DETERMINATION OF RHEOLOGICAL PROPERTIES OF ALTERNATIVE FLOUR SUBSTITUTED DOUGHS

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Abstract: All over the world, healthy foods, functional foods, diet foods and many similar terms are on the agenda. Consumers are offered different types of foods for a healthy diet. To this end, studies to improve the functional properties of bread have gained momentum. One of the ways to improve the functional properties of bread is to use flours with more functional properties than wheat flour. However, the effects of the added flours on the rheology of the dough are also different. The aim of this study is to determine the effects of flours (buckwheat (10-30%), carob (3, 6, 9, and 12%), chickpea (10-50%), oat (10-50%), and barley (10-50%)) in different proportions added to bread flour on the rheological properties of the dough. The Mixolab® (Chopin) instrument was used to determine the rheological properties. A standard protocol for flour analysis was used for the analysis of bread flour and other flour mixtures. Various rheological and other dough properties were determined, such as water holding capacity, development time, stability, amylase activity, and degree of flour retrogradation. Using the obtained Mixolab® curve, C1 values for water retention and stability, C2 values for protein quality, C3 values for starch gelatinization, C4 values for amylase activity, and C5 values for degree of starch degradation were measured. C1 changed between 1.05 and 1.16 Nm, C2 between 0.33 and 0.58 Nm, C3 between 1.22 and 2.13 Nm, C4 between 0.96 and 1.98 Nm, and C5 between 0.95 and 2.81 Nm depending on the flour ratio and type used. As a result of the tests, it was determined that the most suitable flour for bread flour profile is 30% barley flour, 20% oat flour, 9% carob flour and 20% buckwheat flour, separately for each added flour.

Keywords: Buckwheat, Carob, Chickpea flour, Functional food, Rheology

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1. Introduction

Wheat is one of the cereals used for bread making. However, breads made from wheat flour are considered nutritionally inadequate (Sabonis and Tzia, 2009). Partial replacement of wheat flour with non-wheat flour increases the nutritional quality and flavour of baked goods. In recent years, consumers focus on a healthy diet and their demands for a variety of foods have necessitated the fortification of wheat flour with various components. To fortify wheat flour with important nutrients such as protein, fiber, calcium, iron, vitamin E, and polyphenols, fortification studies are being conducted with pseudocereals such as amaranth, quinoa, and buckwheat, (Rodriguez-Sandoval et al., 2012) and with legumes such as chickpeas, lentils, and soybeans (Sabonis and Tzia, 2009). In addition, in underdeveloped or developing countries where wheat cultivation is low, wheat flour is replaced by flour from other cereals such as maize and rice for economic reasons (Elkhalifa and El-Tinay, 2002).

For decades, oats are underutilized and usually grown for feed. However, oats are a good source of starch (55-59%), protein (15-26%), lipids (3-11%), and bioactive compounds such as β-glucan (3-8%) (Duque et al., 2020). Oats (Avena nuda L.) are considered a high-quality health food due to their cholesterol-lowering and antidiabetic properties (Ho et al., 2016; Martínez-Villaluenga and Peñas, 2017), and thus are widely used to make healthy snacks and even meals (Shukri et al., 2021). Barley flour is a good source of soluble fiber, arabinoxylans, and phenolic compounds (Moza and Gujral, 2017) and is nutritionally superior to wheat flour in terms of its bioactive composition (Moza and Gujral, 2018). Chickpea is the third most important legume in the world in terms of total production. Chickpea is a valuable source of protein, carbohydrates, fiber, and many essential vitamins and minerals (El-Sohaimy et al., 2020). Carob flour, obtained from the fruits of Ceratonia siliqua L. after removal of the seeds and subsequent roasting, has gained interest due to its remarkable composition, which has a preventive effect against various diseases. It is characterized by a high content of sugars, dietary fiber (~11%), minerals, and low protein (3-4%) and fat (0.2-0.8%) content, and a high content of phenolic compounds and vitamins (Papageorgiou et al., 2020). Buckwheat (Fagopyrum esculentum) is a pseudocereal that contains proteins with high biological value, as well as fiber, minerals, and flavonoids (Brites et al., 2019).
Replacing wheat flour with flour obtained from other raw materials changes both the rheological properties of the dough and the quality of the baked product. It is known that flours obtained from products other than wheat are not able to form the gluten network responsible for trapping the gas produced during fermentation (Arendt et al., 2002; Gallagher et al., 2003). Among different rheological techniques, Mixolab® has been likely used in many studies for probing dough behavior during processing conditions (Hadnadev et al., 2011). Mixolab® is a device developed by the technology company Chopin for measuring the rheological properties of doughs, which can be used to determine both the starch properties and the physical properties of doughs such as stability and strength. Mixolab® kneads the dough between two kneading arms and simultaneously subjects it to temperature changes. At the same time, the torque (Nm) achieved in the kneading arms is measured in real time (Anonymous, 2005).

There are a limited number of methods for determining the suitability of wheat flour for different end uses (Angioloni and Collar, 2011). The Mixolab® instrument is used to determine the rheological properties of gluten-free oat-based products (Duta and Culetu, 2015), to evaluate the quality of bread wheat (Şahin et al., 2014), and to determine the nutritional and rheological properties of grape seed flour (Mironeasa et al., 2012). Moreover, the Mixolab® instrument is used to determine the effects of additives on the kneading and baking properties of dough (Huang et al., 2010), the rheological properties of gluten-free flours from buckwheat and rice flour (Torbica et al., 2010), the rheological properties of the particle size of the dough in chestnut flour. In determining the effects on the behavior of flour (Moreira et al., 2010), determining the effects of hydrocolloids (Rosell et al., 2007), assessing the suitability of flour in terms of cake quality (Kahraman et al., 2008), determining the effects of enzymes on rheology (Bonet et al., 2006) was used.

An example of a Mixolab® diagram is shown in Figure 1. The diagram consists of five parts. In the first phase, the dough temperature is kept constant at 30°C and the dough’s kneading properties, such as stability, elasticity and water retention, are measured. In this phase, the torque (Nm) exerted by the pallets increases until the deformation of the dough starts and reaches the maximum. The stability of the dough is expressed as the time (min) that the dough resistance remains above the torque of 1.1 ± 0.05 Nm (Anonymous, 2005). In terms of consistency and stability of the dough, it is desirable that the dough reaches a torque of 1.1 Nm and remains in this range for a long period of time. The longer this period, the stronger the protein structure (Rosell et al., 2007). In the second phase, the temperature is gradually increased from 30°C to 60°C. As the temperature increases, protein denaturation begins and the resistance of the dough to the pallets decreases. The angle determined in this phase indicates the slope of the curve drawn from the end of the C1 period at 30°C to the end of the C2 period at 60°C and is used to evaluate the rate of protein attenuation by heat (Anonymous, 2005). In the third phase, when the temperature is increased to 90°C, there is an increase in consistency due to gelatinization of starch, which increases the torque acting on the pallets. At this stage, the starch molecules swell, absorb water, and displace the amylase molecules from the structure, causing an increase in viscosity (Kahraman et al., 2008). The β-angle determined at this stage indicates the slope of the curve between C2 and C3 and indicates the gelation rate (Anonymous, 2005). The steeper this angle is, the lower the dough viscosity, i.e., the harder the dough is, and in the opposite case, the dough is considered softer or liquid. In the fourth stage, where the temperature is kept constant, there is a decrease in consistency due to amylolytic activity. The γ-angle shows the slope of the curve between C3 and C4 and indicates the enzymatic degradation rate (Anonymous, 2005). Based on this value, an idea of the amylose activity of the product can be obtained. In the fifth stage, the temperature is gradually lowered from 90°C to 50°C. In this range, the gelling starch starts to liquefy and the retrogradation of the starch is detected here.

![Figure 1. Typical Mixolab® diagram for dough](image)

There are studies in which the rheological properties of wheat flour, wholegrain wheat flour, buckwheat flour, amaranth flour, rice flour, corn flour, soybean flour (Hadnadev et al., 2011), sprouted whole wheat flour (Liu et al., 2017), oat dough (Huang et al., 2010), finger millet (Sharma et al., 2017), pearl millet-based composite flour (Awolu, 2017), millet flour (Makout et al., 2016), yellow pea flour (Dabija et al., 2017), Cannabis sativa L. skimmed flour (Apostol et al., 2015), grape epicarp flour (Oprea et al., 2018), cassava flour (Manano et al., 2021), milk thistle flour (Bojiňská et al., 2020), defatted mustard seeds (Mironeasa and Codină, 2017) triticale, rye, hulless barley, rice, maize (Sabovićs et al., 2011), flaxseed flours (Codină et al., 2019), tomato seed flours (Mironeasa and Codină, 2019), legume flours (Bojiňská et al., 2021), quinoa and potato flours (Rodriguez-Sandoval et al., 2012), hulless barley flours (Moza and Gujral, 2018), grape seed flour (Mironeasa et al., 2012), hemp flour (Svec and Hruskova, 2015), chestnut flour (Moreira et al., 2012), Agaricus bisporus (Zhang et al., 2019) were determined using Mixolab®. However, there are no literature data on the determination of rheological...
properties of chickpea flour and carob flour using Mixolab®. For barley, oat, and buckwheat flours, there are few data.

The aim of this study was to evaluate the effects of adding 5 flours (barley, oats, chickpea, buckwheat and carob) to wheat flour in different amounts on dough formation and rheological properties using the Mixolab® device (gluten, kneading index, amylase activity, retrogradation, water removal from the flour). In examining the literature data, studies on the determination of rheological properties using the Mixolab® are limited. In this study, alternative flours are added to wheat flour and their effects on the rheological behavior of the dough are investigated. In addition, some chemical compositions of the alternative flours are determined.

2. Material and Methods

2.1. Materials

Wheat flour (0.7 < % ash ≤ 0.8) was provided by Birsan Birlik Gıda San. AŞin Tokat, Turkey. Alternative flours (carob, chickpea, oat, barley, and buckwheat) were purchased from a local company. All flours were stored in a cool and dry environment until use. All chemicals used in this study were from Sigma Chemical Company (MO, USA) or Merck KGaA (Germany) or Alfa Aesar GmbH and Co KG (Germany).

2.2. Methods

The dry matter of the flours were determined by the gravimetric method (AOAC, 2000). The total carbohydrate content of the samples was determined by the phenol-sulfuric acid method (Geater and Fehr, 2000). The micro-kjeldahl method was used to analyse the nitrogen content of the samples (AOAC, 2000). Crude protein content was estimated using a conversion factor of 5.75. Neutral detergent fiber were determined with the Ankom Fiber Analyser (Ankom Technology Corp., Macedon, NY, USA), following the Ankom Technology Method. Total fat were determined with Ankom Fat Analyser (Ankom Technology Corp., Macedon, NY, USA), following the Ankom Technology Method.

2.2.1. Determination of rheological behaviour using Mixolab®

Dough rheological investigations were performed by Mixolab® (Chopin, Tripette et Renaud, Paris, France), which simultaneously determines dough characteristics during the process of mixing at a constant temperature, as well as during the period of constant heating and cooling. All the measurements were performed using the modified Mixolab® 'Chopin' protocol (ICC No. 173) which parameters are presented in Table 1.

The flour mixtures used in the study are as follows. 4 different ratios of barley flour, 10, 20, 30 and 50 percent, were added to wheat flour. 3 different ratios of buckwheat flour, 10, 20 and 30 percent, were added to wheat flour. 4 different ratios of carob flour, 3, 6, 9 and 12 percent, were added to wheat flour. 4 different ratios of chickpea flour, 10, 20, 30 and 50 percent, were added to wheat flour. 4 different ratios of oat flour, 10, 20, 30 and 50 percent, were added to wheat flour. The amount of flour required for the analysis was calculated by the Mixolab® software based on the values entered for flour mixture moisture and water absorption.

2.2.2. Statistical analysis

SPSS statistical program (SPSS, Inc., Chicago, IL, USA) was used, variance analysis of the results (ANOVA) was performed and the differences between the groups were assessed statistically at a 95% confidence interval by the Duncan multiple comparison test.

3. Results and Discussion

Five different alternative flours were added to wheat flour. The composition of the added flours is shown in Table 2. Protein content varies between 4.6-7.5% (P=0.05). The fat content ranges from 0.5-7.5%. Oat flour has the highest fat content, while buckwheat flour has the lowest fat content. There is a statistically significant difference between the fat content of the flours (P<0.05). The fiber content is high in all added flours, with carob flour standing out as the flour with the highest fiber content at 40.1%. Chickpea and oat flour are characterized by their high protein and fat content, while chickpea and carob flour are characterized by their high fiber content. The high fiber content and composition are similar to those reported in the literature (Papaefstathiou et al., 2018; Papageorgiou et al., 2020).

The Mixolab® is an instrument that is used to determine the rheological quality of flour and to more accurately describe its behavior during bread making. The Mixolab® technique allows the complete characterization of the flours in terms of quality of proteins by determining their water absorption, stability, elasticity, and weakening properties; starch behavior.
during gelatinization and retrogradation; consistency modification when adding additives and enzymatic activity of the proteases, amylases, etc. (Stoenescu et al., 2011). This device provides, a complex analysis of the rheological properties of wheat flour dough, considering dough behavior during mixing, protein coagulation, heating-up behavior at enzyme activity intensification, and starch gelatinization and retrogradation during the final cooling (Blandino et al., 2015). In this study, five alternative flours (barley, oats, carob, buckwheat, and chickpea) were mixed with wheat flour in different ratios, and the rheological properties of the obtained mixtures were determined separately using the Mixolab® instrument (Table 3).

Table 2. Chemical composition of flours

<table>
<thead>
<tr>
<th></th>
<th>Wheat Flour</th>
<th>Chickpea Flour</th>
<th>Buckwheat Flour</th>
<th>Carob Flour</th>
<th>Oat Flour</th>
<th>Barley Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>13.20±0.24</td>
<td>9.60±0.30</td>
<td>8.50±0.40</td>
<td>6.90±0.56</td>
<td>10.20±0.60</td>
<td>10.55±1.02</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>11.00±0.40</td>
<td>22.00±0.86</td>
<td>12.00±0.90</td>
<td>4.60±0.14</td>
<td>9.60±0.34</td>
<td>8.20±0.11</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>0.50±0.10</td>
<td>4.80±0.62</td>
<td>0.50±0.10</td>
<td>0.70±0.15</td>
<td>7.50±0.20</td>
<td>1.50±0.05</td>
</tr>
<tr>
<td>T. Carbohydrate</td>
<td>76.00±2.60</td>
<td>61.00±1.45</td>
<td>74.00±2.14</td>
<td>89.00±2.60</td>
<td>54.20±1.58</td>
<td>61.50±1.60</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>1.10±0.10</td>
<td>19.00±1.20</td>
<td>10.20±0.78</td>
<td>40.10±1.50</td>
<td>13.80±0.76</td>
<td>14.40±0.72</td>
</tr>
</tbody>
</table>

\(a,b=\) indicate statistical differences at the P<0.05 level of the samples in the same line.

Table 3. Mixolab analysis results of flours

<table>
<thead>
<tr>
<th></th>
<th>Development Time</th>
<th>Stability Time</th>
<th>C2 Torque (Nm)</th>
<th>C3 Torque (Nm)</th>
<th>C4 Torque (Nm)</th>
<th>C5 Torque (Nm)</th>
<th>(\alpha)</th>
<th>(\beta)</th>
<th>(\gamma)</th>
<th>Water Absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(min)</td>
<td>(min)</td>
<td>(Nm)</td>
<td>(Nm)</td>
<td>(Nm)</td>
<td>(Nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley Flour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10%</td>
<td>5.58±0.2</td>
<td>850±0.1</td>
<td>0.80±0.1</td>
<td>1.95±0.0</td>
<td>1.87±0.1</td>
<td>2.69±0.1</td>
<td>-0.96</td>
<td>0.398</td>
<td>-0.55</td>
<td>60.59±0.1</td>
</tr>
<tr>
<td>20%</td>
<td>5.18±0.1</td>
<td>842±0.1</td>
<td>0.50±0.1</td>
<td>2.04±0.1</td>
<td>1.94±0.1</td>
<td>2.81±0.2</td>
<td>-0.99</td>
<td>0.414</td>
<td>-0.32</td>
<td>61.99±0.1</td>
</tr>
<tr>
<td>30%</td>
<td>4.70±0.3</td>
<td>925±0.1</td>
<td>0.50±0.1</td>
<td>2.06±0.1</td>
<td>1.95±0.1</td>
<td>2.79±0.1</td>
<td>-0.80</td>
<td>0.574</td>
<td>-0.36</td>
<td>63.69±0.1</td>
</tr>
<tr>
<td>50%</td>
<td>4.25±0.1</td>
<td>11.0±0.1</td>
<td>0.50±0.1</td>
<td>2.13±0.1</td>
<td>1.98±0.1</td>
<td>2.70±0.1</td>
<td>-0.86</td>
<td>0.704</td>
<td>-0.44</td>
<td>66.89±0.1</td>
</tr>
</tbody>
</table>

Barley flour was added to wheat flour in four different amounts (10, 20, 30 and 50%). The dough development time, which was 4.70 for the control flour, increased with the addition of barley flour. As the amount of barley flour added increased, the dough development time began to decrease. After the addition of 30% barley flour, the dough development time falls below that of the control flour. The addition of buckwheat and chickpea flour increased the dough development time at all rates. The addition of carob flour gradually increased the dough development time. The addition of oat flour up to 30% increased the dough development time, which tended to decrease at higher addition rates. It is believed that the decrease in dough development times is due to the free phenolic substances in the alternative flours. This can be explained by the ability of the phenolic compounds to react with the sulfhydryl groups of the gluten protein or to increase the rate of sulfhydryl-disulfide exchanges in the protein. For example, the addition of phenolic acids to dough reduces mixing time, tolerance, elasticity, and bread volume (Han and Koh, 2011).
The development time of buckwheat, barley and oat flour was higher than reported in the literature (Hadnadev et al., 2011; Huang et al., 2010; Sabovics et al., 2011). The term expressed as "water holding capacity" in Table 3 is the amount of water taken by the samples until they reach a torque of 1.1 Nm during kneading (Şahin et al., 2014). In Mixolab® charts, C1 (initial maximum consistency (Nm)) is used to determine the water absorption; torque at the end of the holding time at 30 °C (Nm) capacity. The water-holding capacity of the control flour, which was 58.9%, increased for all alternative flour additives. The highest increase, 85.4%, was measured in the mix with 30% buckwheat. The development time refers to the time elapsed until the dough first begins to form, and the composition of the dough affects it (Rosell et al., 2007).
The fact that the fiber content of all added flours is higher than that of wheat flour is thought to be the main reason for the increase in water-holding capacity. Dough stability is defined as the time the dough that stands the applied force after reaching its maximum torque. Also, it refers to the resistance of dough to kneading (Rosell et al., 2007). Dough stability (min) or time until the loss of consistency is lower than 11% of the maximum consistency reached during the mixing. The dough stability time of the samples are shown in Table 3 and Figure 2-6. The stability value was found to be 8.97 min for the control flour. Barley and oat flours were found to increase dough stability time, while buckwheat, carob, and chickpea flours decreased stability time. This decrease is probably due to the gummy substances (locust bean gum, natural hydrocolloids, etc.) in buckwheat, carob and chickpea flours. Protein destabilization occurs when the temperature rises from 30°C to 90°C during Mixolab® analysis. This part, which is expressed by the C2 value, is related to the protein content of the dough (Rosell et al., 2007). The C2 value is a data showing the degree of weakening of the proteins in the dough during kneading. In other words, C2 (minimum consistency (Nm)) can be explained as the minimum value of torque produced by the dough passage while being subjected to mechanical and thermal constraints. In this region, the torque value decreases with kneading and heating. The value of C2 torque was determined to be 0.49 Nm for the control flour. Except for the addition of barley flour, all flours decreased the C2 torque value. Depending on the amount added, the C2 torque value increased up to 0.58 Nm when barley flour was added. The highest decrease was observed with carob flour, and it was found to decrease to 0.33 Nm on average. It is assumed that the gum substances in the structure of carob flour cause this situation. It is desirable that the proteins in the dough do not weaken during kneading and maintain the network structure (Cappelli et al., 2020).

The second part of the Mixolab® curve contains the C3, C4 and C5 values, α-, β- and γ-angles. The C3 value is defined as the resistance of the dough to the kneading arms together with the gelatinization of the starch. In other words, C3 (peak torque (Nm)) can be explained as the maximum torque produced during the heating stage. The C3 torque value, which was 1.78 Nm for wheat flour, increased to 2.13 Nm with the addition of barley flour and to 1.93 Nm with the addition of carob flour. While the addition of buckwheat, chickpea and oat flours decreased the C3 torque value, the largest decrease was
The addition of carob flour to the mixture did not result in a significant change in the α-angle. The β angle is determined, where the temperature is increased to 90°C. This angle value gives the slope of the curve between C2 and C3 and shows the gelation rate. The steeper this angle, the higher the dough viscosity. In other words, the closer the slope is to 1, the higher the dough viscosity resulting the formation of harder dough. The β-angle was determined to be 0.572 for the control flour. It was found that the addition of barley, carob, and oat flours to the mixture caused a gradual increase in the β-angle, while chickpea and buckwheat flours gradually decreased the β-angle. Angle γ shows the slope of the curve between C3 and C4 and gives the rate of enzymatic degradation and gives an idea about the amylase activity of the product. While the γ-angle in the control flour was measured to be 0.012, the values closest to the control flour were determined in the mixtures with 20% oat flour and 10% chickpea flour. Mixtures with the addition of barley and buckwheat are the flours that reduce the γ-angle the most.

4. Conclusion

The addition of alternative flours to wheat flour is becoming more popular by the day due to the nutritional benefits of various plant sources. However, these raw materials other than wheat flour often have negative effects on the technological properties of flour, dough and final product. The results of this study show that the addition of alternative flours to wheat flour at different rates and their effects on rheological properties. The analysis of all rheological data shows that the addition of alternative vegetable flour has an influence on the rheological properties of the dough. Considering all the data, the amounts of addition that would improve or not affect the rheological properties when added to wheat flour were determined to be 30% for barley flour, 20% for buckwheat flour, 6% for carob flour, 20% for chickpea flour, and 30% for oat flour. All added flours increased the water-holding capacity of the dough. While the addition of barley, oat, and chickpea flours increased the dough stability time, buckwheat and carob flours reduced the C2 torque value, which determines the degree of protein weakening, more than other flours. The C3 torque value, an indicator of the degree of gelatinization of starch, decreased more in buckwheat and carob flours with higher content of gummy substances and increased in barley flours. Considering the degree of retrogradation ((C5-C4)/C5*100), it was found that the addition of barley and oat flour reduced the degree of retrogradation of the control flour to a minimum, while the decrease was greatest for buckwheat, carob and chickpea flours. Due to the growing world population and the resulting insufficient raw material resources, the search for alternative products continues day by day. Wheat flour is mixed with various flours to improve its nutritional and technological properties. In this context, it is necessary to
study all the positive and negative effects of adding alternative herbal flours to wheat flour. This study represents an innovation in the literature by determining the effects of the addition of carob and chickpea to wheat flour on rheological properties. It is intended to support the literature in determining the effects of the addition of barley, oats, and buckwheat to contribute to product manufacturing processes such as bread with the rheological data obtained.

**Author Contributions**
Concept: A.C. (50%) and T.Y. (50%), Design: A.C. (50%) and T.Y. (50%), Supervision: A.C. (50%) and T.Y. (50%), Data collection and/or processing: A.C. (50%) and T.Y. (50%), Data analysis and/or interpretation: A.C. (50%) and T.Y. (50%), Literature search: A.C. (50%) and T.Y. (50%), Writing: A.C. (50%) and T.Y. (50%), Critical review: A.C. (50%) and T.Y. (50%). Submission and revision. All authors reviewed and approved final version (50%) and T.Y. (50%).

**Conflict of Interest**
The authors declared that there is no conflict of interest.

**Ethical Consideration**
Ethics committee approval was not required for this study because of there was no study on animals or humans.

**References**


