.0627	0.99976	0.73410	9460.58057
6	136.05	0.0689	0.99976	0.74446	9916.49345
7	143.90	0.0689	0.99976	0.76593	10591.1772
8	143.90	0.0693	1	0.76690	10649.4953
9	146.28	0.0712	1	0.78121	11136.4851
10	136.05	0.0814	1	0.79010	11613.8588
11	141.64	0.0814	1	0.80357	12153.2902

Impact

Furthermore, the GA technique suggested the optimal combination of parameters to achieve an improved surface finish and MRR. Therefore, this experimental study proved, based on the aforementioned observations, that the proposed methodologies can determine the optimum machining parameters which would be significantly beneficial in the manufacturing industries.

Conclusion

The cutting parameters in the turning of the magnesium alloy AZ91D were optimized using evolutionary techniques in this experiment. Through single and multi-objective GA, the RSM quadratic model has been constructed to forecast and evaluate mathematical solutions for achieving optimal cutting parameters. The following conclusions were derived using an evolutionary approach.

- Taguchi methodology implies optimum cutting parameters of 110m/min cutting speed, 0.05mm/rev feed rate, 1mm depth of cut to attain minimum surface roughness of 0.63µm and 150m/min cutting speed, 0.15mm feed rate, 1mm depth of cut to reach maximum MRR of 19550 mm^3/min.
- The minimum surface roughness attained was 0.4789m by optimized cutting parameters of 127m/min cutting speed, 0.05mm/rev feed rate, 0.5mm depth of cut, and the maximum MRR of 23059 mm3/min was attained by optimized cutting parameters of 148m/min cutting speed, 0.15mm feed rate, and 1mm depth of cut with a single objective GA approach.
- GA yields better results of about 34.04% lesser surface roughness and 15.2% higher MRR values when compared with Taguchi method.
- The most optimal values achieved in multi objective optimization NSGA-II are 0.7341 µm surface roughness and 9460 mm^3/min MRR for the cutting parameters of 140.73 m/min cutting speed 0.06 mm/min feed rate and 0.99 mm depth of cut.
- Multi objective NSGA-II optimization provides several non-dominated points on Pareto Front model that can be utilized as decision making for choice among different objectives.

Conflict of interest

There is no conflict of interest to declare.

Acknowledgments

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ASSESSMENT OF INVESTMENT AND INNOVATION IMAGE OF THE REGIONS OF UKRAINE IN TERMS OF SUSTAINABLE TRANSFORMATIONS

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Abstract
The article examines the existing methods of assessing the innovation and investment image of the region and proposes an author's methodology that considers the development of the region's production and infrastructure to form a strategic policy to create and apply innovation and economic potential of the region. The research hypothesis is based on the use of the method of distance from the standard, allowing to obtain relatively objective and fair assessments of the innovation and investment image of the region, considering the leading indicators of enterprises, ensuring their differentiation by industry. Enterprises, industries, and regions in the top rankings are considered more attractive to investors in terms of innovation, economic and social development. To confirm the hypothesis, the method of assessing the innovation and investment image of the regions was considered, which begins with an empirical, theoretical study and ends with the practicality of use. A method for determining the rating of business activity of innovation-active and investment-attractive enterprises considering their territorial and sectoral affiliation has been developed. The study has a significant economic and regional impact: using this method of assessing the innovation and investment image of the regions allows investors to evaluate and select the region in which the investment proposal will consider the features and priorities of economic development of the region and industry trends.

Keywords
industrial development; investment and innovation image; modelling; region; strategic planning; sustainable transformation.

Introduction

The region's sustainable development is determined by the stability of the functioning of structure-forming industries [1–3]. Assessment of the innovation and investment image of the region in the conditions of sustainable market transformations acquires certain features and significance, as it combines innovation and investment components of development. This assessment is a logical continuation of evaluating its investment environment, investment and innovation potential, and investment attractiveness, which scientists pay considerable attention to [4,5].

In market conditions, the investment resources of any country region are one of the essential factors in its innovative development [6–8]. The region's domestic investment potential is becoming insufficient to ensure the required level of investment, so its positive image is the most critical competitive advantage in the international investment market.

This component of the effective functioning of the regional economy has significant potential in the form of land and labour resources, natural resources, intellectual property and more. Primary and secondary markets of investment resources of Ukraine can be integrated into the world economic space because this is what forms the attractive investment climate of its regions and their innovation and investment image.

The study aims to study the existing methods and develop recommendations for improving the methodology for determining the rating of business activity of innovative and investment-attractive enterprises in regions.

The goal involves the consistent implementation of the following tasks:

- to characterize the existing methods of assessing the investment attractiveness of the region
- to conduct a rating assessment of the regions of Ukraine for 2020
- identify factors that affect the innovation and investment image of the regions
- identify the principal areas of innovation and investment image of the regions
- to propose methods and criteria for assessing the investment attractiveness of the external environment for the activities of regional complexes
- to evaluate the methodology and analyze the results

Theoretical Basis

The innovation and investment image of the country is determined by the policy of innovation and investment development and factors of innovation and investment attractiveness of the regions. That is, the closest to the concept of innovation and investment image of the area is investment attractiveness, so it is the method of its evaluation was chosen for in-depth analysis, the results of which are shown in Figure 1.

Almost all these methods consider investment indicators (components). Still, the innovation factor as an independent is considered only in the evaluation methodology of Nechyporuk [9], so other methods cannot fully characterize the innovation and investment image of the region.

Among scientists, the assessment of the image of the regions of Ukraine is often reduced to the ranking of areas by the level of attractiveness to investors.

The introduction of the rating system for enterprises of different regional complexes is a means of intensifying the activities of enterprises, encouraging them to participate in investment and innovative regional competitions, and tenders open additional sources of investment for successful enterprises.

Any investment project has a thematic focus; its most significant effect is achieved in regions with the best investment environment. Therefore, the assessment and forecasting of its investment attractiveness play a vital role in substantiation of the region's innovation and investment development policy.

The rating of regions should be carried out using indicators from investors' point of view, which are most important when deciding on the place and volume of investment. The input data needed to calculate the integrated rating of the region are data on:

1. Economic development of the region: real sector (revenues of local budgets per capita, UAH; volume of investments in housing construction per capita, UAH; share of unprofitable enterprises, %); foreign economic activity (volume of imports per capita, dollars; foreign direct investment per capita, dollars).
2. Market infrastructure: business services (availability and number of insurance institutions, banks, and leasing companies); transport (density of roads; the volume of passenger traffic); telecommunications.
3. Financial infrastructure (availability of financial institutions).

4. The state of human resources (number of registered unemployed; mortality per 1,000 people).
5. Activities of local authorities in the field of private entrepreneurship of small and medium-sized businesses [10–12].

A feature of the use of indicators of variation in the socio-economic development of the regions of Ukraine is the scope of variation ($R = Xmax - Xmin$); this applies to the objectives of interregional comparisons within a particular project. The region's investment attractiveness is determined by its investment potential, which can be assessed using a set of macroeconomic indicators: profit margins, inflation, economic growth, etc.

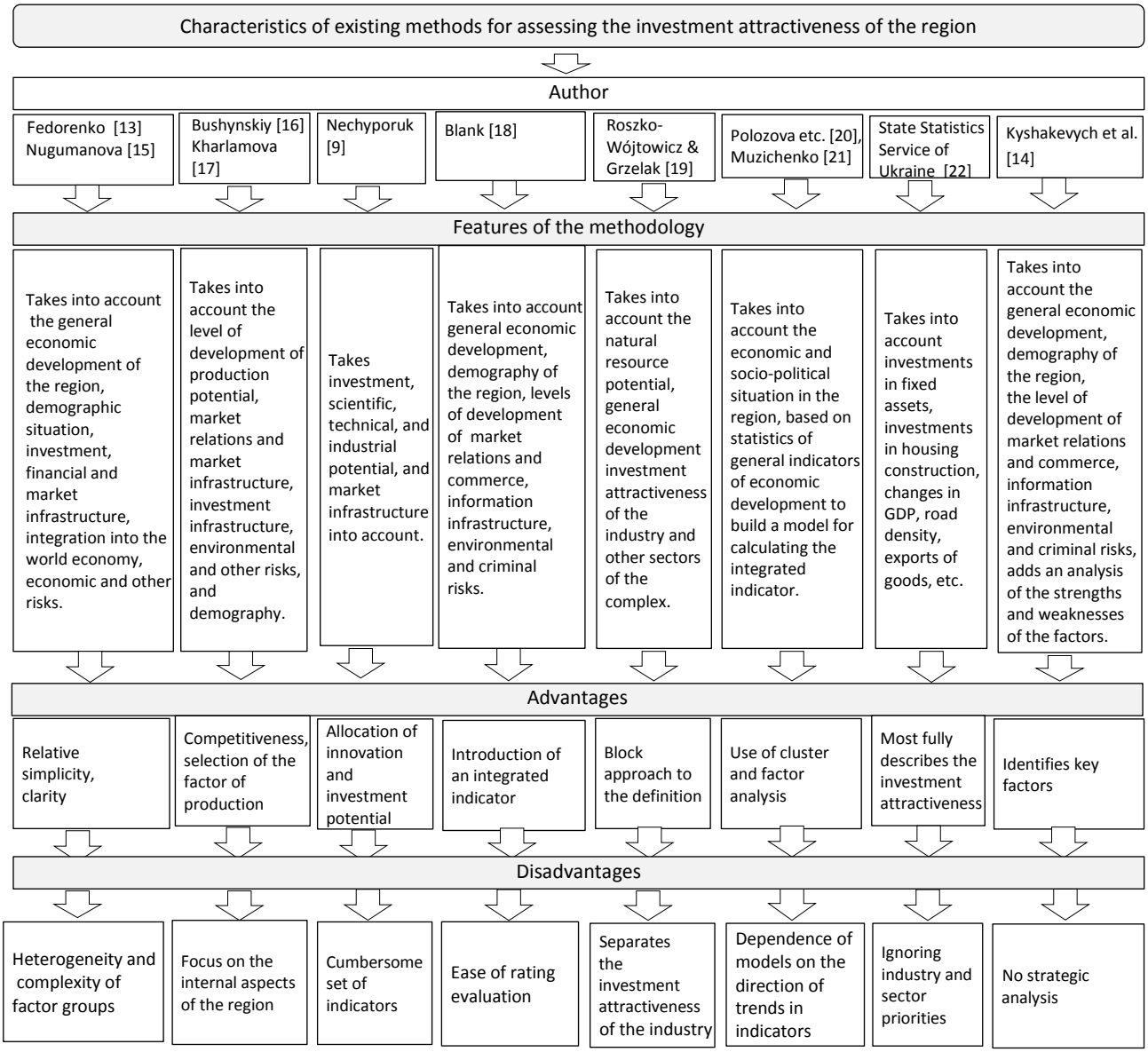


Figure 1. Characteristics of existing methods for assessing the investment attractiveness of the region.
Source: developed by the authors based on [*9,13–22]*

The concepts of "region" and "investment attractiveness" are correlated mainly on the economic basis: if the internal preconditions for the economic development of the region are weak, its investment attractiveness for investors, especially foreign ones, will be lower.
In most methods [14–22] to determine the investment attractiveness of regions, the overall priority of the group of economic indicators is 60-80%. But its level is significantly influenced by infrastructural, socio-political,

technological, and legal factors. This methodology was used to determine the rating of individual regions of Ukraine in 2020, Table 1.

Table 1. Rating assessment of the regions of Ukraine for 2020. *Source: edited by the authors based on [23]*

Regions	**The results of calculations by groups of indicators**										Rating score	Place
	Economic development (25%)		Infrastructure development (22%)		Financial infrastructure (25%)		Human resources (13%)		Entrepreneurship and local government (15%)			
Vinnytsya	0.299	8	0.383	3	0.012	21	0.369	21	0.375	18	0.335	3
Volyn	0.287	10	0.235	17	0.085	7	0.442	4	0.423	12	0.251	12
Dnipropetrovsk	0.298	7	0.255	13	0.143	3	0.267	25	0.309	24	0.248	14
Donetsk	0.185	24	0.232	19	0.029	14	0.413	10	0.293	25	0.176	25
Zhytomyr	0.197	21	0.208	21	0.015	20	0.405	13	0.380	16	0.257	11
Zakarpattya	0.329	2	0.665	1	0.012	24	0.465	2	0.384	15	0.219	19
Zaporizhzhya	0.290	9	0.187	22	0.053	11	0.379	19	0.336	23	0.206	23
Ivano-Frankivsk	0.316	5	0.315	10	0.009	25	0.434	6	0.367	21	0.222	17
Kyiv	0.231	16	0.451	2	0.263	2	0.404	14	0.405	13	0.283	8
Kirovograd	0.186	23	0.355	7	0.053	10	0.380	17	0.477	4	0.274	10
Luhansk	0.170	25	0.220	20	0.140	4	0.451	3	0.342	22	0.180	24
Lviv	0.319	3	0.339	8	0.021	18	0.354	23	0.389	14	0.301	5
Mykolayiv	0.258	14	0.233	18	0.126	5	0.398	15	0.372	19	0.212	22
Odesa	0.297	6	0.327	9	0.058	9	0.386	16	0.430	11	0.251	13
Poltava	0.264	13	0.186	23	0.012	23	0.368	22	0.367	20	0.221	18
Rivne	0.242	15	0.260	12	0.065	8	0.418	9	0.436	10	0.344	2
Sumy	0.188	22	0.240	15	0.029	16	0.409	12	0.437	9	0.236	15
Ternopil	0.265	12	0.383	4	0.040	12	0.437	5	0.443	7	0.289	7
Kharkiv	0.211	20	0.298	11	0.029	15	0.285	24	0.482	3	0.306	4
Kherson	0.269	11	0.166	24	0.021	17	0.419	8	0.456	5	0.215	21
Khmelnytskiy	0.213	19	0.353	6	0.039	13	0.410	11	0.441	8	0.297	6
Cherkasy	0.221	18	0.247	14	0.012	22	0.369	20	0.378	17	0.231	16
Chernivtsi	0.318	4	0.369	5	0.019	19	0.815	1	0.498	2	0.279	9
Chernihiv	0.230	17	0.237	16	0.106	6	0.424	7	0.448	6	0.218	20
city of Kyiv	0.801	1	0.131	25	1.000	1	0.380	18	0.523	1	0.777	1

According to the analysis, groups and individual regions achieved their rating due to various factors: Kyiv is twice ahead of the nearest competitor – for a combination of almost all aspects; the indicator of Rivne region is mainly due to industries and agriculture – the region has the second rating point for economic development; Vinnytsia region – uses its natural potential and therefore undergoes significant structural changes in the regional economy. Kharkiv and Lviv regions also hold leading positions. Donetsk and Luhansk regions remain the least developed in economic development (Table 2). The downgrade of these oblasts is due to the high increase in receivables from enterprises and accounts payable to the budget.

Among agrarians, the Volyn region is in the lead, having increased its rating by several points compared to last year's result. The adverse development trend of this region has changed to a positive one due to the high development of the financial sector and human resources.

In recent years, the change of outsider regions and members of the leading growth group was not due to the improvement of the situation in the region but due to the deterioration of other regions.

Let's define the level of innovation and investment by the main factors of the attractiveness of individual regions grouped into four groups: priority, high, medium, and low attractiveness. To determine the total location of the region indicators of capital investment were selected; the share of sold innovative products in the total volume of sold industrial products and the importance of exports of goods.

Table 2. The level of investment and innovation attractiveness of the regions of Ukraine as of 01.01.2021.
Source: edited by the authors based on [23].

Groups of regions by the level of investment and innovation attractiveness	**Regions**	**Region's place**		**Region's place by indicator**		
		2019	2020	The volume of capital investments	The share of sold innovative products in total	The volume of exports of goods per capita
Priority	city of Kyiv	1	1	1	21-22	1
	Donetsk	15	2	17	1	9
	Kirovograd	3	3	14	2	8
	Dnipropetrovsk	7	4	2	23-24	2
	Poltava	8	5	4	23-24	5
	Zaporizhzhya	10	6	10	9	4
High enough	Kharkiv	11	7	8	3-4	18
	Sumy	14	8	18	8	11
	Mykolayiv	5	9	16	6	3
	Luhansk	23	10	25	3-4	25
	Khmelnytskyi	24	11	11	17-19	20
	Odesa	18	12	6	10-11	16
Medium	Chernihiv	13	13	13	5	12
	Zhytomyr	20	14	12	15-16	17
	Volyn	2	15	5	17-19	15
	Cherkasy	16	16	19	10-11	14
	Vinnytsya	17	17	7	12-14	13
	Kyiv	6	18	3	12-14	6
Low	Ternopil	22	19	15	12-14	21
	Rivne	25	20	23	25	22
	Lviv	12	21	9	15-16	10
	Ivano-Frankivsk	21	22	20	21-22	19
	Chernivtsi	19	23	24	20	24
	Zakarpattya	9	24	22	17-19	7
	Kherson	4	25	21	7	23

Data analysis Table 2 showed that the country's western regions have the lowest investment and innovation attractiveness, i.e., they are not attractive enough for foreign investors.
The main directions of formation of innovation and investment image of the regions of Ukraine are:

- liberalization of investment activity, development of the market of goods and services
- regulation of investment activity by creating a stable regulatory framework
- ensuring the sustainable functioning of state and regional government and the administrative system
- elimination of legislative restrictions on the operation of foreign capital to a competitive investment environment
- improvement of financial support of innovation processes and innovation-oriented investments priority ones
- fight against corruption
- minimization of political risks

The issue of the formation of innovation and investment image of the territory should be considered regarding the existing environment, its transformational changes, and existing trends in the region. Therefore, the method of assessing the investment attractiveness of the external environment for the activities of regional complexes allows:

- first, to recommend to the regional authorities a mechanism of assistance for purposeful management of the general business climate in their region
- secondly, to give the enterprises complex analytical tools for assessing the relationship with regional offices. This, ultimately, allows you to make management decisions on further developing innovation and investment activities of complexes and economic systems

It is recommended to use the following criteria to assess the investment attractiveness of the external environment:

- tax burden above (below) the critical assessment and the availability of benefits for enterprises in the complex
- social security of employees, the degree of social tension, cultural and educational levels, and opportunities to increase them
- opportunities to obtain loans, interest rates
- the presence of laws and regulations that stimulate or, conversely, reduce the capabilities of the complex in the development of production
- the existence of regulations that encourage investment and protect the rights of investors
- degree of development of means of communication and means of communication
- availability of necessary resources for production (in% of cost): labour resources, energy, raw materials.
- a significant factor determines the degree of "importance" of each environment sector

Quantitative assessment of the favourable environment of the complex and the assessment of its impact on the environment is determined by calculating the coefficients: the favourable environment and the impact of the external environment.

One of the central tasks of the region's innovation and investment development policy is to ensure its complexes' investment attractiveness and components. When the financial reporting of units, in particular enterprises, does not fully characterize their activities, the level of reliable public information about innovative enterprises in a form accessible to investors increases. Therefore, it is advisable to openly publish regular ratings of their investment attractiveness, which becomes a reliable source of information for potential investors.

Considering the above provisions, a method for determining the rating of business activity of innovation-active and investment-attractive enterprises, which considers their territorial and sectoral affiliation, has been developed. Depending on the size of the enterprise, the complex is divided into three groups: the first-large enterprises, the second-medium and the third-small.

The practice of rating companies is quite common. Thus, according to Forbes, the newspaper annually publishes a list of the TOP-100 largest companies in Ukraine [24], and the domestic magazine "Finance and Economics" (TOP-200 Ukrainian companies by revenue) the largest companies in the world by capitalization or value multiplied the number of shares issued at their market price. The latter is determined in the case of their turnover in the securities market. But for most Ukrainian issuing companies, it is impossible to estimate the capitalization rate in this way; their shares do not have market quotations. Therefore, it is advisable to calculate the rating within the regions without it.

It is recommended to choose the indicator of the volume of sold products (gross income) as the primary selection criterion, which reflects the usefulness of manufactured goods for buyers with their established solvency and contains products shipped and released to consumers in value terms. This indicator determines the feasibility of the enterprise's economic activity, informing the investor about the dynamics of the indicator, the volume and industry affiliation of the enterprise.

The second important indicator is the profitability of sales, which is an indicator of the efficiency of the enterprise and is calculated as the ratio of balance sheet profit to revenue from sales (in%). Another indicator of the efficiency of the enterprise - is the return on assets, which is a classic, most often used to assess the efficiency of use of funds and is calculated as the ratio of balance sheet profit to the average value of assets. At negative values of these indicators, the enterprises are excluded from a rating.

To assess the enterprise's activities is also used and labour efficiency, which is analogous to productivity but is calculated instead of produced based on sold products, as the ratio of revenue from sales to the average number of employees.

Methods

The research hypothesis is based on the use of the method of distance from the standard of Bakanov & Sheremet [25], which allows for obtaining relatively objective and fair assessments of the innovation and investment image of the region, considering the leading performance indicators of enterprises, ensuring their differentiation by industry. Enterprises, sectors, and regions that fall into the top rankings are considered more attractive to investors in terms of innovation, economic and social development.

To confirm the hypothesis, consider the methodology for assessing the innovation and investment image of the regions, which begins with an empirical, theoretical study and ends with the practicality of the use Figure 2.

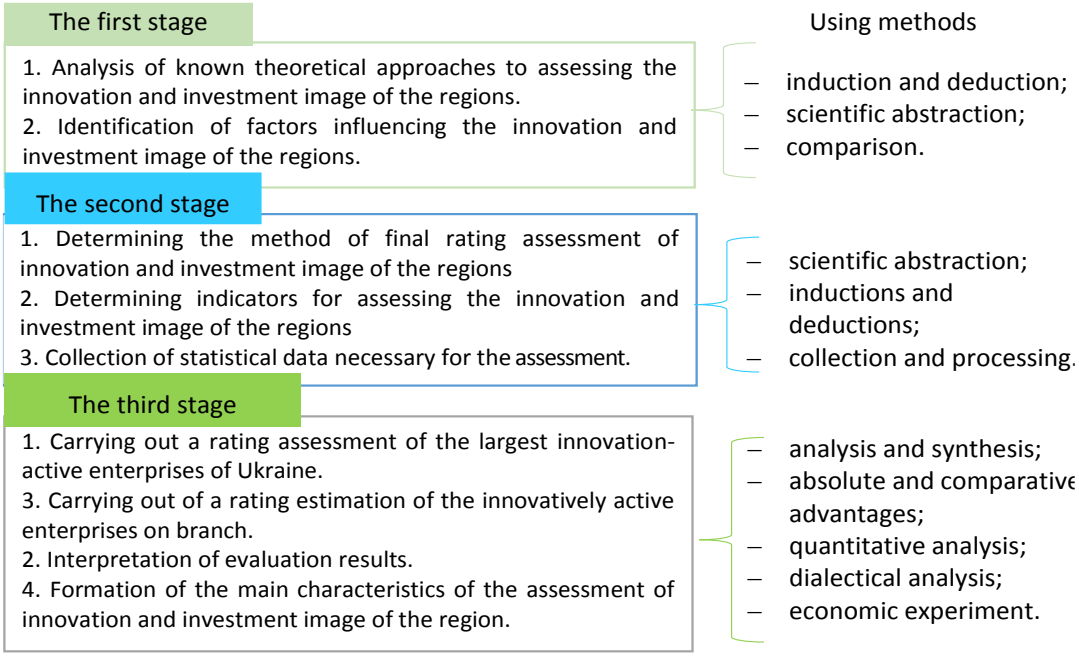


Figure 2. Algorithm of research methodology. *Source: own development.*

To determine the final rating, the standard method of Bakanov & Sheremet [25] or the method of distance from the standard, the essence of which is to find the coefficients of deviation from the standard, their subsequent generalization and weighting on the coefficient of significance.

The initial data are given in the form of a matrix a_ij, where the rows contain the numbers of indicators ($i = 1,2,3, \dots n$), and in the columns – the numbers of enterprises ($j = 1,2,3, \dots t$).

For each indicator is the maximum value and is entered in the column of the conditional reference company (m + 1).

The initial indicators of the matrix a_{ij} are standardized as the ratio to the corresponding indicator of the reference enterprise by the formula [25]:

$$x_{ij} = a_{ij} / max\ a_{ij}, \quad (1)$$

where x_{ij} – standardized indicators of the state of the j-th enterprise.

The value of the rating of each enterprise of the regional complex:

$$Rj = \sqrt{(1 - x_{1j})^2 + (1 - x_{2j})^2 + \dots + (1 - x_{nj})^2} \quad (2)$$

where Rj is the rating of the J-th enterprise. The company with the lowest value of Rj has the highest rating. $x_{1j}, x_{2j}, \dots, x_{nj}$ – standardized indicators of the j-th enterprise.

Experiment

To conduct a rating assessment, the 60 largest enterprises of Ukraine in different regions and industries were surveyed according to the 2020 rating. Enterprises that had negative values on rating indicators were excluded from the rating.

The results of the rating assessment based on the results of 2020 by groups of enterprises of industrial complexes of the regions of Ukraine are shown in Table 3.

Table 3. Final rating assessment of the largest innovative enterprises of Ukraine for 2020p.
Source: calculated by the authors based on data [24].

Name of Company	Branch	Revenue, MUAH	Balance sheet profit, MUAH	Number of employees, persons	Assets, MUAH	Profitability of sales, %	Return on assets, %	Labour efficiency, %	Rating points, Rj	Place
Interpipe	metallurgy	29000.0	21400.0	2 420	2198.5	73.79	97.33	11.98	121.23	1
Roshen	food industry	24000.0	2500.0	10 000	3001.8	10.42	83.28	2 40	82.82	2
Carpathian Petrochemical	chemical industry	13600.0	1900.0	2693	2505.9	13.97	75.82	5.05	76.04	3
Ferrexpo	metallurgy	38900.0	10400.0	9000	15472.9	26.74	67.21	4.32	71.11	4
Ukrnaftoburnya	fuel and energy sector	84000	2800.0	169	9495.2	33.33	29.49	49.70	65.02	5
Metinvest	metallurgy	278000.0	8800.0	66000	13454.0	3.17	65.41	4.21	64.52	6
Amic	fuel and energy sector	8100.0	4200.0	2122	1055.3	51.85	397.99	3.82	63.93	7
«Yuzhniy Gok» Mining and Processing Plant	metallurgy	25200.0	8900.0	6500	22763.9	35.32	39.10	3.88	51.35	8
Carlsberg	alcohol and beer	8200.0	1400.0	1493	2862.3	17.07	48.91	5.49	50.73	9
Philip Morris	FMCG	25600.0	346.0	503	10571.4	1.35	3.27	50.89	49.94	10
Kernel	agro	93000.0	3200.0	14000	7664.4	3.44	41.75	6.64	41.21	11
Kyivstar	connection	22200.0	9000.0	3023	86957.0	40.54	10.35	7.34	41.12	12
Foxtrot	retail	13100.0	274.0	4623	679.4	2.09	40.33	2.83	39.38	13
Alliance Oil	wholesale trade. fuel and energy sector	7800.0	54.0	212	466.3	0.69	11.58	36.79	37.32	14
METRO Cash & Carry	retail	19800.0	1300.0	3200	3668.7	6.57	35.44	6.19	35.27	15
DTEK Energy	fuel and energy sector	76800.0	1800.0	44000	5011.2	2.34	35.92	1.75	34.95	16
JTI International Company	FMCG	14300.0	698.0	462	4038.6	4.88	17.28	30.95	34.30	17
new post	postal services	13500.0	783.0	27334	2288.0	5.80	34.22	0.49	33.56	18
Eridon	agro	21200.0	738.0	824	4200.3	3.48	17.57	25.73	29.87	19
Concern Galnaftogaz	fuel and energy sector	41100.0	2700.0	28000	9575.9	6.57	28.20	1.47	27.76	20
Farmak	pharma	6500.0	1000.0	2698	4046.2	15.38	24.71	2.41	27.76	20
Odessa Regional Energy Company	fuel and energy sector	7800.0	81.0	291	949.0	1.04	8.54	26.80	26.87	22
Kryukiv Carriage Building Plant	engineering	8500.0	829.0	5630	3278.0	9.75	25.29	1.51	25.82	23
Krivoj Rog's Iron-Ore Combine	metallurgy	6600.0	1600.0	7425	13062.0	24.24	12.25	0.89	25.81	24
BaDM	wholesale trade. pharma	35500.0	2900.0	2799	12737.3	8.17	22.77	12.68	25.72	25

International Airlines of Ukraine	transport	26900.0	1600.0	1 600	9225.7	5.95	17.34	16.81	23.26	26
Sandora	food industry	10400.0	548.0	3 230	2405.8	5.27	22.78	3.22	22.30	27
TEDIS Ukraine	wholesale. FMCG	49700.0	356.0	2 321	4492.3	0.72	7.92	21.41	21.55	28
MHP	agro	53100.0	5600.0	28 000	32468.8	10.55	17.25	1.90	18.86	29
Lemtrans	transport	14100.0	440.4	866	11308.8	3.12	3.89	16.28	15.69	30
ADM Ukraine	agro	23100.0	9.0	149	5867.3	0.04	0.15	155.03	15.40	31
Venta LTD	wholesale trade. pharma	13000.0	56.0	802	4923.9	0.43	1.14	16.21	15.22	32
Novokramatorsk Machine-Building Plant	engineering	6700.0	671.0	8475	5548.6	10.01	12.09	0.79	14.29	33
Eramov Systems	IT	8100.0	440.0	8300	3163.1	5.43	13.91	0.98	13.64	34
Kharkivnergozbut	fuel and energy sector	6800.0	31.0	520	2567.4	0.46	1.21	13.08	12.09	35
Optima-Pharm	wholesale trade pharma	31200.0	256.0	2420	11379.5	0.82	2.25	12.89	11.95	36
ATB	retail	104900.0	4400.0	49259	35652.1	4.19	12.34	2.13	11.83	37
Epicenter K	retail	45700.0	3700.0	25325	44525.4	8.10	8.31	1.80	10.22	38
Imperial Tobacco	FMCG	7700.0	167.0	700	6175.4	2.17	2.70	11.00	10.21	39
SoftServe	IT	7000.0	4.2	7193	38.7	0.06	10.82	0.97	9.86	40
Comfy	retail	13600.0	15.0	3267	4331.8	0.11	10.17	4.16	9.73	41
DniproAzot	chemical industry	7000.0	274.0	3412	2749.3	3.91	9.97	2.05	9.48	42
Eva	retail	12900.0	375.0	10420	4460.9	2.91	8.41	1.24	7.65	43
Mykolayiv.alumin a plant	metallurgy	8600.0	264.0	1500	5491.0	3.07	4.81	5.73	6.41	44
Nibulon	agro transport	27700.0	1400.0	6164	33840.8	5.05	4.14	4.49	6.2	45
Dniprospetsstal	metallurgy	8300.0	78.0	4800	2138.4	0.94	3.65	1.73	2.74	46
Tavria Plus	retail	7500.0	64.0	3922	2088.1	0.85	3.07	1.91	2.26	47
Bayadere	alcohol and beer	6600.0	22.0	4000	2913.4	0.33	0.76	1.65	0.96	48

According to the results of the calculations in Table 3 it is seen that the highest rating was received by enterprises: 1. Interpipe (metallurgy) Dnipropetrovsk region; 2. Roshen (food industry), Kyiv; 3. Karpatnaftohim (chemical industry) Ivano-Frankivsk region; 4. Ferrexpo (metallurgy) Poltava region; 5. Ukrnaftoburinnya (fuel and energy sector), Kyiv; 6. Metinvest (metallurgy) Donetsk region; 7. Amic (fuel and energy sector), Kyiv; 8. «Yuzhniy Gok» Mining and Processing Plant (metallurgy) Dnipropetrovsk region; 9. Carlsberg (alcohol and beer) Zaporizhzhia region; 10. Philip Morris (Fast Moving Consumer Goods), Kyiv. The obtained results confirm the level of investment and innovation attractiveness of the regions of Ukraine, where the priority ones are Kyiv, Donetsk, Dnipropetrovsk, Poltava and Zaporizhia regions Table 2.

According to Table 3, it is also appropriate to identify metallurgy as the most attractive industry. Next, we will assess the largest innovative enterprises in the metallurgical industry of Ukraine for 2020 Table 4. Table 4 shows that Interpipe Corporation has the highest value in terms of indicators in metallurgy, so relative to others, it has the highest rating and is a benchmark.

A similar calculation can be performed for other industries to obtain reference companies in the industry.

Among the priority sectors of Ukraine and regional delimitations, it is expedient to single out the resort and recreational sphere and tourism, agro-industrial complex, processing industry, etc., in more detail Table 5.

This division of industries by region allows potential investors to consider other industries as priorities for investment according to personal preferences.

Using this rating methodology concerning innovation-active and investment-attractive enterprises of specific regional complexes allows obtaining relatively objective and fair assessments, considering the leading performance indicators of enterprises, ensuring their differentiation by industry. The methodology uses official reporting materials and documents and is quite simple to perform calculations.

When applying the rating assessment methodology, it was established that it is necessary to apply the tax burden indicator for a comprehensive description of innovation and investment activities of a particular region complex. This is because an equal value of the rating of enterprises in one region may bear different tax burdens, skillfully using tax imperfections or benefits. There is no definition of a single tax burden and official statistical accounting of this indicator at the state and regional levels, nor is there a generally accepted methodology for determining

it. Only scattered measures indirectly give a partial idea of the total tax burden. Recently, other countries and Ukraine have begun to use a methodology for determining the share of taxes in GDP, which is quite simple, straightforward and can be widely used in economic calculations, but does not allow to study of the tax burden at the micro level when comparing enterprises different industries and sectors of the economy.

Table 4. Final rating assessment of the largest innovative enterprises of the metallurgical industry of Ukraine for 2020p. *Source: own development.*

Company	**Profitability of sales, %**	**Return on assets, %**	**Labour efficiency, %**	**Rating, points** $R_j = \sqrt{(1-x_{1j})^2+(1-x_{2j})^2+\ldots+(1-x_{nj})^2}$	**Place**
Metinvest	3.17	65.41	4.21	$\sqrt{(1-3.17)^2+(1-65.41)^2+(1-4.21)^2} = 64.52$	3
Ferrexpo	26.74	67.21	4.32	$\sqrt{(1-26.74)^2+(1-67.21)^2+(1-4.32)^2} = 71.11$	2
Interpipe	73.79	97.33	11.98	$\sqrt{(1-73.79)^2+(1-97.33)^2+(1-11.98)^2} = 121.23$	1
«Yuzhniy Gok» Mining and Processing Plant	35.32	39.10	3.88	$\sqrt{(1-35.32)^2+(1-39.10)^2+(1-3.88)^2} = 51.35$	4
Mykolayiv. alumina plant	3.07	4.81	5.73	$\sqrt{(1-3.07)^2+(1-4.81)^2+(1-5.73)^2} = 6.41$	6
Dniprospetsstal	0.94	3.65	1.73	$\sqrt{(1-0.94)^2+(1-3.65)^2+(1-1.73)^2} = 2.74$	7
Krivoj Rog's Iron-Ore Combine	24.24	12.25	0.89	$\sqrt{(1-24.24)^2+(1-12.25)^2+(1-0.89)^2} = 25.81$	5
Reference company – Interpipe (Dnipropetrovsk region)	73.79	97.33	11.98	121.23	

Table 5. Distribution of industries by region. *Source: own development.*

Industry	**Regions**
Heat energy	Donetsk, Kharkiv, Kyiv, Ivano-Frankivsk, Lviv, Zaporizhzhya, Odesa regions
Electricity	Donetsk, Luhansk regions
Coal industry	Zaporizhzhya, Donetsk, Ivano-Frankivsk regions
Chemical Industry	Donetsk, Ivano-Frankivsk, Zakarpattya regions.
Mining and chemical industry	Zaporizhzhya, Poltava, Kherson, Chernihiv, Dnipropetrovsk regions
Automotive industry	Kyiv, Lviv, Sumy, Kharkiv, Odesa regions
Sewing industry	Kharkiv, Ivano-Frankivsk, Lviv, Odesa regions
Fur industry	Kyiv, Kharkiv, Poltava, Odesa, Luhansk, Lviv regions
Meat industry	Donetsk, Kharkiv, Chernihiv, Lviv, Chernivtsi, Rivne regions.
Cement industry	Odesa, Zaporizhzhya, Zakarpattya regions.
Resort and recreational sphere and tourism	Maritime: Odesa, Nikolaev, Donetsk regions. River: Kyiv, Dnipropetrovsk, Zaporizhzhya regions. City public: Kyiv, Dnipropetrovsk, Kharkiv regions
Transport infrastructure	Chernivtsi, Kharkiv, Ivano-Frankivsk, Dnipropetrovsk, Donetsk, Lviv, Cherkasy, Volyn, Zaporizhzhya, Mykolayv, Kherson, Odesa, Chernihiv, Sumy, Poltava, Kirovograd regions
Machine-building complex (manufacture of computers, electronic and optical products, machinery and equipment, electrical equipment, vehicles)	Dnipropetrovsk, Zaporizhzhya, Kirovograd, Luhansk, Mykolayv, Odesa, Poltava, Sumy, Kharkiv, Kherson, Cherkasy regions

Results and discussion

Thus, the assessment of the regional rating showed that the allegations of "local growth points" are currently not valid at the regional level. Differentiation of rates of local, central, and regulatory taxes in part directed to regional budgets can be considered as reserves of the development of innovation and investment processes in regions.

In developing this methodology, we tried to consider the shortcomings, in our opinion, of the existing methods [9,15–22].

All methods for determining the assessment of the investment attractiveness of the region, shown in Figure 1, contribute to obtaining the corresponding economic result. However, they are cumbersome, since they require, for calculation, the collection of a large amount of information from various aspects of activity and grouping according to the impact on the investment attractiveness of the regions.

Firstly, it has already been noted that the presented methods, except for [9], do not consider the innovative component.

Secondly, while applying the rating assessment methodology, it was established that the tax burden indicator should be used for a comprehensive description of the innovation and investment activity of a particular complex of the region. This is because an equal value of the rating of enterprises in one region may bear different tax burdens, skillfully using tax imperfections or benefits. There is no definition of a single tax burden and official statistical accounting of this indicator at both the state and regional levels, nor is there a generally accepted methodology for determining it. Only scattered measures indirectly give a partial idea of the total tax burden.

Recently, other countries and Ukraine have begun to use a methodology for determining the share of taxes in GDP [26], which is quite simple, straightforward and can be widely used in economic calculations, but does not allow for to study of the tax burden at the micro level when comparing enterprises different industries and sectors of the economy.

Third, improved indicators, thereby eliminating heterogeneity [13,15], cumbersome set of indicators [9] or vice versa, excessive simplicity [18], paying attention only to internal indicators [16,17] or disregard for sectoral and sectoral priorities [22], separating only the investment attractiveness [19] and the dependence of models on the direction of trends in indicators [20,21].

Groups of methods not included in the demonstrative figure are based on conducting surveys and questionnaires among economic entities in a particular region. Such surveys can be grouped according to the sectoral affiliation of the surveyed subjects; however, they require significant labor and financial resources.

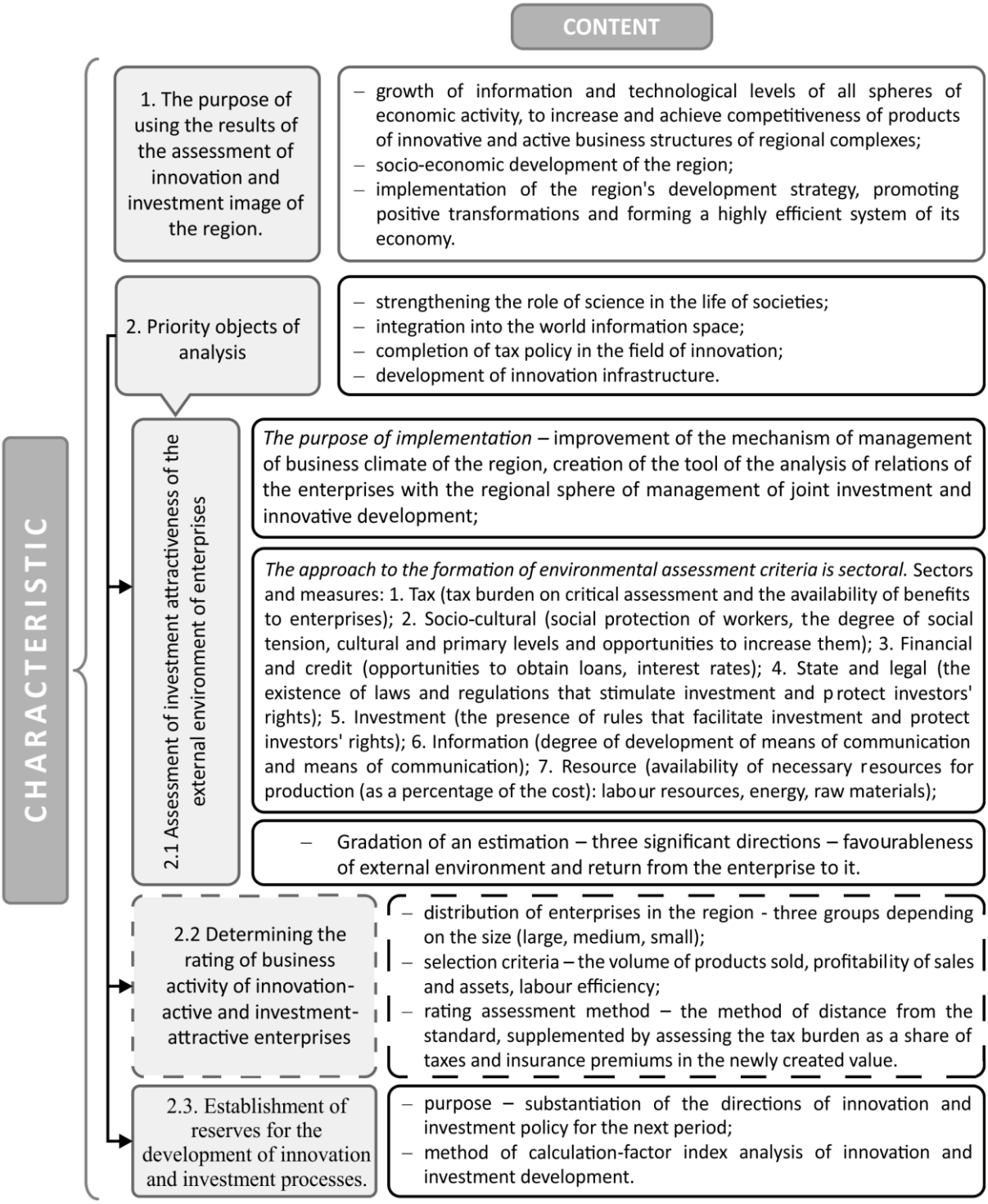
The method proposed by our authors bypasses the listed shortcomings of the methods listed above, since it is based on the officially recognized Forbes Rating, including all significant indicators of enterprises and does not require cumbersome calculations and resource costs.

Accordingly, the use of the results of the assessment of the innovation and investment image of the region in the context of sustainable transformations should be aimed at solving specific problems. The main essential characteristics of the proposed method of assessing the innovation and investment image of the region are shown in Figure 3.

Thus, the main advantage of the proposed method is:

- introduction of the division of enterprises in the region into three groups depending on the size (large, medium, small), which facilitates perception and is understandable to all stakeholder groups
- definition of selection criteria: the volume of products sold, profitability of sales and assets, labour efficiency
- application of the rating assessment method - the method of distance from the standard, which, unlike others, is supplemented by the assessment of the tax burden as a share of taxes and insurance premiums in the newly created value.

The use of this method of assessing the innovation and investment image of the regions allows domestic and foreign investors to evaluate and choose the region for which the investment proposal will consider the features and priorities of economic development and trends in the industry.



**Dashed line highlights the block that concerns our development*

Figure 3. Proposed method of assessing the innovation and investment image of the region.
Source: own development.

Impact

Economic Impact

It is determined that the allegations of "local growth points" currently have no good basis at the regional level. Differentiation of rates of local, central, and regulatory taxes in part directed to regional budgets can be considered as reserves for the development of innovation and investment processes in regions. The main essential characteristics of the assessment of innovation and investment image of the region are offered.

Most often, five indicators are distinguished for rating:

1. coefficient of manoeuvrability (normative value 0.1)
2. the coefficient of total solvency (regulatory value 2.0)
3. direct turnover ratio of invested capital (regulatory value > 2.5)
4. the coefficient of profitability of sales (normative value is determined by the discount rate of NVU)
5. the coefficient of return on invested capital (coefficient of economic profitability is not limited by standards).

The study selected the profitability indicators of sales, return on assets, and labour efficiency for the rating assessment.

The return on assets is calculated as the ratio of profit from ordinary activities to taxation and the average annual value of assets. Calculating the return on assets (used assets of the enterprise) is the opposite indicator of return on equity (used liabilities of the enterprise). In contrast to those proposed by the authors is considered appropriate because the return on assets is formed under the influence of all internal and external factors; reserves to increase the rate can be found in all business areas.

The labour efficiency indicator is calculated as the ratio of the volume of tangible and intangible goods produced and the amount of labour expended. This indicator was chosen because it means an increase in the number of goods produced without increasing labour costs, i.e., implies the degree of self-improvement of economic workers and is responsible for increasing actual product and income; therefore, it is a significant indicator of economic growth in general.

Regional Impact

The article has a significant regional impact: the level of investment and innovation attractiveness of regions is determined, and those that are not attractive to foreign investors are identified. The main directions of the formation of innovation and investment image of the regions of Ukraine are determined, which consider the existing environment, its transformational changes, and existing trends in the region. A method for determining the rating of business activity of innovation-active and investment-attractive enterprises, which considers their territorial and sectoral affiliation, has been developed.

Conclusion

The high level of innovation and investment attractiveness indicates the positive development of both the region and the country. Investments can be attracted only if investors are confident in the stability and economic growth. Today, such investment conditions have not been created, so their involvement remains low and, in some regions, even absent.

The development of production and infrastructure of the region is the primary goal of the policy in the strategic direction of creating and applying innovations, providing socio-economic and legislative guarantees for the constant reproduction and effective use of the scientific and economic potential of the region. This is considered by the proposed assessment. The use of the proposed method for assessing the region's investment and investment image allows investors to evaluate and select a region in which the corresponding investment project considers the region's specifics and critical areas of development and the development trends of the relevant industries.

From this point of view, the assessment of the innovation and investment image of the region in the conditions of sustainable transformation becomes a logical component of the sustainable policy of innovation and investment development of the region, and the proposed method of its implementation is quite convenient and transparent.

Conflict of interest

There is no conflict of interest to declare.

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