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Development and Sensory Evaluation of Instant Coffee Fortified with Bone-Derived Collagen

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Abstract—**This study revealed the development of instant coffee-collagen fusion powder using collagen extracted from Ethiopian cattle bones and Ethiopian Arabica coffee. Different coffee – to- collagen formulation (90:10 – 60:40 ratios) were prepared and evaluated for physic chemical and sensory attributes of developed product. The result revealed high solubility (97-99%) and slightly acidic pH values (5.22-5.65).increasing collagen level improved protein, SNF and mineral content of the developed product. sensory attributes also in acceptable level, while increasing collagen levels negatively affected flavor and consistency. Among the formulations, the 85:15 coffee-collagen ratio showed the highest overall acceptability (6.86 ± 1.43).overall the study revealed that cattle bone collagen can successfully be incorporated into instant coffee to enhance nutritional value while supporting sustainable utilization of slaughter house by-products**.

Index Terms—**Instant coffee-collagen, cattle bone, sensory attributes, Ethiopian Coffee Arabica**

I. INTRODUCTION

Coffee is the second most important commodity in the global market next to oil and serves as a major source of foreign currency earnings for more than 80 tropical countries, including Ethiopia (Worku, 2023). According to reported data, approximately 10.78 million tons of coffee beans were produced worldwide from about 4.3 million hectares of land in 2022 (FAOSTAT, 2024). The two dominant coffee species cultivated globally are *Coffea Arabica* and *Coffea canephora* (Robusta) (Langi et al., 2023). Ethiopia is widely recognized as the origin and center of genetic diversity of Arabica coffee (*Coffea arabica L.*), and it ranks among the leading producers globally (Worku, 2023). It is currently the fifth-largest coffee producer and the third-largest producer of *Arabica* coffee after Brazil and Colombia. In 2022, Ethiopia produced about 496,200 tons of coffee from approximately 741,850 hectares of land (FAOSTAT, 2024).

Coffee plays a critical role in the Ethiopian economy, contributing 25–30% of total foreign currency earnings, around 10% of government revenue and employment for about 25% of the population. Beyond its economic value, coffee also holds deep sociocultural importance in Ethiopia, particularly through traditional coffee ceremonies associated with social events such as marriage arrangements and conflict resolution (Worku, 2023). Traditionally, coffee is consumed as a brewed beverage prepared by extracting roasted and ground coffee using hot water. However, in recent decades, instant coffee, produced through spray drying or freeze drying of coffee extract, has gained widespread popularity due to its convenience (Vareltzis et al., 2020; (Souza et al., 2023)).

On the other hand, Ethiopia possesses one of the largest livestock populations in Africa, with millions of cattle, sheep, goats, and poultry (Birhanu, 2019). These livestock resources contribute significantly to food supply and income generation. Meat is a highly valued product due to its rich protein content, essential amino acids, and micronutrients. However, meat processing and slaughtering operations generate a substantial amount of by-products, which account for approximately 60% of the live animal weight (Sharma et al., 2021). Although often treated as waste, slaughterhouse by-products are rich sources of proteins, bioactive compounds, and essential nutrients (Glimaker, 2023). Improper handling leads to environmental pollution, but effective utilization can create economic value and support environmental sustainability (Sharma et al., 2021).

One of the most important components of these by-products is collagen, which constitutes about 25–30% of total protein in animals (Halson et al., 2023). Collagen is a structural protein that provides strength and support to connective tissues such as skin, bones, and cartilage. Through partial hydrolysis, collagen can be converted into gelatin or hydrolyzed collagen, which are widely used in food and beverage industries, pharmaceuticals, cosmetics, and biomedical applications (Dille et al., 2021). Collagen has several functional and health benefits, including improving skin elasticity and reducing aging effects, supporting joint and bone health, providing antioxidant and antihypertensive properties and Enhancing nutritional quality of foods products (Hashim et al., 2015; Lee et al., 2019; Musayeva et al., 2022). Recent studies have demonstrated the incorporation of collagen into various food products such as sausages, beverages, and functional drinks (Hashim et al., 2015; Musayeva et al., 2022).

Coffee contains caffeine, a natural stimulant responsible for its characteristic bitterness and physiological effects. While caffeine has recognized medicinal and stimulating benefits, excessive intake may lead to adverse health effects such as sleep disturbances, irritation, cardiovascular complications, nausea, and other physiological disorders (Rezk et al., 2018). The recommended caffeine concentration for therapeutic purposes ranges from 5–20 mg/L in solution (Rezk et al., 2018). Furthermore, the safe upper limit of daily caffeine intake recommended is 400 mg per day for a healthy adult, 200 mg per day for pregnant and breastfeeding women (Olechno et al., 2021) and 100 mg per day for children (Rezk et al., 2018). In Ethiopia, where coffee consumption is deeply rooted in daily life and cultural practices, there is a high likelihood of cumulative caffeine intake exceeding recommended levels, particularly among frequent consumers. This situation highlights the need for innovative approaches to moderate caffeine concentration while maintaining the sensory quality of coffee.

At the same time, cattle bones and other slaughterhouse by-products are largely underutilized in Ethiopia, despite being rich in collagen (13.68–26.14 g/100 g) (Zhang et al., 2021). These materials are often discarded, leading to environmental pollution, loss of valuable nutrients and missed economic opportunities (Jayathilakan et al., 2012). Although collagen has been incorporated into some beverages globally, challenges remain due to its thermal sensitivity, as it begins to degrade at high temperatures (Bozec and Odlyha, 2011). Since coffee is typically brewed at 90–96°C, it is feasible to incorporate collagen without significant degradation (Musayeva et al., 2022). Therefore this study is initiated for the utilization of collagen in the processing of instant coffee to improve sensory characteristics.

II. MATERIALS AND METHODS

2.1. Experimental Area

The experiment was conducted at Federal Technical and Vocational Training Institute, Addis Ababa and Ethiopian standard and conformity institute from March to September, 2024.

2.2. Raw Material Collection

About 5 kg of labeled Coffee Arabica was purchased from Addis Ababa super market. This coffee variety was selected because it is the most variety produced in Ethiopia and has a principal taste and flavor that consumers prefer more than Coffee Robusta, and has high commercial value (Langi et al., 2023; Worku, 2023). Hence, collagen supplementation probably helps to improve the quality more. The received coffee was inspected and cleaned for further process.

The other raw material for the experiment was collagen which was extracted from cattle bones. Non contaminated Cattle bones (10 pc) was collected from certified slaughter house in Addis Ababa and received with care to laboratory. In the laboratory it was checked for any physical defects and stored at refrigerator temperature of 4 ^{0}C until collagen extraction process. Furthermore other ingredients was purchased and used in processing of instant coffee-collagen fusion product.

2.3 Sample Preparation

2.3.1.Preparation of coffee powder

Coffee powder was prepared according to the method mentioned in Langi et al., (2023). Briefly, cleaning and washing the collected coffee sample, roasting at 235 ^{0}C for 14 minute in an oven with frequent stirring. The roasted coffee bean had grind into a fine powder with coffee grinder machine. Coffee powder was packed in polyethylene bag and used for the preparation of instant coffee-collagen fusion product.

2.3.2. Preparation of collagen extract

Collagen extraction was carried out following the method indicated in Fatimah et al., (2023). Briefly, the collected cattle bone was cleaned and washed, removal of fat depose, cooking in autoclave at 125 ^{0}C and 0.15 MPa to soften the bone structural components and loosen collagen for 45 minutes. Following to cooking it was poured into bigger pot for collagen extraction by heating over the stove to boil for four hours and cool down to remove bones from liquid extract. Then the extract was cooled and allowed to gelling in refrigerator to separate fat part which is solidified on the top. All of the fat residues was removed and the collagen part was stored in refrigerator at 5 °C until further application or it was liquefied with heat application for direct supplementation in instant coffee processing. Figure 1 shows flow diagram of collagen processing from cattle bone.

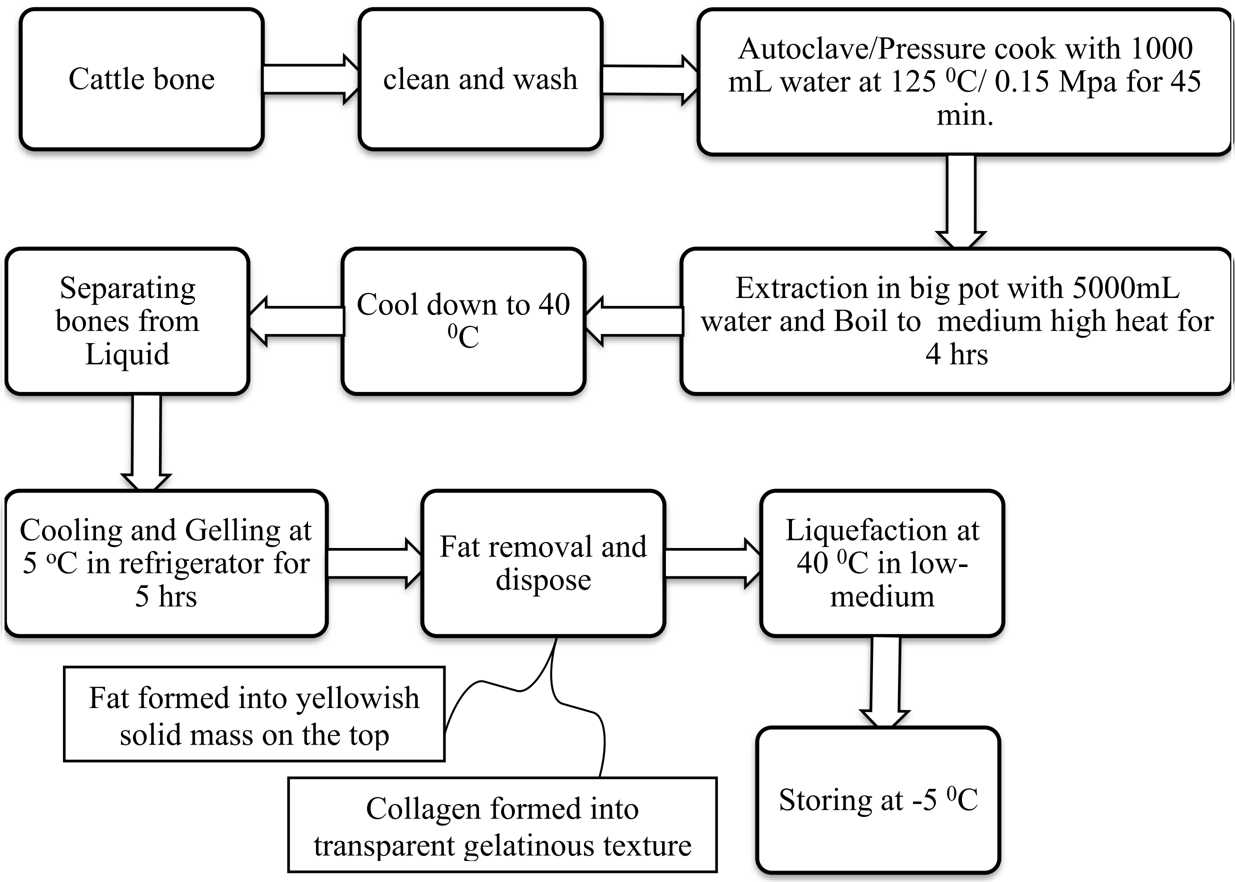


Figure 1: Collagen extraction process from cattle bones

2.2.3.Preparation of instant coffee-collagen fusion

Instant coffee-collagen fusion product was prepared from coffee extract and the extracted collagen according to the formulation ratio shown on table (2) under design of experiment. Preparation follows the method indicated in Langi et al., (2023) for the processing of instant coffee. Briefly, the coffee powder and other ingredients was boiled and extracted with hot water in the ratio of 1:8 w/v at 93 ± 3°C for 90 min and wait to cool. The extract was filtered with filter cloth and liquefied collagen was added to filter as the specified ration and again boil the mix at 50 °C for 30 min. The mix was filtered and spray dried at temperature of 180 °C to prepare instant coffee-collagen fusion powder product. The prepared product was stored at 4 °C for studying sensory attributes. Figure 2 shows instant coffee-collagen fusion powder processing flow diagram.

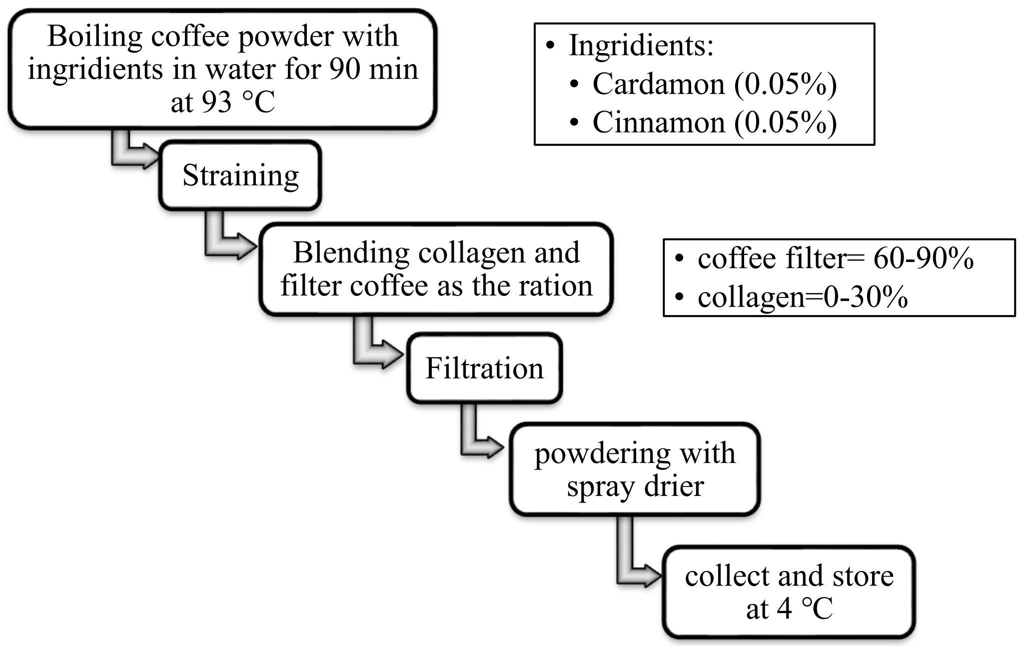


Figure 2: Instant coffee-collagen fusion product processing flow diagram

2.3.4 Experimental Design

The experiment was conducted using a Completely Randomized Design (CRD) consisting of seven treatment formulations and three independent replications for each treatment. The formulations were prepared by varying the ratio of Ethiopian instant coffee extract and liquefied bovine collagen to determine the optimum formulation based on physicochemical and sensory characteristics. Each treatment was prepared independently under identical processing conditions and randomly assigned to minimize experimental error. Three batches were produced for each formulation, resulting in a total of 21 experimental units (7 treatments × 3 replications). The treatment combinations used in the study are presented in Table 1.

Table 1. Completely Randomized Design (CRD) for Instant Ethiopian Coffee–Collagen Fusion

Treatment	Coffee Extract (%)	Liquefied Collagen (%)	Replication 1	Replication 2	Replication 3
T1	90	10	S1	S2	S3
T2	85	15	S1	S2	S3
T3	75	25	S1	S2	S3
T4	71	29	S1	S2	S3
T5	67	33	S1	S2	S3
T6	64	36	S1	S2	S3
T7	60	40	S1	S2	S3

Each experimental unit was evaluated for physicochemical properties, including pH, solubility, ash content, protein, fat, lactose, solids-not-fat (SNF), density, and salt content. Sensory evaluation was conducted using a 9-point hedonic scale to assess colour, aroma, flavour, consistency, and overall acceptability. The collected data were statistically analyzed using one-way analysis of variance (ANOVA) under the Completely Randomized Design, and treatment means were separated using

Duncan's Multiple Range Test (DMRT) at a significance level of $p < 0.05$.

2.3.5. Physico chemical analysis

Physic chemical analysis was carried out by ultrasonic Lacto scan, ash content was analysed by using muffle furnace, pH were analysed by heavy duty pH meter, Solubility by hand refract meter.

2.3.6. Sensory property of instant coffee-collagen fusion

Instant coffee-collagen fusion product was evaluated for sensory attributes (color, taste, aroma and overall acceptability) with 9 hedonic scale by participating 20 untrained panelists. Instant coffee-collagen fusion product brew was prepared according to the method described in (Langi et al., 2023) and served for panelists at room temperature. The sample was brewed by dissolving the prepared product in hot water with the ration of instant coffee-collagen fusion powder to water (1:120) w/v for each experimental runs.

2.3.7. Data Analysis

The gathered data was subjected to optimization process with SPSS software. The optimisation process was carried out to identify the best formulation ratio of coffee filter to collagen extract in processing of instant coffee-collagen fusion product.

III. RESULTS AND DISCUSSION

Table 2. Physicochemical properties of Instant Coffee Collagen Fusion formulations (Mean ± SD)

Formulation	Solubility (°Brix)	Ash (%)	pH	Temperature (°C)	Fat (%)	Density	Lactose (%)	SNF (%)	Protein (%)	Salt (%)
71:29:00	98	32	5.47 ± 0.01	34.43 ± 4.74	0.11 ± 0.03	3.46 ± 0.35	0.45 ± 0.07	0.94 ± 0.09	0.34 ± 0.03	0.07 ± 0.01
60:40:00	97	40	5.65 ± 0.01	31.90 ± 0.17	0.12 ± 0.03	5.08 ± 0.44	0.75 ± 0.07	1.37 ± 0.12	0.50 ± 0.05	0.11 ± 0.01
75:25:00	97	33	5.46 ± 0.01	32.00 ± 0.44	0.14 ± 0.01	3.05 ± 0.18	0.46 ± 0.02	0.83 ± 0.05	0.30 ± 0.02	0.07 ± 0.01
85:15:00	98	30	5.22 ± 0.01	31.83 ± 0.06	0.14 ± 0.01	3.00 ± 0.02	0.45 ± 0.00	0.83 ± 0.01	0.30 ± 0.00	0.06 ± 0.00
90:10:00	99	44	5.35 ± 0.01	32.00 ± 0.00	0.05 ± 0.03	3.59 ± 0.36	0.53 ± 0.05	0.96 ± 0.09	0.35 ± 0.03	0.07 ± 0.01
64:36:00	98	28	5.45 ± 0.01	32.47 ± 0.06	0.12 ± 0.02	3.62 ± 0.09	0.54 ± 0.02	0.98 ± 0.03	0.36 ± 0.01	0.16 ± 0.14
67:33:00	98	32	5.48 ± 0.02	32.40 ± 0.10	0.12 ± 0.01	3.23 ± 0.02	0.48 ± 0.01	0.88 ± 0.01	0.32 ± 0.00	0.07 ± 0.00

Values are expressed as mean ± standard deviation (n = 3).

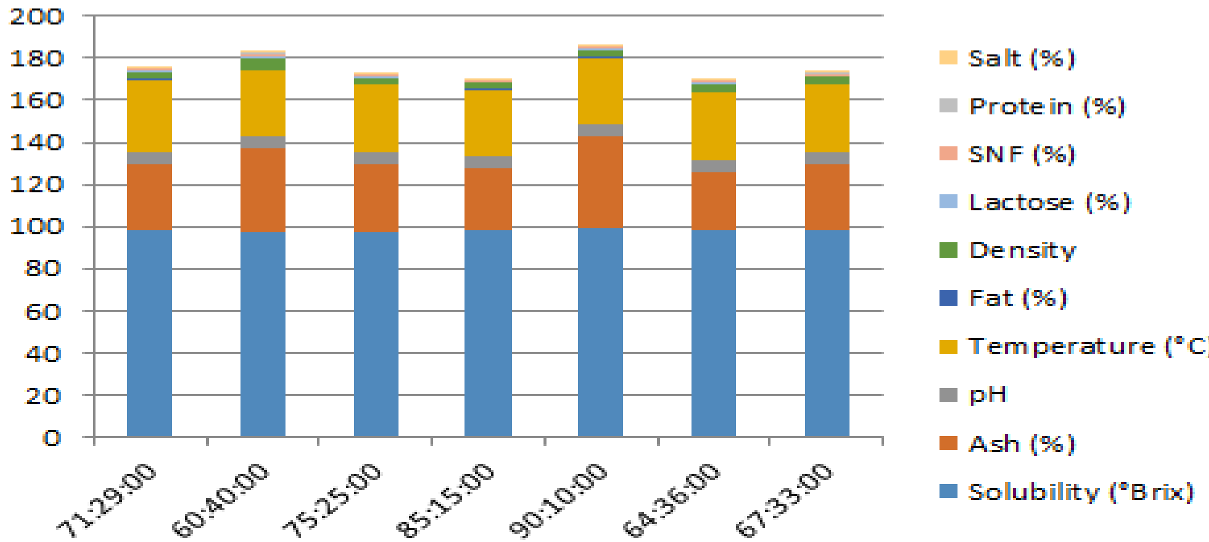


Figure-3 showing the physic chemical properties of coffee addition with different levels of collagen

3.1 Physicochemical Properties of Instant Coffee–Collagen Fusion

The physicochemical characteristics of instant coffee–collagen fusion formulations prepared at different coffee-to-collagen ratios are presented in Table 2. The results showed noticeable variations among treatments, indicating that the incorporation level of collagen influenced several compositional attributes of the final product.

The pH values ranged from 5.22 to 5.65, indicating that all formulations were slightly acidic in nature, which is typical for coffee-based beverages. The highest pH was observed in the 60:40 formulation (5.65), while the lowest was recorded in the 85:15 formulation (5.22). The observed variation suggests that increasing coffee concentration tends to slightly reduce acidity buffering, whereas higher collagen levels may contribute to marginal pH stabilization due to protein buffering effects.

Total soluble solids (TSS) ranged from 97 to 99 °Brix, indicating high solubility and good reconstitution properties of all formulations. The relatively small variation among treatments demonstrates that collagen incorporation did not negatively affect the instant dissolution characteristics of the product. High solubility is an important quality attribute for instant beverage powders, ensuring uniform dispersion in hot water.

Ash content varied from **28 to 44%**, reflecting differences in total mineral content among formulations. The highest ash content was observed in the 90:10 formulation, while the lowest was recorded in the 64:36 formulation. This variation may be attributed to differences in mineral composition between coffee extract and collagen source materials.

Protein content increased with higher collagen incorporation, ranging from 0.30% to 0.50%, confirming the successful enrichment of coffee with collagen. The 60:40 formulation exhibited the highest protein content (0.50%), indicating that collagen addition effectively enhances the nutritional value of the beverage. Similarly, solids-not-fat (SNF), lactose, density, and salt content also increased with higher collagen levels, particularly in formulations containing ≥30% collagen. This trend demonstrates that collagen incorporation improves the overall nutritional composition of the product.

Fat content remained low across all treatments (0.05–0.14%), indicating that collagen addition had minimal influence on lipid content. Temperature values recorded during analysis showed minor variation, confirming consistent experimental conditions.

Overall, the physicochemical results demonstrate that collagen incorporation improves nutritional composition without significantly compromising solubility and stability of the instant coffee formulation.

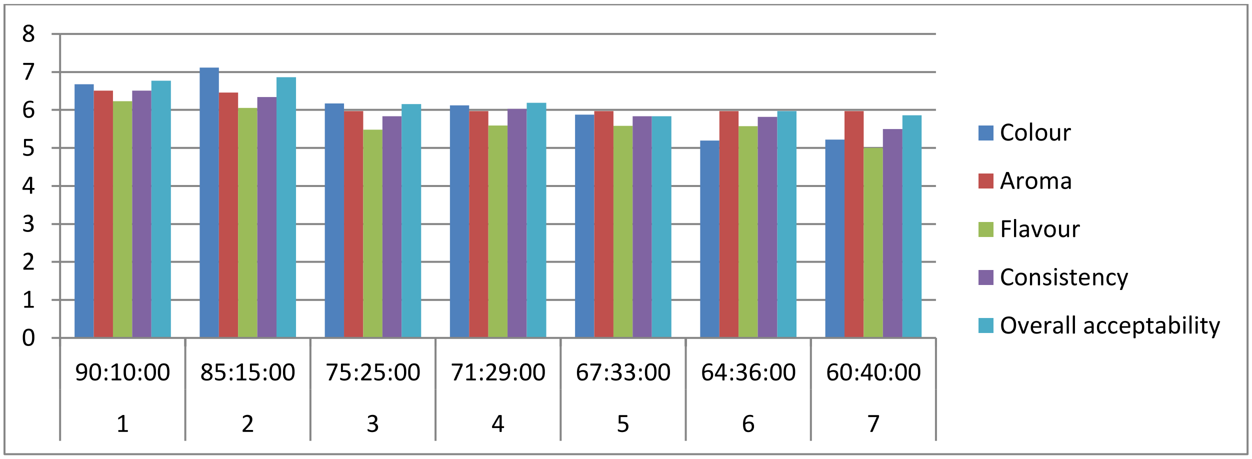
3.2 Sensory Properties of Instant Coffee–Collagen Fusion

The sensory evaluation results are presented in Table 3. The addition of collagen significantly influenced most sensory attributes, including colour, flavour, consistency, and overall acceptability ($p < 0.05$), while aroma was not significantly affected.

Table 3. Sensory evaluation of instant coffee-collagen fusion formulations (Mean ± SD, n = 20)

Run	Coffee : Collagen (%, w/w)	Colour	Aroma	Flavour	Consistency	Overall acceptability
1	90:10	6.68 ± 1.71^{ab}	6.51 ± 2.11^{a}	6.23 ± 1.69^{a}	6.51 ± 1.54^{a}	6.77 ± 1.71^{b}
2	85:15	7.11 ± 1.64^{a}	6.46 ± 1.85^{a}	6.05 ± 1.99^{a}	6.34 ± 1.66^{ab}	6.86 ± 1.43^{b}
3	75:25	6.17 ± 1.77^{bc}	5.97 ± 1.90^{a}	5.48 ± 2.00^{a}	5.83 ± 1.80^{ab}	6.15 ± 1.89^{ab}
4	71:29	6.12 ± 1.85^{bc}	6.20 ± 1.85^{a}	5.59 ± 1.87^{a}	6.03 ± 1.86^{ab}	6.19 ± 1.87^{ab}
5	67:33	5.88 ± 1.59^{c}	6.00 ± 1.93^{a}	5.58 ± 1.79^{a}	5.83 ± 1.79^{ab}	5.83 ± 1.68^{a}
6	64:36	5.19 ± 2.02^{c}	6.18 ± 1.51^{a}	5.57 ± 2.04^{a}	5.82 ± 1.57^{ab}	5.97 ± 1.64^{a}
7	60:40	5.22 ± 1.79^{c}	5.64 ± 2.00^{a}	5.00 ± 2.09^{a}	5.50 ± 1.92^{b}	5.86 ± 1.67^{a}

Values are mean ± standard deviation (n = 20). Mean values within the same column not sharing the same superscript letter differ significantly ($p < 0.05$).



Colour

Colour scores ranged from 5.19 to 7.11, with the highest value observed in the 85:15 formulation. Moderate collagen incorporation maintained acceptable colour characteristics similar to conventional instant coffee. However, higher collagen levels resulted in reduced colour intensity and lower consumer preference. This may be due to dilution of coffee pigments and changes in visual perception caused by protein addition. Similar findings have been reported in protein-enriched beverage systems, where increased protein content affects colour perception and acceptability (Gueguen & Jacob, 2014).

Aroma

Aroma scores ranged from 5.64 to 6.51, with no significant differences among treatments. This indicates that collagen incorporation does not substantially influence coffee aroma. The neutral odour profile of collagen likely explains its minimal impact on volatile aroma compounds responsible for coffee fragrance. Similar observations have been reported in protein-fortified beverages where aroma perception remains largely unchanged despite protein addition (Zhang et al., 2021; Amyyoony et al., 2023).

Flavour

Flavour scores decreased gradually with increasing collagen concentration, ranging from 5.00 to 6.23. The highest flavour score was observed in the 90:10 formulation, while the lowest was recorded in the 60:40 formulation. The decline in flavour acceptability may be attributed to protein-associated aftertaste and dilution of characteristic coffee flavour compounds at higher collagen levels. Similar trends have been reported in protein-enriched dairy and beverage systems (Rashidinejad et al., 2022).

Consistency

Consistency scores ranged from 5.50 to 6.51, indicating moderate variation among treatments. The 90:10 formulation exhibited the highest consistency score, suggesting better mouthfeel and consumer preference. In contrast, higher collagen concentrations slightly reduced perceived texture quality. Protein enrichment can alter viscosity and mouthfeel, which may influence consumer perception of beverage smoothness (Wijesekara & Ekaette, 2025).

Overall Acceptability

Overall acceptability scores ranged from 5.86 to 6.86, with the highest value recorded in the 85:15 formulation. This formulation was statistically comparable to the control, indicating that moderate collagen addition does not adversely affect consumer acceptance. However, formulations containing higher collagen levels (≥30%) showed reduced acceptability, primarily due to declines in flavour and colour perception.

IV. CONCLUSION

This study showed the incorporation of collagen extracted from cattle bones into instant coffee and its effect on sensory properties. The sensory evaluation results revealed that incorporation (5-10%) maintained desirable sensory attributes comparable to conventional instant coffee. the formulation with 85:15 coffee – to- collagen ratio showed the highest overall acceptability, indicating that optimal blending can enhance product quality while improving nutritional value.

Aroma was found not much significant effect on addition of collagen. In contrast, flavor and consistency showed decline level with addition of collagen. This approach also contributes to environmental sustainability by reducing waste and supports the development of functional beverages in Ethiopia.

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Photos showing the research work

Figure 1: The prepared cattle bones and instant collagen powder

Figure 2: Roasted and prepared of instant coffee

Figure 3: Collagen coffee fusion instant coffee

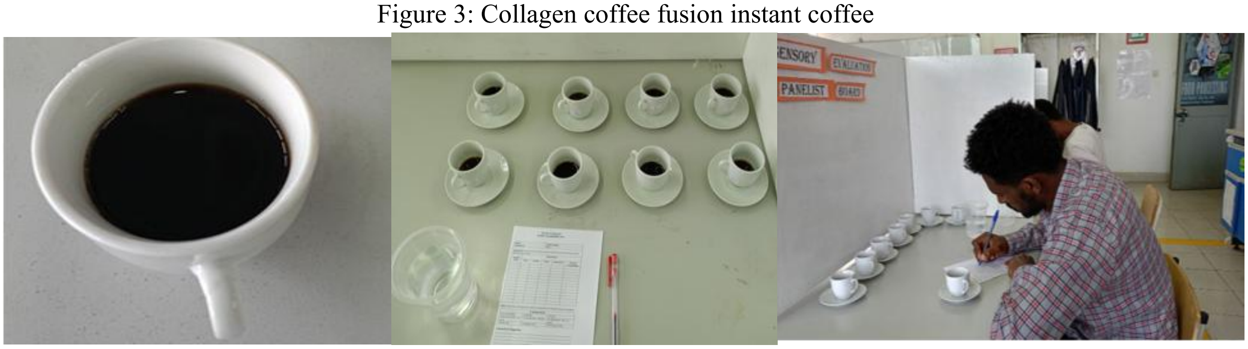

Figure 4: Sensory evaluation